EUROPEAN MULTICOLLOQUIUM OF PARASITOLOGY

Program & Abstract Book

EMOP XI

Cluj-Napoca, Romania
July 25-29, 2012
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Editorial

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XIth European Multicolloquium Of Parasitology

Cluj-Napoca – Romania
July 25th-29th, 2012

PROGRAM
&
ABSTRACT BOOK

IMPORTANT NOTICE

The abstracts included in this book are the proceedings of the "XIth European Multicolloquium of Parasitology", as provided by the authors.

Editorial

Dear Colleagues,

Welcome to Cluj-Napoca, Romania! Please find enclosed the program and the abstracts of communications to be presented to the XIth European Multicolloquium of Parasitology (EMOP XI, Cluj-Napoca, Romania, July 25th-29th, 2012).
The organizers of the 11th "European Multicolloquium of Parasitology" in Cluj-Napoca, Romania, aim at including it into the coordinates of the European traditionalism, already adopted at the previous multicolloquia in other European centers.
In the contemporary world, dominated by significant changes in all aspects of life - social, economic, scientific, and technical etc., from the smallest living organisms to mammals and humans, the scientific research in the medical field and in the field of biology has turned towards new main directions. In the universe of living organisms, parasites must be tackled and studied from evolutionary, co-evolutionary, and joint perspectives.
The re-defined medical research, based on interdisciplinary arguments shall ensure: a) a deepening of the investigations in the fields of molecular and parasite-ultrastructure biology, further directed towards those of ecology and eco-parasitology; b) a reassessment of the conflict between the host and the parasitic species, in which each partner is under possible changes; c) the monitoring of the diseases caused in the host (both in humans and animals) by parasitic species, with modified, and often insufficient, protective capacities.
For a correct diagnosis of parasitic diseases it is necessary for the trans-disciplinary scientific research to upgrade the diagnostic methods, supported by observations from nanology, immunology, genetics, eco-parasitology and cryoparasitology. The modern transportation means favor the displacement of both parasites and their biological vectors, expanding their developmental areas and introducing parasitic diseases to naïve regions of Earth. As a consequence, the epidemiological parameters of parasitoses in humans and animals are continuously changing, further complicating the diagnosis.
The problems encountered in prophylaxis and therapy impose the disclosure of new molecules, representing an alternative to classical treatments by use of natural, organic products, such as those used in plant based phyto-therapy. These types of compounds lack side effects and withdrawal periods for food products of animal origin (meat, milk, eggs etc.).
A relatively novel, but very important branch of parasitological research deals with development of antigen isolation techniques. In this branch, the improvement of isolation methods to obtain highly immunogenic antigens suitable for vaccine development has to be a priority, especially when it comes to aggressive, zoonotic parasites.
Similarly, investigations should be carried on, to clarify the therapeutic potential of parasitic products in human medicine, such as in cancer therapy, as well as the potential beneficial role of antagonistic parasitic relationships, i.e. hyper parasitism.
The organization of high level scientific events, in which relevant scientific and academic international partners take part, at regular intervals, as well as the support of joint publications (editing, co-editing proceedings volumes and books) and joint editing of internationally ranked journals and periodicals, may lead to the international spreading and acknowledgement of Parasitology.
We would like to wish you a fruitful congress.

Santiago Mas-Coma	**Monica Junie**	**Vasile Cozma**
(President of European Federation of Parasitologists)	(Vice-president of National Organising Committee)	(President of National Organising Committee)

Committiees

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The Honorary president of EMOP XI is Doina Codreanu-Bălcescu.

Summary

Map

1. Aula Magna
2. Life Sciences Institute (Navy-Blue and Green Amphitheatres)
3. A3 Amphitheatre
4. A4 Amphitheatre
5. Blue Amphitheatre
6. Biodiversity Center
7. Library
8. Lunch-Zone
9. Main Entrance

Detailed Program

24.07.2012, TUESDAY

18:00 - 20:00 **Registration and secretariat** - Grand Hotel Napoca
20:00 - 22:00 **Official Reception*** - Grand Hotel Napoca

25.07.2012, WEDNESDAY

DAY 1

Grand Hotel Napoca (25.07)

08:00 - 10:30 **Registration and secretariat**

09:00 - 10:15 **Opening Ceremony**
09:00-09:10 / **Welcome Speech**
Prof. Dr. Dr. Honoris Causa Santiago Mas-Coma, President of the European Federation of Parasitologists
09:10-09:15 / **Welcome Speech**
Prof. Dr. Vasile Cozma, President of the EMOP XI Local Organizing Committee
09:15-09:20 / **Rector's message: University of Medicine and Pharmacy "Iuliu Haţieganu" Cluj-Napoca**
Assoc. Prof. Dr. Alexandru Irimie
09:20-09:25 / **Rector's message: University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca**
Prof. Dr. Doru Pamfil
09:25-09:55 / **Speech of local and national authorities**
09:55-10:05 / **Anniversary of 100 years from Prof. Dr. Ernest M. Ungureanu's Birth**
Prof. Dr. Eugen Tarcoveanu (Iaşi, Romania)
10:05-10:15 / **Tribute to Professor Alexandru Niculescu**
Prof. Dr. Ion Didă (Bucharest, Romania), Assist. Prof. Dr. Eugenia Avram (Bucharest, Romania)

10:15 - 10:30 Coffee break** - Grand Hotel Napoca

10:30 - 12:30 **Plenary Session (PS01)**
Chairmen: *Prof. Dr. Santiago Mas-Coma*, *Prof. Dr. Jean Dupouy-Camet*, *Prof. Dr. Vasile Cozma*. Secretary: *Dr. Andrei Daniel Mihalca*
10:30-11:30 / **Neglected Tropical Diseases: action roadmap for the present decade**
Dr. Lorenzo Savioli (World Health Organization, Geneva, Switzerland)
11:30-12:30 / **Trends in parasitic diseases: past, present and future**
Prof. Dr. David J. Rogers (Oxford, UK)

12:30 - 13:30 Lunch** - Grand Hotel Napoca

Life Sciences Institute (25.07)

12:30 - 18:00 **Registration and secretariat**

Aula Magna Mihail Şerban (25.07)

13:45 - 16:00 **New insights in tick-borne diseases control (SY00) (Sponsored by Merial)**
Chairmen: *Dr. Steffen Rehbein, Dr. Mihai Cernea.* Secretary: *Dr. Ovidiu Şuteu*
Keynote presentations:
13:45-14:15 / **The dynamic of tick-borne pathogen transmission**
Dr. Muriel Vayssier-Taussat (Maisons Alfort, France)
14:15-14:45 / **Common tick-borne rickettsioses for humans in Europe**
Dr. Cristina Socolovschi (Marseille, France)
14:45-15:15 / **A new approach in tick control in dogs**
Dr. Guillaume Convert (Lyon, France)
15:15-16:00 / Discussion

16:00 - 16:15 Coffee break** - At venues

16:15 - 18:00 **Immunopathology of parasitic infections (SY15)**
Organiser: *Prof. Dr. Maria Adela Valero (Valencia, Spain)*
Chairmen: *Prof. Dr. Manuel Fresno, Assist. Prof. Dr. Nicodim Fiţ.* Secretary: *Dr. Mihaela Niculae*
Keynote presentations:
16:15-16:45 / **Immunological aspects in *Trypanosoma cruzi* infection**
***Prof. Dr. Manuel Fresno* (Madrid, Spain)**
Authors: Fresno M., Cuervo H., Sanoja C., Carbajosa S., Santi-Rocca J., Guerrero N, Gironès N.
16:45-17:15 / **Host-parasite interaction in experimental fascioliasis**
***Prof. Dr. Maria Adela Valero* (Valencia, Spain)**
Oral presentations:
17:15-17:30 / **Prostaglandin E_2 as a Cestodes Immunomodulator**
Kutyrev A., Scharsack J.P., Biserova N.M., Kurtz J.
17:30-17:45 / **Relationship between an inflammatory mucosal T Cell response and susceptibility of sheep to *Teladorsagia circumcincta* infection**
Venturina V.M., Gossner A.G., Hopkins J., Taylor D.W.
17:45-18:00 / **Parasites and cancers: parasite antigens as possible targets for cancer immunotherapy**
Darani H.Y., Yousefi M.

Navy Blue Amphitheatre (25.07)

16:15 - 18:00 **Drug resistance in parasites (SY26)**
Chairmen: *Dr. Ian Sutherland, Assoc. Prof. Dr. Irina Brumboiu*. Secretary: *Dr. Mihai Cernea*
Keynote presentations:
16:15-16:45 / **Anthelmintic resistance in nematodes infecting livestock: How to win the battles and prolong the war**
Dr. Ian Sutherland (Palmerston North, New Zeeland)
16:45-17:15 / **Actual aspects in the evaluation of etiological therapies**
Assoc. Prof. Dr. Irina Brumboiu (Cluj-Napoca, Romania)
Oral presentations:
17:15-17:30 / **Genetic variability in β-tubulin isotype 1 in benzimidazole resistant/susceptible *Haemonchus contortus* from sheep population in Rawalpindi, Pakistan**
Irum S., Stear S., Qayyum M., Mirza B.

17:30-17:45 / **Resistance to several classes of antihelmintics is not associated in parasitic nematodes: why should it be?**
Cabaret J.
17:45-18:00 / **Comparative evaluation of drug resistance tests in equine strongylidosis**
Cernea M., Cernea L.C., Cozma V., Madeira de Carvalho L.

Green Amphitheatre (25.07)

16:15 - 18:00 **Veterinary parasitology (SY25/1)**
Organiser: ***Prof. Dr. Claudio Genchi (Milano, Italy)***
Chairman: ***Prof. Dr. Marina Spînu, Dr. Menelaos Lefkaditis.*** Secretary: *Dr. Daniel Mărcuțan*

Oral presentations:
16:15-16:30 / **Laboratory mice experimental infestation with *Trichinella spiralis* larvae from frozen pork meat**
Iacob O., Răileanu G., Niță C.
16:30-16:45 / **Deepening the molecular epidemiology of human and pig ascariasis**
Cavallero S., Snabel V., Otranto D., Latrofa M.S., Perrone V., D'Amelio D.
16:45-17:00 / **Clinical signs and clinicopathological findings in dogs with *Uncinaria stenocephala* infestation**
Lefkaditis M.A., Koukeri S.E, Eleftheriadis T.G.
17:00-17:15 / **The use of deltamethrin on farm animals: our experience on flea control of small ruminants**
Papadopoulos E., Farmakis G.
17:15-17:30 / **Evidence for the vertical transmission of *Babesia canis* in a litter of Central Asian Shepherd pups - the case study**
Mierzejewska E., Rodo A., Welc-Faleciak R.
17:30-17:45 / **Immunological changes in experimentally induced acute eimeriosis of lambs**
Spînu M., Cozma V.
17:45-18:00 / **Hematological and biochemical parameters in Carpathian goats that were digestive and pulmonary infested on pasture**
Iacob O., Solcan G., Pavel G.

Blue Amphitheatre (25.07)

16:15 - 17:15 **IV Symposium on bird schistosomes and cercarial dermatitis (SY08)**
Organiser: ***Prof. Dr. Petr Horák (Prague, Czech Republic)***
Chairmen: ***Prof. Dr. Petr Horák, Assist. Prof. Dr. Viorica Mircean*****.** Secretary: *Dr. Tatiana Băguț*

Keynote presentation:
16:15-16:45 / **Cercarial dermatitis: crucial moments in transmission of bird schistosomes**
Prof. Dr. Petr Horák (Prague, Czech Republic)

Oral presentations:
16:45-17:00 / ***Trichobilharzia regenti*: neuropathogenic effect of the most harmful species of bird schistosomes**
Lichtenbergová L., Horák P.
17:00-17:15 / **Pathogenic impact of bird schistosome *Trichobilharzia regenti* on immunomodulated murine host**
Chanová M.

17:15 - 18:00 **Epidemiology of zoonoses (SY23/1)**
Organiser: ***Prof. Dr. Ioan Stelian Bocșan (Cluj-Napoca, Romania)***
Chairmen: ***Prof. Dr. Ioan Stelian Bocșan, Assist. Prof. Dr. Viorica Mircean*****.** Secretary: *Dr. Tatiana Băguț*

17:15-17:30 / **Human cryptosporidiosis in France: case notification and genotyping during the 2009-2011 period through the Anofel Cryptosporidium Network**
Villena I., Kapel N., Guyot K., Dutoit E., ANOFEL Cryptosporidium National Network
17:30-17:45 / ***Toxoplasma gondii* genotypes in humans and animals in Serbia**
Vujanic M.
17:45-18:00 / **Anatomo-clinical picture of the trichinellosis of the domestic pig experimentally infected with *Trichinella britovi***
Popescu L., Bărbuceanu F., Chițimia L., Didă I., Predoi G.

Biodiversity Centre (25.07)

13:45 - 16:00 **Meeting of SEEEP (Association of South Eastern and Eastern European Parasitologists)**
Organiser: ***Prof. Dr. Albert Marinculic (Zagreb, Croatia)***

16:00 - 16:15 Coffee break** - At venues

16:15 - 17:15 **Amoebae and free living amoebae (SY06)**
Chairmen: ***Dr. Hélène Yera, Prof. Dr. Herve Pelloux*****.** Secretary: *Dr. Violeta Briciu*

Keynote presentation:
16:15-16:45 / **Diagnosis of *Acanthamoeba* keratitis**
Dr. Hélène Yera (Paris, France)
Authors: Yera H., Dahane N., Maubon D., Dupouy-Camet J.

Oral presentation:
16:45-17:00 / **Serodiagnosis of extra-intestinal amoebiasis: validation of a new ELISA kit in non-endemic setting**
Beyls N., Faure O., Stahl J.P., Rogeaux O., Pelloux H.
17:00-17:15 / **Clinical and laboratory status of enteric symptomatic amebiasis in patients**
Shirbazou S., Khedmat H., Izadi M., Nakhaei F., Hosseini M. J.

17:15 - 18:15 **Parasites of insects with economic importance (SY22)**
Organisers: ***Prof. Dr. Liviu Mărghitaș (Cluj-Napoca, Romania), Dr. Silvio Erler (Cluj-Napoca, Romania)***
Chairmen: ***Prof. Dr. Robin Moritz, Assist. Prof. Dr. Dan Dezmirean*****.** Secretary: *Dr. Silvio Erler*

Keynote presentations:
17:15-17:45 / **The use of modern genetical tools for breeding parasite resistant honeybees**
***Prof. Dr. Robin Moritz* (Halle, Germany)**
17:45-18:15 / **Pollinator decline - possible causes and interactions in bumblebees**
Dr. Silvio Erler (Cluj-Napoca, Romania)

A3 Amphitheatre (25.07)

16:15 - 18:00 **Allergy and zoonoses (SY24)**
Organiser: ***Prof. Dr. Fabrizio Bruschi (Pisa, Italy)***
Chairmen: ***Prof. Dr. Fabrizio Bruschi, Dr. Ioana Colosi.*** Secretary: *Dr. Petrică Ciobanca*

Keynote presentations:
16:15-16:45 / **Allergy and zoonoses**
Prof. Dr. Fabrizio Bruschi (Pisa, Italy)

16:45-17:15 / **Ascarids of human importance: where are we now?**
Prof. Dr. Celia Holland (Dublin, Ireland)
17:15-17:45 / **Immunoregulation by zoonotic helminths and its effect on allergic diseases**
Dr. Elena Pinelli (Bilthoven, Netherlands)

Oral presentations:
17:45-18:00 / **Chronic intraperitoneal helminth infection disrupts short-term memory as well as neurotransmitter and cytokine levels in the hippocampus**
Morales-Montor J., Picazob O., Besedovsky H., C. Guzmán, Hernández-Bello R., López-Griego L., Pavón L., Becerril E.L., Morenof J., Nava-Castroa K., Camacho-Arroyo I.

A4 Amphitheatre (25.07)

16:15 - 18:00 **Meeting of the Romanian Association of Parasitologists (APR)**
Chairmen: *Prof. Dr. Doina Codreanu-Bălcescu, Prof. Dr. Ioan Cironeanu*. Secretary: *Dr. Cristian Magdaș*

MV Hall (25.07)

Session 1 **Chairman: *Dr. Sándor Sikó Barabási*.** Secretary: *Dr. Ioana Matei*

13:45 - 16:00 **Multidisciplinary analysis of Chagas disease (SY02.P)**

Poster presentations:
SY02.P.01 **Humoral response to the C-terminal conserved region of the mucin-associated surface protein (MASP) family of *Trypanosoma cruzi***
De Pablos L.M., Díaz Lozano I.M., Zulantay I., Jercic M.I., Ying A., Apt W., Osuna A.
SY02.P.02 **Distinctive modulation of cytokine production in oral and intraperitoneal *Trypanosoma cruzi* infection triggered by Cl and Y strains**
Kuehn C.C., Oliveira L.G.R., Miranda M.A., Peixoto R.V., Artigas P., Prado Jr. J.C.
SY02.P.03 **Anti-inflammatory protective actions of melatonin against heart damage during the chronic phase of Chagas' disease**
Oliveira L.G.R., Kuehn C.C., Miranda M.A., Peixoto R.V., Flores R., Artigas P., Prado Jr. J.C.

Leishmaniases and their vectors: present and future (SY03.P)

Poster presentations:
SY03.P.01 **Lipidomic of *Leishmania donovani* and *Leishmania infantum*: from promastigote to amastigote**
Azzouz S., Vandenbroucke I., Petavy A.F.
SY03.P.02 **Comparison of three antigen-based ELISAs in the diagnosis of mediterranean visceral leishmaniasis**
Ben Abda I., Lakhal S., Mekki S., Ben Sghaier I., Amri F., Aoun K., Bouratbine A.
SY03.P.03 **Clinical criteria and PCR-RFLP in the identification of tunisian cutaneaous leishmaniasis forms and *Leishmania* species involved**
Ben Abda I., N. Bousslimi, R. BenMously, J. Bettaieb, R. Ben Abdallah, E. Siala, O. Souissi,N. Zallagua, Z. Harrat, A. Bouratbine, K. Aoun
SY03.P.04 **In vitro leishmanicidal activity of a new benzimidazole derivative complexed with methyl-beta-cyclodextrin**
Rojas-Aguirre J., Bilbao-Ramos P., Dea-Ayuela M.A., Hernández-Luís F., Bolás-Fernández F.
SY03.P.05 **Lymphoproliferative response, pre and post-infection, in BALB/c mice immunized with the recombinant chimeric protein L25a-HSP70M2 of *Leishmania braziliensis***
Rodríguez Borges V.M., González García A.C., Bourgon Rodríguez M.G., Pou Barreto C., Martínez E., Valladares B.
SY03.P.06 **Cytokine profiles, histopathology and parasite load of experimental canine leishmaniasis**
Cortes S., Peleteiro C., Cristóvão J., Nunes M., Rolão N., Campino L., Maia C.
SY03.P.07 **In vitro biological behavior of *Leishmania infantum*, *L. major* and *L. infantum*/*L. major* hybrids from mediterranean basin**
Cortes S., Albuquerque A., Canudo J., Campino L.
SY03.P.08 **In vivo evaluation of the leishmanicidal activity of ursolic acid from *Erica* spp.**
Dea-Ayuela M.A., Bilbao-Ramos P., Ruiz H.K., Serrano-López D.R., López-Castillo C., Torrado J.J., Blanquer A., Bolás-Fernández F.
SY03.P.09 **Asparagopsis taxiformis: a novel anti-*Leishmania* therapy?**
Bruno F., Castelli G., Piazza M., Vitale F., Armeli Minicante S., Morabito M., Genovese G.
SY03.P.10 **Analysis of homology between the only known nucleoside transporter from *Leishmania braziliensis* and other higher eukaryotes**
Fumero Brito L., González Hernández A.J., Rodríguez Borges V.M., González García A.C., Valladares B.
SY03.P.11 **Nucleoside transporter from *Leishmania braziliensis*. Comparative analysis of sequences between this protozoa and other Trypanosmatids**
González Hernández A.J., Fumero Brito L., Rodríguez Borges V.M., González García A.C., Valladares B.
SY03.P.12 **Polimerase Chain Reaction and Real Time PCR for diagnosis of infection in dogs by *Leishmania infantum* using different biological samples**
Camara Alves L., do Nascimento Ramos R.A., do Nascimento Ramos C.A., de Araujo F.R., de Souza Pimentel D., de Sá Santos E.M., da Gloria Faustino M.A., de Azevedo Oliveira G.M.
SY03.P.13 **Quantification of *Leishmania infantum* DNA skin of naturally infected dogs using Real-Time Polymerase Chain Reaction**
Camara Alves L., do Nascimento Ramos R.A., do Nascimento Ramos C.A., de Araujo F.R., de Andrade Santana M., de Sá Santos E.M., da *Gloria Faustino M.A., Arruda N.*
SY03.P.14 **Canine leishmaniasis treatment in Portugal**
Maia C., Campino L.
SY03.P.15 **Feline leishmaniasis in Portugal**
Maia C., Campino L.
SY03.P.16 **The potential use of apathogenic Trypanosmatids as alternative source of antigen for serological screening of leishmaniasis in dogs**
Martinkovic F.
SY03.P.17 **Evaluation of in Vitro anti-leishmanial activity of *Pergularia tomentosa* L. (Asclepidaceae) from Bushehr Plains, Southwest Iran**
Fouladvand M., Barazesh A., Naeimi B., Sartavi K.
SY03.P.18 **Leishmaniasis in Morocco: ten years of cases reported**
Ouanaimi F., Kahime K., Boussaa S., Boumezzough A.
SY03.P.19 **Histopathological changes in liver and spleen of canine visceral leishmaniasis due to *Leishmania infantum***
Sarkari B., Manochehri Ardakani R., Jannesar R., Moshfea A.A., Postforosh A.
SY03.P.20 **Genotypes of *Leishmania* spp. imported to Poland**
Szostakowska B., Lass A., Pietkiewicz H., Szulta J., Nahorski W.L., Felczak-Korzybska I., Golian J., Wroczyńska A., Kowerzanow J., Borys S., Sulima M., Myjak P.
SY03.P.21 **Apoptotic activity of some natural and synthetic stilbene and terphenyl compounds against *Leishmania infantum* promastigotes**
Castelli G., Bruno F., Piazza M., Vitale F., Colomba C., Tolomeo M.

Plasmodium, *Anopheles* and malaria (SY04.P)

Poster presentations:

SY04.P.01 **Elimination of malaria in the Russian Federation**
Bogdanova E.

SY04.P.02 **Genetic characteristics of *Plasmodium vivax* apical membrane antigen-1 gene in isolates from Iran**
Rastaghi A.R.E., Nahrevanian H., Hoseinkhan N.

SY04.P.03 **Importance of socio-cultural context in reducing malaria infection**
Shahandeh K., Basseri H.R.

SY04.P.04 **Acute renal failure during severe malaria: a case report**
Siala E., Abid Z., Ben Abdallah R., Ben Abda I., Zallega N., Aoun K., Bouratbine A.

Toxoplasma and toxoplasmosis (SY05.P)

Poster presentations:

SY05.P.01 **Seroprevalence of toxoplasmosis among arthritis rheumatoid patients**
Rezavand B., Alishiri G.H., Sadraei J., Manafi M.R., Hossini M.J., Bagheri J.O.

SY05.P.02 **Positive association of *Toxoplasma gondii* infection and *Anisakis simplex* parasitism in chronic urticaria**
Cuéllar C., Fernández-Fígares V., Rodero M., Valls A., De Frutos C., Daschner A.

SY05.P.03 **ELISA and IFA in testing the serologic profile of toxoplasmosis among a sample of municipality workers in Dubai – UAE**
Khalil M.M., Sultan D.M., Hamad M.

SY05.P.04 **Effect of bee venom on *Toxoplasma gondii* tachyzoites in vitro**
Rezavand B., Piranfar V., Khoobdel M., Bagheri O., Joneidi N., Izadi M.

SY05.P.05 **Toxoplasmosis and immunosupression: reactivation in a case of dual (genetic and transplantation-induced) immunodeficiency**
Zivkovic T., Vasiljevic Z., Ristic G., Vujanic M., Ivovic V., Djurkovic- Djakovic O.

SY05.P.06 **Infection of human nervous cells by different strains of *Toxoplasma gondii* in vitro: analysis of neuronal cytokine and chemokine expression profiles**
Mammari N., Riahi H., Gatet M., Dardé M.L., Courtioux B.

SY05.P.07 **Seroprevalence of *Toxoplasma gondii* antibodies and profile of CD4+ counts in HIV/AIDS paitents in North of Iran 2009-2010**
Meigouni M., Daryani A., Sharif M., Rafiei A., Baba Mahmoudi F., Mirabi A.M.

SY05.P.08 ***Toxoplasma gondii* evolves a new architecture for the multi-aminoacyl-trna synthetase complex**
Murat J.B., Van Rooyen J., Belrhali H., Hakimi M.A., Pelloux H.

SY05.P.09 **Validation of the Elecsys® Toxo IgG Avidity Assay for toxoplasmosis: new insights in evaluation of the time of infection?**
Murat J.B., L'Ollivier C., Fricker Hidalgo H., Franck J., Pelloux H., Piarroux R.

SY05.P.10 **Role of *Toxoplasma gondii* infection in serum level of testosterone**
Shirbazou S., Abbasian L., Talebi Meymand F.

SY05.P.11 **Resistance induction of *Toxoplasma gondii* strain by sulfadiazine pressure**
Doliwa C., Escotte-Binet S., Geers R., Aubert D., Villena I.

IV K.E. Mott Symposium on schistosomiasis and foodborne trematodiases (SY07.P)

Poster presentations:

SY07.P.01 **Fasciolosis in Mexican pediatric patients**
Calderón-Romero L., Romero-Cabello R., Romero-Feregrino R., Tay-Zavala J.

SY07.P.02 **Characterization of *Fasciola hepatica* strains from sheep susceptible and resistant to anthelmintics using mitochondrial DNA markers**
Martínez-Valladares M., Manga-González M.Y., Rojo-Vázquez F.A.

SY07.P.03 **Molecular cloning and characterization of cathepsin L3 from *Fasciola hepatica***
Zawistowska-Deniziak A., Wasyl K., Bien J., Wesolowska A., Grodzik M., Baska P., Wedrychowicz H.

SY07.P.04 **Liver fluke phenotypic characterization in Andean human endemic areas: altiplanic versus valley patterns**
Pérez-Crespo I., Khoubbane M., Artigas P., Peixoto R., Debenedetti A., Mas-Coma S., Valero M.A.

SY07.P.05 **Uterus development correlated with egg-shedding in *Fasciola hepatica***
Pérez-Crespo I., Panova M., Khoubbane M., Peixoto R., Artigas P., Debenedetti A., Mas-Coma S., Valero M.A.

SY07.P.06 **Efficacy of triclabendazole pour-on against *Fasciola hepatica* in cows in tropical Mexican region**
Jiménez Y.K., Alonso D.M.A., Quiroz R.H.

SY07.P.07 **Amplification and evaluation of a phage display clone as immunogen against *Fasciola hepatica* in sheep**
Quiroz R.H., Perez J.L., Alonso R., Gayosso V.A., Villa M.A., Figueroa C.A., Cruz M.I., Hernández G.K., Flores V.L.M.

SY07.P.08 **Frequency and intensity of adult and immature *Fasciola hepatica* in Veracruz, México**
Paz F.C.P., Corro M.M.D., Cruz M.I., Quiroz R.H.

SY07.P.09 **Fascioliasis in cattle from Western Argentina: study of hematological and biochemical parameters**
Sidoti L., Mera y Sierra R.L., Gerbeno L., Fantozzi C., Sohaefer N., Spongia S., Neira G.

Intestinal and larval echinococcoses (SY10.P)

Poster presentations:

SY10.P.01 **Specific anti-hydatid IgG subclasses for the diagnosis of primary infection and relapses of cystic echinococcosis**
Ben Abid M., Galai Y., Nouira R., Ben Abda I., Bouchoucha S., Bouratbine A., Aoun K.

SY10.P.02 **Mitochondrial sequence diversity of *Echinococcus multilocularis* isolates from humans in Southern Germany**
Ebi D.A., Barth T., Dinkel A., Schroer S., Mackenstedt U., Romig T.

SY10.P.03 **Cross reaction between the crude hydatid cyst fluide (Hcf) antigens of human and animals origin (mice, sheep, cattle) in response to human IgG class, IgG subclasses and IgM antibodies**
Fallah M., Shamsi M., Khosravi A., Maghsood A.

SY10.P.04 **Use of the recombinant antigen 2B2t in a commercial immunochromatographic test for the diagnosis of cystic echinococcosis**
Hernandez-Gonzalez A., Brunetti E., Tamarozzi F., Meroni V., Genco F., Junghanss T., Stojkovic M., Delgado J.M., Soriano F.M., Siles-Lucas M.

SY10.P.05 **Genotypes of Echinococcus granulosus vomplex in Central-Eastern Europe**
Šnábel V., Kuzmina T., Calma C., Georgescu S.O., Szénási Z., Emets A., Neghina R.

SY10.P.06 **Inter- and intraspecific diversity of *Echinococcus* spp. in Southern Kenya**
Wassermann M., Addy F., Zeyhle E., Mbae C., Mackenstedt U., Romig T.

SY10.P.07 **Cystic echinococcosis in slaughtered cattle in Sardinia: a retrospective epidemiological study and spatial analysis**
Brundu D., Aloi D., Rolesu S., Piseddu T., Masala G.

SY10.P.08 **A retrospective study on burden of human echinococcosis based on hospital discharge records from 2001 to 2009 in Sardinia, Italy**
Mastrandrea S., Stegel G., Brundu D., Piseddu T., Ledda S., Masala G.

Session 2 **Chairman: *Dr. Ioniță Mariana*.** Secretary: *Dr. Ioana Matei*

Detailed Program

16:15 - 18:00 **Vectors and vector-borne diseases (SY14.P)**

Poster presentations:

SY14.P.01 **Discrimination of *Culicoides* of *Avaritia* subgenus by Multiplex PCR**
Sarvašová A., Goffredo M., Kočišová A., Bocková E.

SY14.P.02 **First detection of tick-borne encephalitis virus in *Ixodes ricinus* ticks collected in France since the 70s**
Umhang G., Devillers E., Demerson J.M., Caillot C., Schaeffer J., Boué F., Moutailler S.

SY14.P.03 **Some epidemiological and clinico- biological aspects in human neuroboreliosis**
Costache D., Costache C., Bogdan A.T.

SY14.P.04 **Current distribution and predicted range expansion of the sand fly vector *Phlebotomus neglectus* (Diptera, Psychodidae) in the Eastern Mediterranean**
Ivovic V., Djokic V., Vujanic M., Djurkovic-Djakovic O.

SY14.P.05 **Sandflies of the subgenus *Larroussius* in the region of El Haouz, Morocco**
Kahime K., Ouanaimi F., Boussaa S., Boumezzough A.

SY14.P.06 **Prevalence of ixodid ticks on cattle and sheep in Sistan and Baluchestan provinces, Iran**
Mirzaie M., Khedri J.

SY14.P.07 **The presence and genetic variability of *Anaplasma phagocytophilum* and candidatus *Neoehrlichia mikurensis* in rodents as reservoir hosts in Slovakia**
Pangrácová L., Stanko M., Víchová B., Mošanský L., Bona M., Fričová J., Peťko B., Derdáková M.

SY14.P.08 **Breed characteristics of host influence the intensity of tick infestation in ruminant livestock**
Sajid M.S., Saqib M., Iqba, Z., Khan M.N.

SY14.P.09 **Questing ticks (Ixodida) collected during expedition in Croatia 2011**
Stanko M., Bona M., Mošanský L., Víchová B., Peťko B.

SY14.P.10 **Detection and prevalence of sibling species of the *Anopheles maculipennis* complex (Diptera: Culicidae) in the Republic of Moldova**
Sulesco T., Toderas I., Toderas L.

SY14.P.11 **Enzymatic and functional characterization of the *Leishmania major* Protein Disulfide Isomerase (Lmpdi) as a drug target**
Ben Khalaf N., DeMuylder G., Ratnam J., Kean-Hooi Ang K., Arkin M., McKerrow J., Chenik M.

SY14.P.12 ***Leishmania major* large Rab GTPase (LmRAB) protects BALB/c mice against a *L. major* challenge and is highly Immunogenic in immune leishmaniasis individuals**
Chamakh-Ayari R., Garnaoui A., Markikou W., Aoun K., Chenik M.

SY14.P.13 **Serologic screening of vector-borne infections in dogs from Western Romania: current status**
Ilie M.S., Imre K., Hotea I., Imre M., Sorescu D., Andrei S., Ilie A., Morariu S., Morar D., Dărăbuș G.

SY14.P.14 **Olfactometry phyto preferendum for ixodid ticks in the steppe landscape of Armenia**
Rukhkyan M.

SY14.P.15 **Detection of naturally infected vector ticks by different species of *Babesia* and *Theileria* agents in three different enzootic parts of Iran**
Abdigoudarzi M., Nam-Avari M.M, Habibi G.

SY14.P.16 **First cases of *Bartonella bovis* infection in cattle from Poland**
Welc-Falęciak R., Mierzejewska E., Grono K.

SY14.P.17 **New data on *Dermacentor reticulatus* expansion in Central Europe**
Mierzejewska E., Kowalec M., Bajer A.

SY14.P.18 ***Loa loa* and *Mansonella perstans* infection associated with fatal progressive malignancy**
Pavlovic M., Dakic Z., Milosevic I., Dulovic O., Ofori-Belic I., Milosevic B., Mitrovic S., Dzamic A.

SY14.P.19 **A new Real Time PCR based on OmpA gene for specific *Rickettsia conorii* detection and quantization**
Blanda V., Di Marco V., Scimeca S., D'Agostino R., Torina A.

SY14.P.20 **The promising use of *Babesia bigemina* Apical Membrane Antigen-1 in a new ELISA diagnostic test**
Torina A., Blanda V., Agnone A., Di Marco V., Caracappa S., La Farina M., Sireci G., Albanese I.

SY14.P.21 **Prevalence and abundance of *Culicoides imicola*, *C. obsoletus* and *C. pulicaris* in Palermo province, Italy**
Torina A., Scimeca S., Blanda V., D'Agostino R., Ferrara MC., Di Marco V.

Reproduction, developmental biology and ultrastructure of parasites (SY17.P)

Poster presentations:

SY17.P.01 **TEM Study of the tegument of adult *Maritrema feliui* (Digenea: Microphallidae)**
Świderski Z., Bakhoum A.J.S., Montoliu I., Feliu C., Gibson D.I., Miquel J.

SY17.P.02 **Ultrastructure of the early embryonic stages of *Maritrema feliui* (Digenea: Microphallidae)**
Świderski Z., Bakhoum A.J.S., Montoliu I., Feliu C., Gibson D.I., Miquel J.

SY17.P.03 **Pro-inflammatory changes in the intestine determine number and protein composition of *Heligmosomoides polygyrus* L4**
Donskow-Łysoniewska K., Bien J., Brodaczewska K., Doligalska M.

SY17.P.04 **The infection biology of a diplozoid found on *Labeo umbratus* Smith, 1841 in the Vaal Dam, South Africa**
Dos Santos Q.M., Avenant-Oldewage A.

SY17.P.05 **A comparative study of the second internal transcribed spicer (ITS2) of ribosomal DNA of species *Haemonchus contortus* and *H. placei* (Nematoda: Trichostrongylidae)**
Kuchboev A.E., Abramatov M.B., Khalilov I.M., Abdurakhmanov I.Y., Azimov D.A.

SY17.P.06 **Darkling beetles (Coleoptera) as intermediate hosts of Spiruroidea (Nematoda) parasites of Murinae (Rodentia) in El Hierro (Canary Islands, Spain)**
Montoliu I., Abreu-Acosta N., Villa M., Foronda P.

SY17.P.07 **Molecular and morphometric analysis of heterophyid trematodes; collected from freshwater fishes in Nan Province, Thailand**
Namchote S., Krailas D., Boonmekam D., Plai-Ngam C., Nakai W.

SY17.P.08 **Synanthropization of sand flies as a factor in increasing their epidemiological significance in the former USSR**
Bogdanova E.

SY17.P.09 **Spermatological characters of the Trypanorhyncha, with new data on the little-studied Superfamily Tentacularioidea**
Świderski Z., Marigo A.M., Bakhoum A.J.S., Eira C., Miquel J.

SY17.P.10 **Ultrastructure of vitellogenesis and vitellocytes in the trypanorhynch cestode *Aporhynchus menezesi*, a parasite of the velvet belly lanternshark, *Etmopterus spinax***
Świderski Z., Miquel J., Marigo A.M., Feliu C., Gibson D.I.

SY17.P.11 **Identification and classification of proteins expressed in the protozoan parasites *Pentatrichomonas hominis* using two-dimensional gel electrophoresis**
Fwu-Mann K., Kuo-Yang H, Tang P.

SY17.P.12 **Cercarial infections of freshwater mollusks at Pasak Cholasid Reservoir, Thailand**
Chuanprasit C., Krailas D., Namchote S., Dechruksa W., Pimkan S., Phuttharuksa K., Thongsuntud N.

SY17.P.13 **The neuro-muscular system in fresh-water furcocercaria. Comparative study**
Tolstenkov O., Terenina N., Akimova L., Prokofiev V., Gustafsson M.

Biodiversity, health and environment (SY19.P)

Poster presentations:

SY19.P.01 **Sequence diversity in the galectin loci from *Teladorsagia circumcincta***
Donskow-Łysoniewska K., Belch A.C., Murphy L., Stear M.J.

SY19.P.02 **Report of a new genotype of *Ehrlichia* species from cattle and cervids**
Gajadhar A., Scandrett B., Lobanov V., Al-Adhami B., Wobeser G., Campbell J.

SY19.P.03 **Detection of the most important species of *Cryptosporidium* for human health in river water of Iran by Gp60 primer**
Mahmoudi M.R., Jahantab S., Haghighi A., Kazemi B.

SY19.P.04 **Random preliminary screening of an expressed sequence tag library of *Dicrocoelium dendriticum***
Martínez-Ibeas A.M., Perteguer M.J., González-Lanza C., Garate T., Manga-González M.Y.

SY19.P.05 **Stages of interspecific and intraspecific interactions between helminthes**
Tarassovskaya N.E., Zhumabekova B.K., Syzdykova G.K., Shaimardanov Z.K.

SY19.P.06 **Detection and molecular characterization of *Cryptosporidium* species in recreational waters of Shahr-E-Kord District of Iran using nested-PCR-RFLP method**
Kourosh M.N.

Parasites of wildlife (SY20.P)

Poster presentations:

SY20.P.01 **Prevalence of antibodies to *Neospora caninum* and *Toxoplasma gondii* in red foxes (*Vulpes vulpes*) from Slovakia**
Čobádiová A., Reiterová K., Turčeková Ľ., Smrčo P., Oravec M., Dolinská M.

SY20.P.02 **Position of *Pholeter gastrophilus*, *Braunina cordiformis* and *Ogmogaster antarcticus*, parasites from cetaceans, in the molecular phylogeny of the Digenea**
Fraija N., Crespo E.A., Raga J.A., Aznar F.J., Fernández M.

SY20.P.03 **Gastrointestinal parasites in northern fur seals (*Callorhinus ursinus* L.) on St. Paul Island, Alaska**
Kuzmina T., Lyons E., Spraker T., Gelatt T., Kharchenko V.

SY20.P.04 **Antimalarial therapy and clinical manifestation of *Plasmodium relictum* infection in gyr falcons (*Falco rusticolus*)**
Königová A., Molnár L., Molnárová M., Ptáček M., Silvanose C.

SY20.P.05 **Metastrongilids of wild boars in Uzbekistan**
Kuchboev A.E., Umarov D.K., Karimova R.R., Ruziev B.K.

SY20.P.06 **Molecular evidence for the existence of two further sibling species of the *Contracaecum rudolphii* complex (Nematoda: Anisakidae) from the spotted shag, *Phalacrocorax punctatus*, a species of cormorant endemic to New Zealand**
Mattiucci S., Webb S.C. Paoletti M., Nascetti G.

SY20.P.07 **Prevalence of endoparasites in brown bears (*Ursus arctos*) from natural habitats in Romania**
Mircean V., Chivu R., Jurj R., Dumitrache M.O., Cozma V.

SY20.P.08 **The helminth fauna of wild ungulates animals in natural ecosystems of the Inner Tien-Shan**
Shermatov S.M.

SY20.P.09 **Study on the ectoparasites of chukar partridge, *Alectoris chukar* from Shaqlawa District, Kurdistan Region, Iraq**
Zhala O., Khoshnaw I., Shamall M., Abdullah A.

SY20.P.10 **The helminthes fauna of anural amphibians in Kazakhstan**
Tarassovskaya N.E., Zhumabekova B.K., Syzdykova G.K.

SY20.P.11 **Incidence and genetic characterisation of *Toxoplasma gondii* in red foxes (*Vulpes vulpes*) in Slovakia**
Turčeková Ľ., Hurníková Z.

SY20.P.12 **Molecular characterization of *Contracaecum rudolphii* (Nematoda: Anisakidae) from *Phalacrocorax carbo sinensis* from Sicily**
Costa A., Cavallero S., D'Amelio S., Alio V., Seminara L., Caracappa S.

Veterinary parasitology (SY25.P)

Poster presentations:

SY25.P.01 **A sensitive and specific PCR based method for identification of *Cryptosporidium* sp. using new primers from 18S ribosomal RNA**
Bairami Kuzehkanan A., Rezaeian M., Zeraati H., Mohebali M., Meamar A.R., Babaei Z., Kashi L., Heydarnezhadi M., Rezaie S.

SY25.P.02 **Seasonal prevalence of gastrointestinal nematodes in sheep at El-Beheira province, Egypt: eggs and third larval stage characterizations**
Bazh Eman K.A., Otify Y.Z., Rwash A.A., Menshawi Soad M.G.

SY25.P.03 **Identification of *Echinococcus multilocularis* in canids of Iran**
Beiromvand M., Razmjou E., Akhlaghi L., Fattahi Massom S.H., Mobedi I., Meamar A.R., Oormazdi H., Motevalian A.

SY25.P.04 **Serological survey of animal toxoplasmosis in Dakar and in Sine-Saloum (Senegal)**
Blaga R., Mediannikov O., Perret C., Thomas M., Demoncheaux J.P.[4], Tine R., Diarra M., Scandola P., Vallee I. , Guillot J., Villena I., Aubert D., Davoust B.

SY25.P.05 **Toxoplasmosis and trichinellosis: an epidemiological survey of pig population in Madagascar**
Rakotoharinome M., Andriamanivo H., Blaga R., Perret C., Lacour S.A., Grasset-Chevillot A., Mace P., Thomas M., Villena I., Aubert D., Boireau P., Porphyre V.

SY25.P.06 **Study of gastrointestinal nematodes in Sicilian sheep**
Caracappa S., Disclafani R., Marineo S., Piazza A., Cavallaro N., Licitra F., Torina A.

SY25.P.07 **Coproparasitological investigation in dogs from Southern Romania, with focus on cestodes**
Costin I., Enăchescu V., Ciopasiu R., Ioniță M., Mitrea I.L.

SY25.P.08 ***Neospora caninum* in dogs from Bucharest area, Romania: screening for seroconversion by indirect fluorescent antibody test**
Enăchescu V., Ioniță M., Bărboi G., Mitrea I.L.

SY25.P.09 **Molecular biology methods for detection and identification of *Demodex* mites**
Garbacewicz A., Sobieraj K., Lewin T., Grytner- Zięcina B.

SY25.P.10 **Molecular characterization of *Babesia* parasites from dogs in Banat region using PCR-RFLP**
Imre M., Ilie M.S., Imre K., Hotea I., Morariu S., Dărăbuș G.

SY25.P.11 **Detection and molecular characterization of *Babesia canis* and *Babesia vogeli* from naturally infected romanian dogs**
Ioniță M., Mitrea I.L., Pfister K., Hamel D., Buzatu M.C., Silaghi C.

SY25.P.12 **Strongylids (Nematoda; Strongylida) in equids at the Askania-Nova Biosphere Reserve, Ukraine: analysis of biodiversity of parasite community**
Kuzmina T., Kharchenko V., Zvegintsova N., Yakovlev E.

SY25.P.13 **A study on parasites of pigeon (*Columba livia domestica*) in Urban Area of Moldava n/Bodvou, Eastern Slovakia**
Komorová P., Kočišová A., Hurníková Z., Ondrejková A., Hapl E., Letková V.

SY25.P.14 **Evaluation of sodium acetate acetic acid formalin (SAF) versus 10 % formalin as preservative in the detection of intestinal parasites in stool**
Galal L.A., Yones D.A.

SY25.P.15 **Therapeutic approaches in canine demodecosis**
Mederle N., Sorescu D., Dărăbuș G., Morariu S., Marius I., Tilibas E.

SY25.P.16 **Canine toxocariasis in South East of Iran**
Mirzaie M.

SY25.P.17 **The prevalence, abundance, and distribution of small strongyles (Nematoda, Stongylidae) in horses from western Romania**
Morariu S., Tolliver S.C., Bogdan A.T., Dărăbuș G., Ilie M., Imre K., Imre M., Lyons E.T.

SY25.P.18 ***Cryptosporidium* spp. in fecal samples of canaries (*Serinus canaria*) in Brazil**
Nardi A.R.M., Guiguet Leal D.A., C.M.B. Oliveira, Branco N., Allegretti S.M., Franco R.M.B., Guaraldo A.M.A.

SY25.P.19 ***Leucosporidium* spp. a new pathogen species or a new name for a known agent of systemic candidiasis in farm-reared red-legged partridge (*Alectoris rufa*)?**
Lanteri G., Marino F., Cosenza M., Martuscelli L., Reale S., Bivona M., Macrì B.

SY25.P.20 **Molecular study of *Cryptosporidium* infection in cattle in Mashhad, Iran**
Sadrebazzaz A., Farhoodi M.

SY25.P.21 ***Thysanosoma actinioides* in sheep from Mendoza Province, Argentina**
Neira G., Denegri G., Deis E., Sidoti L., Cuervo P., Mera y Sierra R.L.

SY25.P.22 **Epidemiological investigation of some fungal diseases in poultry**
Starciuc N., Chihai O.

SY25.P.23 **Reproductive analysis of dairy cows in term of Neospora-associated abortions**
Reiterová K., Špilovská S., Antolová D., Čobádiová A., Pošivák J., Vendeľová E.

SY25.P.24 **Epidemiological overview of the cattle cryptosporidiosis in the regions of Tizi Ouzou and Bouira (Algeria)**
Yahia A., Hamrat K., Zane A., Sbaihi M.

Auditorium Maximum (25.07)

19:30 - 20:30 **Chamber music concert**

Casa Universitarilor (25.07)

20:30 - 22:00 **Merial Welcome Event***

26.07.2012, THURSDAY

DAY 2

Life Sciences Institute (26.07)

08:30 - 18:00 **Registration and secretariat**

Aula Magna Mihail Șerban (26.07)

09:00 - 11:00 **Vectors and vector-borne diseases (SY14/1)**
Organisers: *Prof. Dr. Gad Baneth (Rehovot, Israel), Prof. Dr. Gheorghe Dărăbuș (Timișoara, Romania)*
Chairmen: *Prof. Dr. Robert Farkas, Prof. Dr. Gheorghe Dărăbuș*. Secretary: *Maria Bindea*

Keynote presentation:
09:00-09:30 / **Animal hosts and reservoirs of *Leishmania* species in Israel and the Palestinian Authority**
Prof. Dr. Gad Baneth (Rehovot, Israel)
Authors: Baneth G., Talmi-Frank D., Guthmann Y, King R., Nasereddin A., Abdeen Z., Al-Jawabreh A., Klement E., Jaffe C.L.

Oral presentations:
09:30-09:45 / **Epidemiology of cutaneous leishmaniasis and transmission cycle of *Leishmania tropica* in Southeastern Tunisia**
Bousslimi N., Tabbabi A., Ben-Abda I., Aoun K., Bouratbine A.
09:45-10:00 / **Canine visceral leishmaniasis in Palestine: a nation-wide field survey using ELISA and ITS1-PCR**
Hamarsheh O., Al-Jawabreh A., Nasereddin A., Sawalha S., Al-Jawabreh H., Baneth G., Abdeen Z.
10:00-10:15 / **Epidemiological and clinical features of visceral leishmaniasis in the emerging focus of Kairouan, Central Tunisia**
Aoun K., Habboul Z., Ben Abda I., Rkhami O., BenAlaya N., Amri F., Bouratbine A.
10:15-10:30 / **Polymorphism in HASPB repeats (k26) of East African *Leishmania donovani*: potential effect on vaccination and diagnosis**
Zackay A., Nasereddin A., Takele Y., Tadesse D., Hailu W., Hurissa Z., Yifru S., Diro E., Kassahun A., Hailu A., Jaffe C.L.
10:30-10:45 / **Characterization of the human and mouse immune responses to *Leishmania* excreted/secreted proteins**
Drini S., Ben Khalaf N., Kharmachi H., Garnaoui-Meddeb A., Chenik M.
10:45-11:00 / **Use of Fourier Transform Infrared spectroscopy for characterization of *E. coli* and *B. burgdorferi* sensu stricto species**
Bindea M., Ștefan R., Chirilă F., Cozma V.

11:00 - 11:15 Coffee break** - At venues

11:15 - 13:00 **Vectors and vector-borne diseases (SY14/2)**
Chairmen: *Prof. Dr. Gad Baneth, Prof. Dr. Kurt Pfister*. Secretary: *Maria Bindea*

Keynote presentations:
11:15-11:45 / ***Rickettsia conorii*: an old but newly recognized pathogen in dogs**
Dr. Laia Solano-Gallego (Barcelona, Spain)
11:45-12:15 / **Canine ehrlichiosis - an overview and insights from a recent vaccination study**
Prof. Dr. Shimon Harrus (Rehovot, Israel)

Oral presentations:
12:15-12:30 / **Risk of exposure to ticks and certain tick-borne diseases in recreational areas in Central Europe**
Cuber P., Asman M., Solarz K., Andreassen Å., Strzelczyk J., Trapp G., Szilman P., Szilman E., Vainio K., Dudman S., Ånestad G.
12:30-12:45 / **Prospective study on the transmission risk of *Borrelia burgdorferi* sensu lato from *Ixodes ricinus* ticks to humans in Romania**
Briciu V.T., Lupșe M., Nastase V., Meyer F., Sebah D., Mihalca A.D., Carstina D., Huber I., Fingerle V., Tatulescu D.
12:45-13:00 / **Lyme borreliosis: clinical aspects and diagnosis**
Mihăilescu P., Crețu C.M.

13:00 - 14:00 Lunch** - USAMVCN

13:00 - 14:00 **Meeting of MERC (Middle East Regional Cooperation)**

14:00 - 16:00 **Vectors and vector-borne diseases (SY14/3)**
Chairmen: *Prof. Dr. Bertrand Losson, Prof. Dr. Vasile Cozma*. Secretary: *Dr. Gianluca D'Amico*

Keynote presentations:
14:00-14:30 / **Vector-borne zoonotic diseases in Central and Eastern Europe**
***Prof. Dr. Robert Farkas* (Budapest, Hungary)**

14:30-15:00 / **Ticks and tick-borne pathogens in public city parks**
***Prof. Dr. Kurt Pfister* (Munich, Germany)**
Authors: Pfister K., Poljak S., Silaghi C.

Oral presentations:
15:00-15:15 / **Vector-borne infections in imported dogs - a serological and molecular survey**
Hamel D., Silaghi C., Mihalkov A., Maurer U., Pfister K.
15:15-15:30 / **Vector-borne Infections in Dogs from Kiev, Ukraine**
Hamel D., Silaghi C., Mihalkov A., Zapadynska S., Kudrin A., Pfister K.
15:30-15:45 / **Identification of relapsing fever *Borrelia* species by diagnostic species-Specific PCR based on flagellin gene**
Naddaf S.R., Khajevand M., Ghazinezhad B.
15:45-16:00 / **Differential altitudinal distribution of ticks in dogs of nomadic tribes from Northern Kenya**
D'Amico G., Dumitrache M.O., Siroky P., Domșa C., Balázsi R., Modrý D., Mihalca A.D.

16:00 - 16:15 Coffee break** - At venues

16:15 - 18:15 **Vectors and vector-borne diseases (SY14/4)**
Chairmen: *Prof. Dr. Shimon Harrus, Dr. Laia Solano-Gallego*. Secretary: *Dr. Gianluca D'Amico*

Keynote presentation:
16:15-16:45 / **Emerging vector borne diseases of livestock**
Prof. Dr. Bertrand Losson (Liege, Belgium)

Oral presentations:
16:45-17:00 / **Mosquito (Culicidae) prevalence in the Košická Kotlina Basin - Eastern Slovakia**
Bocková E., Kočišová A.
17:00-17:15 / ***Stomoxys* spp. (Diptera: Muscidae), potential vectors of many different pathogens**
Muenworn V., Baldacchino F., Desquesnes M., Charoenviriyaphap T., Duvallet G.
17:15-17:30 / **Bartonellosis and the human diseases produced by *Bartonella* species**
Drăghici S.
17:30-17:45 / **Barcoding tick species of Romania**
Marosi B.A., Dumitrache M.O., Kalmár Z., Mihalca A.D.
17:45-18:00 / **Detection and typing of *Borrelia burgdorferi* sensu lato species in *Ixodes ricinus* ticks from Romania**
Kalmár Z., Mărcuțan D.I., Dumitrache M.O., Béla M., Oltean M., Gherman C.M., Mihalca A.D., Cozma V.
18:00-18:15 / **Structural evaluation and parasitism of the female reproductive organs of dogs naturally infected by *Leishmania (Leishmania) infantum***
da Silva Junior V.A., Gomes de Oliveira V.V., Barros de Macedo S.R., de Sá Santos E.M., Lyra Maia F.C., Alves L.C.

Navy Blue Amphitheatre (26.07)

09:00 - 11:00 **Intestinal and larval echinococcoses (SY10/1)**
Organisers: *Dr. Thomas Romig (Stuttgart, Germany), Assist. Prof. Dr. Carmen Crețu (Bucharest, Romania)*
Chairmen: *Prof. Dr. Peter Kern, Assist. Prof. Dr. Carmen Crețu*. Secretary: *Dr. Diana Onac*

Keynote presentations:
09:00-09:30 / ***Echinococcus* spp.: epidemiological implication of inter- and intraspecific diversity**
Dr. Thomas Romig (Stuttgart, Germany)
09:30-10:00 / **Hydatidosis in Europe: present situation and future perspectives**
Dra. Maria del Mar Siles Lucas (Salamanca, Spain)

Oral presentations:
10:00-10:15 / **Genetic variability of *Echinococcus granulosus* sensu stricto in Europe**
Casulli A., Interisano M., Sreter T., Chițimia L., Kirkova Z, Pozio E.
10:15-10:30 / ***Echinococcus granulosus* in France: update of the distribution and molecular characterization**
Umhang G., Richomme C., Hormaz V., Boucher J-M., Boué F.
10:30-10:45 / **Laparoscopic vs. open surgery for hepatic hydatid cyst**
Puia I.C., Cristea P.G., Tomuș C.
10:45-11:00 / **Hyadatidosis in Boyer Ahmad District, South of Iran**
Moshfe A.

11:00 - 11:15 Coffee break** - At venues

11:15 - 13:00 **Intestinal and larval echinococcoses (SY10/2)**
Chairmen: *Dr. Thomas Romig, Assist. Prof. Dr. Cosmin Puia*. Secretary: *Dr. Diana Onac*

Keynote presentations:
11:15-11:45 / **Echinococcosis: different clinical pathways for two diseases**
Prof. Dr. Peter Kern (Ulm, Germany)
11:45-12:15 / **Cystic echinococcosis: chronic, complex and still neglected**
Prof. Dr. Enrico Brunetti (Pavia, Italy)

Oral presentations:
12:15-12:30 / **Surgical approach of the hydatic cysts developped in the right hepatic lobe**
Popa C., Ionescu S., Zarafin A., Mastalier B.,Crețu C.M., Popa L.G., Mihăilă D., Simion I.
12:30-12:45 / **Biliary fistulae - important issue in the surgical treatment of the hydatic hepatic cysts**
Popa C., Mastalier B., Ionescu S., Zarafin A., Crețu Ca.M., Popa L.G., Simion I., Zarafin L.
12:45-13:00 / **Genetic variation of *Echinococcus granulosus* in wild boars from Romania**
Onac D., Oltean M., Gyorke A., Cozma V.

13:00 - 14:00 Lunch** - USAMVCN

14:00 - 16:00 **Intestinal and larval echinococcoses (SY10/3)**
Chairmen: *Prof. Dr. Enrico Brunetti, Dr. Sándor Sikó Barabási*. Secretary: *Dr. Petrică Ciobanca*

Keynote presentations:
14:00-14:30 / **Inside of clinical echinococcosis - Romanian experience**
Assist. Prof. Dr. Carmen Crețu (Bucharest, Romania)
Authors: Crețu C.M., Mastalier B., Popa C., Mihăilescu P., Constantin C.M., Tarnea L., Barabas E., Popa L.G.
14:30-15:00 / **Human echinococcosis in North-West Romania: clinic, epidemiology and diagnosis**
Prof. Dr. Monica Junie (Cluj-Napoca, Romania)
Authors: Junie L.M., Ciobanca P., Constantea N., Coroiu Z.

Detailed Program

Oral presentations:
15:00-15:15 / **Hepatic hydatid cyst in pediatrics - a continuous challenge**
Iacob D., Fufezan O., Horvat M.
15:15-15:30 / **Diagnosis of hydatid cyst: imaging, serology and molecular**
Mihăilescu P., Crețu C.M., Mastalier B., Popa L.G., Popa C., Cordos I., Brunetti C.E., Bandi C., Ciochir D., Piccoli L.
15:30-15:45 / **Surgical drainage modalities in hepatic hydatic cyst - the experience of Colentina General Surgery Clinic**
Mastalier B., Popa C., Botezatu C., Eliah W., Deaconescu Violeta, Drăghici C.
15:45-16:00 / **The Albendazol treatment's efficacy in hydatic cysts with different locations**
Dumitru I.M., Rugină S., Dumitru E.

16:00 - 16:15 Coffee break** - At venues

16:15 - 18:00 **Intestinal and larval echinococcoses (SY10/4)**
Chairmen: *Dra. Maria del Mar Siles Lucas, Dr. Zoe Coroiu*. Secretary: *Dr. Petrică Ciobanca*

Keynote presentations:
16:15-16:45 / ***Echinococcus multilocularis* in Eastern Europe generally with special reference to Romania**
Dr. Sándor Sikó Barabási (Sfântu Gheorghe, Romania)
Authors: Sikó Barabási S., Sikó Barabási Z., Cozma V.
16:45-17:15 / ***Echinococcus multilocularis* and alveolar echinococcosis: outlooks and research needs**
Prof. Dr. Tíbor Kassai (Budapest, Hungary)

Oral presentation:
17:15-17:30 / **Current status of echinococcosis in Serbia**
Djurkovic-Djakovic O., Bobic B., Nikolic A., Katic-Radivojevic S., Klun I.
17:30-17:45 / ***Echinococcus multilocularis* detection with Real Time PCR in contrasted endemic regions**
Knapp J., Mouzon L., Umhang G., Grenouillet F., Raoul F., Giraudoux P., Said Ali Z., Millon L.
17:45-18:00 / **EmsB microsatellite approach on the expansion of *Echinococcus multilocularis* endemic areas in France**
Umhang G., Knapp J., Hormaz V., Boué F.

18:00 - 19:00 **WHO-IWGE | Informal Working Group on Echinococcosis (WS03)**
Chairmen: *Prof. Dr. Peter Kern*, *Assist. Prof. Dr. Carmen Crețu*. Secretary: *Dr. Petrică Ciobanca*

Green Amphitheatre (26.07)

09:00 - 11:00 **Biology of the Acanthocephala (SY11)**
Organiser: *Dr. Omar Amin (Scottsdale - AZ, USA)*
Chairman: *Dr. Omar Amin*. Secretary: *Dr. Miruna Oltean*

Keynote presentation:
09:00-09:30 / Variability in the Acanthocephala
Dr. Omar Amin (Scottsdale - AZ, USA)

Oral presentations:
09:30-09:45 / **Acanthocephalans of genus *Echinorhynchus* (sensu lato) in the Baikal Rift Zone**
Baldanova D.R., Hamnueva T.R.
09:45-10:00 / **The discovery of two ligament sacs in a member of the Class Palaeacanthocephala (Acanthocephala)**
Lisitsyna O.I.
10:00-10:15 / **The description and host-parasite relationships of a new species of *Acanthosentis* (Acanthocephala: Quadrigyridae) from the Persian toothcarp, *Aphanius farsicus* (Actinopterygii: Cyprinodontiade) in Iran**
Amin O.M., Gholami Z., Akhlaghi M., Heckmann R.A.
10:15-10:30 / **Acanthocephala of freshwater fishes of Mexico**
Salgado-Maldonado G., Novelo-Turcotte M.T.
10:30-10:45 / **Taxonomy and host parasite relationships of *Polymorphus spindlatus* (Acanthocephala) in its vertebrate hosts in Peru**
Amin O.M.
10:45-11:00 / **The parasite systems of acanthocephalans in the Lake Baikal**
Baldanova D.R.

11:00 - 11:15 Coffee break** - At venues

11:15 - 13:00 **Multidisciplinary analysis of Chagas disease (SY02)**
Organiser: *Prof. Dr. Maria Dolores Bargues (Valencia, Spain)*
Chairmen: *Prof. Dr. Maria Dolores Bargues*, *Dr. Carmen Costache*. Secretary: *Dr. Miruna Oltean*

Keynotes presentations:
11:15-11:45 / **Vector species of the genus *Triatoma*: a molecular approach**
Prof. Dr. Maria Dolores Bargues (Valencia, Spain)
11:45-12:15 / **Congenital Chagas disease: diagnosis, treatment and control**
Prof. Dr. Yves Carlier (Brussels, Belgium)
12:15-12:45 / **Biodiversity, poverty and incidence of Chagas disease in Mexico**
Prof. Dr. Alejandro Cruz-Reyes (Mexico City, Mexico)

Oral presentation:
12:45-13:00 / **Expression of the Mucin-Associated Surface Proteins (MASP) multigene family during the life cycle of the *Trypanosoma cruzi***
De Pablos L.M., Seco-Hidalgo V., Gomez Samblás M.M., Díaz Lozano I.M., Osuna A.

13:00 - 14:00 Lunch** - USAMVCN

14:00 - 16:00 ***Plasmodium*, *Anopheles* and malaria (SY04/1)**
Chairmen: *Dr. Carlo Severini*, *Dr. Gabriela Nicolescu*. Secretary: *Dr. Oana Paștiu*

Keynote presentations:
14:00-14:30 / **Aspects of research work on malaria in the field**
Prof. Dr. Virgilio Do Rosario (Lisbon, Portugal)
14:30-15:00 / **Severe f*alciparum* and *vivax* malaria: new therapeutic approaches**
Prof. Dr. Stephane Picot (Lyon, France)
Authors: Picot S., Bienvenu A.L.

Oral presentations:
15:00-15:15 / **Malaria situation nowadays in Hungary**
Kucsera I., Danka J., Orosz E., Glatz K., Szénási Z.
15:15-15:30 / **Decrease of imported malaria in France during the 2006-2011: epidemiology and clinical data through the French National Reference Centre for Malaria "CNR du Paludisme"**
Houzé S., Thellier M., Kendjo E., Pradines B., Parzy D., Taudon N., Hubert V., Houzé P., Durand R., Le Bras J., Danis M.& correspondents of the malaria national reference centre
15:30-15:45 / **Hazard and risk mapping of malaria disease in Iran: an 11 year trend**
Siavashi M., Mostafavi E., Noori A.

Detailed Program

15:45-16:00 / **Faster PCR Results without compromise**
Rus I. (Filara Biomed)

16:00 - 16:15 Coffee break** - At venues

16:15 - 18:00 **_Plasmodium_, _Anopheles_ and malaria (SY04/2)**

Chairmen: *Prof. Dr. Virgilio Do Rosario*, *Prof. Dr. Stephane Picot*. Secretary: *Dr. Oana Paștiu*

Keynote presentation:

16:15-16:45 / **Molecular epidemiology of *Plasmodium vivax* malaria in different endemic settings**
Dr. Carlo Severini (Rome, Italy)
Authors: Severini C., Menegon M.

Oral presentations:

16:45-17:00 / **A Bayesian-based approach for spatio-temporal modelling of county level prevalence of malaria in Jiangsu Province, China**
Zhang S., Yang G., Xia Z., Zhou S., Wang W., Gao Q., Zhou X.

17:00-17:15 / **Post-arrival screening for malaria in asymptomatic refugees using Real-Time PCR**
Matisz C., Naidu P., Shokoples S., Grice D., Krinke V., Brown S.Z., Kowalewska-Grochowska K., Houston S., Yanow S.K.

17:15-17:30 / **A multiplex Real-Time PCR Assay for detection and quantification of *Plasmodium* spp. infection in malaria vectors**
Sandeu M.M., Moussiliou A., Moiroux N., Massougbodji A., Corbel V., Ndam N.T.

17:30-17:45 / **The role of lectin on interaction between ookinete of *Plasmodium berghei* and midgut epithelial cells of *Anopheles stephensi* by using Nano Particle Quantum Dot**
Basseri H.R.

17:45-18:00 / **Strain and species-transcending immunity induced by exposure to low doses of blood stage malaria parasites - a new paradigm for vaccine development**
Good M.

Blue Amphitheatre (26.07)

09:00 - 11:00 **Veterinary parasitology (SY25/2)**

Organiser: *Prof. Dr. Claudio Genchi (Milano, Italy)*
Chairmen: *Prof. Dr. Claudio Genchi, Prof. Dr. Liviu Ioan Mitrea.* Secretary: *Dr. Anamaria Balea*

Keynote presentations:

09:00-09:30 / **Neonatal porcine isosporosis**
Prof. Dr. Anja Joachim (Vienna, Austria)
Authors: Joachim A., Worliczek H.L.

09:30-10:00 / **Comparative epidemiology of coccidian infections of mammalian livestock and consequences for their control**
Dr. Hans-Christian Mundt (Leverkusen, Germany)
Authors: Mundt H.C., Daugschies A.

Oral presentations:

10:00-10:15 / **Control of cryptosporidiosis of ruminants in Greece**
Papadopoulos E., Giadinis N.

10:15-10:30 / **Molecular characterization of *Cryptosporidium* isolates from pre-weaned calves in western France**
Rieux A., Chartier C., Pors I., Paraud C.

10:30-10:45 / **Seroprevalence of *Toxoplasma gondii* in dogs in Ukraine**
Galat M.V., Soroka N.M., Galat V.F., Subotenko T.O., Blaga R.

10:45-11:00 / **Development of transfection tools in *Neospora caninum*: a pyrimethamine cassette of resistance with Lac-Z as the reporter gene**
Pereira L.M., Baroni L., Yatsuda A.P.

11:00 - 11:15 Coffee break** - At venues

11:15 - 13:00 **Veterinary parasitology (SY25/3)**

Chairmen: *Prof. Dr. Anja Joachim*, *Assist. Prof. Dr. Narcisa Mederle*. Secretary: *Dr. Anamaria Balea*

Keynote presentation:

11:15-11:45 / **The FLOTAC strategy: new approach for the diagnosis of human and veterinary parasites**
Prof. Dr. Giuseppe Cringoli (Naples, Italy)
Authors: Cringoli G., Rinaldi L.

Oral presentations:

11:45-12:00 / **Comparison of different molecular tests for detection of cryptosporidiosis in animals**
Mirhashemi M.E., Zintl A., Lucy F., Mulcahy G., De Waal T.

12:00-12:15 / **Prevalence of *Balantidium coli* in cattle from Roodsar abattoir, East of Guilan, Northern Iran**
Mirzaei M.D., Mohammadyari N.

12:15-12:30 / **Preliminary study of the presence of dog helminth parasites in soil from Timiș County: importance for human health**
Andrei S., Dărăbuș G.

12:30-12:45 / **Occurrence of gastrointestinal nematode parasitism and subclinical mastitis in ewes reared under low input management systems**
Tzanidakis N., Voutzourakis N., Stefanakis A., Brozos C., Kiossis E., Sotiraki S.

12:45-13:00 / **A comparative study between necropsy and biochemistry of hydatidosis in camels (*Camelus dromedarius*) in the Region of Touggourt in Algeria**
Hamrat K., Yahia A., Benaissa M.H.

13:00 - 14:00 Lunch** - USAMVCN

14:00 - 16:00 **Veterinary parasitology (SY25/4)**

Chairmen: *Prof. Dr. Laura Rinaldi*, *Prof. Dr. Dumitru Militaru*. Secretary: *Dr. Cristian Magdaș*

Keynote presentation:

14:00-14:30 / **Ticks and tick borne-disease of dogs in the UK**
Prof. Dr. Richard Wall (Bristol, UK)
Authors: Wall R., Morgan E.R., Smith F.D.

Oral presentations:

14:30-14:45 / **Prevalence of some vector borne diseases in dogs in R. Macedonia**
Stefanovska J., Farkas R., Kochevski Z.

14:45-15:00 / **Babesiosis of dogs in Belgrade area between 2009-2011**
Pavlovic I., Terzin V., Pavlovic M., Petkovic D., Terzin D., Stankovic B.
15:00-15:15 / **Prevalence of erlichiosis, anaplasmosis and boreliosis in dogs in Serbia**
Pavlovic I., Milojkovic N., Curcin L., Kovacevic M., Novak N., Ivanovic O.
15:15-15:30 / **Epidemiology and associated risk factors of ectoparasites infesting goat population of district Toba Tek Singh, Pakistan**
Sajid M.S., Iqbal A., Khan M.N., Khan M.K.
15:30-15:45 / **Detection and monitoring of increasing health risks due to parasite infections in cattle as a result of global climate change**
Brandt C., Demeler J., von Samson-Himmelstjerna G.
15:45-16:00 / **Determining the best sheep protective strategy to combat infection by the nematode *Haemonchus contortus*: is resistance futile?**
Chylinski C., Cortet J., Cabaret J.

16:00 - 16:15 Coffee break** - At venues

16:15 - 18:00 **Veterinary parasitology (SY25/5)**

Chairmen: *Prof. Dr. Richard Wall*, *Assist. Prof. Dr. Olimpia Iacob*. Secretary: *Dr. Cristian Magdaș*

Keynote presentation:
16:15-16:45 / **Sustainable control of gastro intestinal nematodes; use of Mediterranean bioactive plant**
Dr. Smaragda Sotiraki (Thessaloniki, Greece)
Authors: Sotiraki S., Manolaraki F., Arroyo-Lopez C., Hoste H.

Oral presentations:
16:45-17:00 / **Prevalence and risk factors of trichomonads infection in puppies from french breeding kennels**
Grellet A., Robin C., Meloni D., Cian A., Viscogliosi E., Polack B.
17:00-17:15 / **Risk factors of *Pentatrichomonas hominis* infection in puppies from a large breeding kennel and impact on feces quality**
Grellet A., Diallo L., Carrez B., Meloni D., Cian A., Viscogliosi E., Polack B.
17:15-17:30 / **Effect of antiparasitic treatment on the immune response of bovine**
Chihai O., Erhan D., Tălămbuță N., Rusu S., Pavaliuc P., Melnic G., Zamornea M.
17:30-17:45 / **Study of the fertility of *Echinococcus granulosus* cysts in ruminants in the Province of Djelfa (Algeria)**
Hamrat K., Yahia A., Kadi A., Tchenchen A., Ghediri Y., Chana A., Belferde Z., Ibrahimi B., Rebhi M.
17:45-18:00 / **Molecular identification of *Echinococcus multilocularis* in small mammals based on mitochondrial DNA in Razavi Khorasan Province, Iran**
Razmjou E., Beiromvand M., Akhlaghi L., Darvish J., Meamar A.R., Fattahi Massom S.H., Oormazdi H.

Biodiversity Centre (26.07)

09:00 - 11:00 **IV K.E. Mott Symposium on schistosomiasis and foodborne trematodiases / A. Schistosomiasis (SY07/1)**

Organisers: *Prof. Dr. Santiago Mas-Coma (Valencia, Spain), Prof. Dr. David Rollinson (London, UK)*
Chairmen: *Prof. Dr. Guillaume Mitta*, *Prof. Dr. Lidia Lazăr*. Secretary: *Dr. Adriana Jarca*

Keynote presentations:
09:00-09:30 / **Schistosomiasis control in Africa: hopes and needs**
Prof. Dr. David Rollinson (London, UK)
Authors: Rollinson D., Knopp S.
09:30-10:00 / **Dynamic organization of the tegument of schistosome parasites: relevance for vaccine design**
Prof. Dr. Malcolm K. Jones (Brisbane, Australia)
Authors: Jones M.K, Schulte L., Mulvenna J.

Oral presentations:
10:00-10:15 / **The role of protein kinase C and mitogen-activated protein kinase signalling in host detection, invasion and development of *Schistosoma mansoni***
Ressurreicao M., Rollinson D., Page N., Kirk R., Walker A.J.
10:15-10:30 / **Possible role of epigenetic mechanisms in sex chromosome emergence in *Schistosoma mansoni*, a human parasite**
Lepesant J.M.J., Cosseau C., Boissier J., Freitag M., Portela J., Climent D., Perrin C., Zerlotini A., Mitta G., Grunau C.
10:30-10:45 / **A role for epigenetics in the adaptive evolution of a human parasite, *Schistosoma mansoni*, in response to environmental stress**
Lepesant J.M.J., Cosseau C., Roquis D., Boissier J., Rognon A., Mitta G., Grunau C.
10:45-11:00 / **Genetic bases of adaptive evolution of *Schistosoma mansoni* to its intermediate host: clues from next generation sequencing data**
Clément J.A.J., Roquis D., Parrinello H., Boissier J., Rognon A., Mitta G., Grunau C.

11:00 - 11:15 Coffee break** - At venues

11:15 - 13:00 **IV K.E. Mott Symposium on schistosomiasis and foodborne trematodiases / A. Schistosomiasis (SY07/2)**

Chairmen: *Prof. Dr. David Rollinson*, *Prof. Dr. Monica Junie*. Secretary: *Dr. Adriana Jarca*

Keynote presentations:
11:15-11:45 / **Compatibility polymorphism in the interaction between *Biomphalaria glabrata* and *Schistosoma mansoni*: from populations to molecular mechanisms**
Prof. Dr. Guillaume Mitta (Perpignan, France)
Authors: Mitta G., Gourbal B., Theron A.
11:45-12:15 / ***Biomphalaria glabrata* innate immunity: specificity and molecular basis of immune priming response against *Schistosoma mansoni***
Dr. Benjamin Gourbal (Perpignan, France)
Authors: Portela J., Duval D., Rognon A., Coustau C., Mitta G., Théron A., Gourbal B.

Oral presentations:
12:15-12:30 / **Resistance to re-infection with *Schistosoma mansoni* in mice treated with Mefloquine**
El-Sayed S.H., Kamel M.M., Hassan S.I., El-Badry A.A., Mahmoud S.S., Hamam O.A.
12:30-12:45 / **In vitro study: anti-*Schistosoma mansoni* biological activity of volatile oil of *Persea americana* Mill. leaves (avocado) on different parasite stages**
El-Bady A.A., Abdel Aziz I.Z., Abdelrahman E.H.
12:45-13:00 / **Anti-parasitic activity of biomphalysin, the first beta pore forming toxin from *Biomphalaria glabrata***
Portela J., Galinier R., Moné Y., Delbecq S., Mitta G., Gourbal B., Duval D.

13:00 - 14:00 Lunch** - USAMVCN

13:00 - 14:00 **EFP - Meeting of the Executive Board**

Detailed Program

14:00 - 16:00 **IV K.E. Mott Symposium on schistosomiasis and foodborne trematodiases / A. Schistosomiasis (SY07/3)**
Chairmen: ***Dr. Benjamin Gourbal, Prof. Dr. Mihaela Lupşe.*** Secretary: *Bindea Maria*

Keynote presentations:
14:00-14:30 / **Urinary schistosomiasis in Iran: a decade after interruption of transmission**
Assoc. Prof. Dr. Gholamreza Mowlavi (Tehran, Iran)
14:30-15:00 / **Schistosomiasis in Europe**
Prof. Dr. Lawton Scott (Kingston upon Thames, UK)

Oral presentations:
15:00-15:15 / **Preliminary indication of direct predisposing relationship between chronic human *Schistosoma mansoni* infection and hepatocellular carcinoma**
Abd El-Aal A.A., Mahmoud N.S., Abdel Aziz I.Z., El Hamid A., Sabri A.
15:15-16:00 / Discussion

16:00 - 16:15 Coffee break** - At venues

A3 Amphitheatre (26.07)

09:00 - 11:00 **Reproduction, developmental biology and ultrastructure of parasites (SY17/1)**
Organiser: ***Prof. Dr. Zdzisław Piotr Świderski (Warsaw, Poland)***
Chairman: ***Prof. Dr. Libuse Kolarova*****.** Secretary: *Dr. Tatiana Băguț*

Keynote presentation:
09:00-09:30 / **Transmission mechanisms of the hexacanth larvae of taeniids of medical and veterinary significance**
Prof. Dr. Zdzisław Piotr Świderski (Warsaw, Poland)

Oral presentations:
09:30-09:45 / **Metal bioaccumulation and partitioning in the tissues of the endoparasite, *Bothriocephalus acheilognathi***
Gilbert B.M., Avenant-Oldewage A.
09:45-10:00 / **Acetylcholinesterase secretion by *Anisakis simplex* larvae (Nematoda: Anisakidae) - a biological response to changes of environmental conditions**
Podolska M., Nadolna K.
10:00-10:15 / **Life cycle of *Lamproglena clariae* under laboratory conditions**
Madanire-Moyo G.N., Avenant-Oldewage A.
10:15-10:30 / **Human intestinal flukes *Haplorchis taichui* and *Haplorchis pumilio* in their intermediate hosts, freshwater snails family Thiaridae in Thailand**
Krailas D., Dechruksa W., Chotesaengsri S., Pattaradussadee N., Rattanathai P., Namchote S., Koonchornboon T.
10:30-10:45 / **Evaluating the status and identity of snails as intermediate hosts of trematodes of genus *Melanoides* Olivier, 1804 (Gastropoda, Thiaridae, Melanoides) in Thailand**
Dechruksa W., Krailas D., Glaubrecht M.
10:45-11:00 / **What does „regularly deworming of dogs" mean?**
Varga Z. (Pfizer Animal Health)

11:00 - 11:15 Coffee break** - At venues

11:15 - 13:00 **Reproduction, developmental biology and ultrastructure of parasites (SY17/2)**
Chairman: ***Prof. Dr. Zdzisław Piotr Świderski*****.** Secretary: *Dr. Tatiana Băguț*

Keynote presentation:
11:15-11:45 / **Migratory routes of helminths throughout organs of incompatible host**
Prof. Dr. Libuse Kolarova (Prague, Czech Republic)

Oral presentations:
11:45-12:00 / **Early spring: good for parasites and bad for hosts or bad for both?**
Georgieva S., Kostadinova A.
12:00-12:15 / **Living in a lagoon: effect of environmental factors on larval emergence rates of two ppecoelids in *Gibbula adansonii***
Born-Torrijos A., Holzer A.S., Raga J.A., Kostadinova A.
12:15-12:30 / **Density- and time-dependent processes in the population growth of *Gyrodactylus salaris* on Atlantic salmon stocks**
Ramirez R., Østby-Pedersen A., Harris P.D., Bakke T.A.
12:30-12:45 / **Site selection in *Dolops ranarum*, a branchiuran fish parasite**
Avenant-Oldewage A.
12:45-13:00 / **Involvement of subtilisin-like serine proteases Sub3 in the adherence of *Microsporum canis* to human and different animal species epidermis**
Băguț E.T., Baldo A., Mathy A., Cambier L., Cozma V., Mignon B.

13:00 - 14:00 Lunch** - USAMVCN

14:00 - 16:00 **Towards coordinated actions in the management of emerging parasitoses in Europe (RT01)**
Organiser: ***Prof. Dr. Jean Dupouy-Camet (Paris, France)***
Chairman: ***Prof. Dr. Jean Dupouy-Camet*****.** Secretary: *Pharm. Anamaria Cozma*

14:00-14:10 / **Present challenges and forces to address human parasitic diseases in Europe**
Prof. Dr. Jean Dupouy-Camet (Paris, France)
14:10-14:25 / **The European Union reference laboratory for parasites**
Dr. Edoardo Pozio (Rome, Italy)
14:25-14:40 / **The ESCMID study group for clinical parasitology**
Dr. Titia Kortbeek (Bilthoven, Netherlands)
14:40-14:55 / **Aims and actions of the European Veterinary Parasitology College**
Prof. Dr. Anja Joachim (Vienna, Austria) and Prof. Dr. Claudio Genchi (Milano, Italy)
14:55-15:10 / **European working groups on human toxoplasmosis since the '90s till present**
Prof. Dr. Herve Pelloux (Grenoble, France)
15:10-15:25 / **A European think tank coordinating actions in the management of emerging parasitoses**
Prof. Dr. Santiago Mas-Coma (Valencia, Spain)
15:25-16:00 / Discussion

16:00 - 16:15 Coffee break** - At venues

16:15 - 18:20 **Young Scientist Award (YSA)**
Organiser: ***Prof. Dr. Jean Dupouy-Camet (Paris, France)***

16:15-16:20 / Opening

16:20-16:35 / ***Toxoplasma gondii*** **genotypes in humans and animals in Serbia**
Marija Vujanić. National Reference Laboratory for Toxoplasmosis, Serbian Centre for Parasitic Zoonoses, Institute for Medical Research, University of Belgrade, Belgrade, Serbia.

16:35-16:50 / **Endemic *Toxoplasma gondii* Genotype II causes fatal infections in animal hosts in Europe - lessons learnt**
Pikka Jokelainen. Veterinary Pathology and Parasitology, Department of Veterinary Biosciences, Faculty of Veterinary Medicine, University of Helsinki ,Finland.

16:50-17:05 / **Infection by *Alaria alata* mesocercaria in naturally infected wild boars and experimental infection on mice**
Julien Portier. French agency for food, environmental and occupational health and safety (ANSES), Maisons Alfort & University of Reims Champagne-Ardenne, Reims, France

17:05-17:20 / **Prostaglandin E_2 as a cestodes immunomodulator**
Ivan.A. Kutyrev. Institute of General and Experimental Biology, Siberian Branch of Russian Academy of Sciences, Ulan-Ude, Russia

17:20-17:35 / ***Trichinella*** **of wild animals in Ukraine**
Yuliya M. Didyk. Institute of Zoology NAS of Ukraine, Kiev, Ukraine

17:35-17:50 / **New insights into the neglected lungworm *Capillaria aerophila* affecting pets, wildlife and humans**
Angela Di Cesare. Department of Comparative Biomedical Sciences, University of Teramo, Teramo, Italy

17:50-18:05 / **Determining the best sheep protective strategy to combat infection by the nematode *Haemonchus contortus*: is resistance futile?**
Caroline J Chylinski, INRA, UMR 1282 Infectiologie et Santé Publique, F-37380 Nouzilly, France

18:05-18:20 / **Studying tsetse ecology to understand the epidemiology of African trypanosomoses and promote integrated pest management**
Jéremy Bouyer, CIRAD-INRA, Institut Sénégalais de Recherche Agricole, Dakar, Senegal

A4 Amphitheatre (26.07)

09:00 - 11:00 **Biodiversity, health and environment (SY19/1)**

Chairmen: *Prof. Dr. Veena Tandon, Prof. Dr. Boris R. Krasnov*. Secretary: *Dr. Ioana Matei*

Keynote presentations:

09:00-09:30 / **Biodiversity of lung nematodes - Protostrongylidae Leiper, 1926 (emend. Boev et Schultz, 1950) - of animals in some Eastern European countries: Armenia, Bulgaria, Poland, Russia**
Acad. Prof. Dr. Sergei Movsesyan (Moscow, Russia)
Authors: Movsesyan S., Panayotova-Pencheva M., Boyakhchyan G., Demiaszkewicz A.

09:30-10:00 / **Cestode diversity: How much do we know?**
Prof. Dr. Jean Mariaux (Geneva, Switzerland)

Oral presentations:

10:00-10:15 / **The current situation of rare helminthiases in the Russian Federation**
Guzeeva M.

10:15-10:30 / **The use of the *Diplozoon* as a sentinel organism for metal pollution**
Hussain E., Avenant-Oldewage A.

10:30-10:45 / ***Capillaria philippinensis*** **a newly emerging parasitic cause of protein-losing enteropathy in Egypt**
El-Dib N.A.

10:45-11:00 / **Synanthropic dogs and cats as parasitic pollution source of urban ecosystems in Chişinău**
Tălămbuță N., Chihai O., Iacub N.

11:00 - 11:15 Coffee break** - At venues

11:15 - 13:00 **Biodiversity, health and environment (SY19/2)**

Chairmen: *Prof. Dr. Sergei Movsesyan*. *Prof. Dr. Elias Papadopoulos*. Secretary: *Dr. Ioana Matei*

Keynote presentations:

11:15-11:45 / **Influence of climate change on parasitic infections**
Prof. Dr. Birgitta Evengard (Umea, Sweden)

11:45-12:15 / **Ectoparasite diversity: host species, host communities and geography**
Prof. Dr. Boris R. Krasnov (Mitzpe Ramon, Israel)
Authors: Krasnov B., Khokhlova I.S.

Oral presentations:

12:15-12:30 / **Eimeria oocysts in soil and faeces on naturally infected pastures and the role of seasonal effect**
Lassen B., Lepik T., Järvis T., Mägi E.

12:30-12:45 / **Importance of gentle handling of *Eimeria bovis* oocysts recovered from soil samples and timing of oocysts entering the soil on the presence in the following grazing season**
Lepik T., Lassen B., Bangoura B.

12:45-13:00 / **MSD AH Presentation**
Gosu G.C. (MSD Animal Health)

13:00 - 14:00 Lunch** - USAMVCN

14:00 - 16:00 ***Trichinella*** **and trichinellosis (SY13/1)**

Organisers: *Dr. Edoardo Pozio (Rome, Italy), Assist. Prof. Dr. Călin Gherman (Cluj-Napoca, Romania)*
Chairmen: *Prof. Dr. Dante Sam Zarlenga, Dr. Benjamin Martin Rosenthal*. Secretary: *Dr. Miruna Oltean*

Keynote presentations:

14:00-14:30 / **The *Trichinella* genome: what can it tell us and how can we apply it?**
Prof. Dr. Dante Sam Zarlenga (Washington, USA)
Authors: Zarlenga D.S., Wang Z., Mitreva M.

14:30-15:00 / **Genetic variation as a key to understanding the origins of *Trichinella* species, the history and structure of their populations, and the number of independent infections that a given animal is likely to sustain**
Dr. Benjamin Martin Rosenthal (Washington, USA)

Oral presentations:

15:00-15:15 / **Induction of protective immunity in mice to *Trichinella spiralis* using a 30 mer peptide of the 43 kDa *T. spiralis* antigen or TSL-1 antigens with defined adjuvants**
Ortega-Pierres G., Fonseca-Liñán R., Araceli-Vaquero V., Quiñonez-Bastidas G.N., Castillo-Alvarez A., Mendoza-Hernández G., Goldbaum F., Aguilar-Faisan L., Bermúdez-Cruz R.M., Villegas-Sepúlveda N., Ruiz-Pérez F.

15:15-15:30 / **Trichinellosis in Croatia**
Marinculić A.

15:30-15:45 / **Incidence of *Trichinella* infection in pork and wild boar samples in Transylvania - features on the investigation methods**
Mihaiu M., Dan S.D., Lăpuşan A., Jecan C., Ciupa A.

15:45-16:00 / **The anti-tumor potential of *Trichinella* experimental infection**
Oltean M., Sevastre B., Györke A., Gherman C., Irimie A., Cozma V., Tăbăran F.

16:00 - 16:15 Coffee break** - At venues

MV Hall (26.07)

Session 1 **Chairman: *Assist. Prof. Dr. Narcisa Mederle*.** Secretary: *Dr. Zsuzsa Kalmar*

14:00 - 16:00 **Amoebae and free living amoebae (SY06.P)**

Poster presentations:

SY06.P.01 **Frequency of *Entamoeba histolytica* and *Entamoeba dispar* prevalence among patients with gastrointestinal complaints in Chelgerd City, Southwest of Iran**
Pestehchian N., Nazary M., Haghighi A., Salehi M., Yosefi H.

Taeniasis/cysticercosis: identification and control strategies (SY09.P)

Poster presentations:

SY09.P.01 **Seroprevalence of human cysticercosis among blood donors in UAE**
Sultan D.M., Al-Amiri A., Al-Marzouqi M.H.

Update on dirofilariasis knowledge (SY12.P)

Poster presentations:

SY12.P.01 **Detection of *Dirofilaria immitis* in dogs from Mendoza Province, Argentina**
Gerbeno L., Sidoti L., Cuervo P., Rinaldi L., Neira G., Mera y Sierra R.

SY12.P.02 **Canine filarioses in Central Poland**
Masny A., Salamatin R., Cielecka D., Lewin T., Golab E.

SY12.P.03 **Human *Dirofilaria repens* infection in Poland**
Cielecka D., Zarnowska-Prymek H., Masny A., Salamatin R., Wesołowska M., Golab E.

***Trichinella* and trichinellosis (SY13.P)**

Poster presentations:

SY13.P.01 **Screening for immunostimulant activity of chitosan during *Trichinella spiralis* infection in mice**
Brodaczewska K., Donskow-Lysoniewska K., Doligalska M.

SY13.P.02 **A new driver - development a specific algorithm of severity with clinical and biological character in trichinosis**
Costache D., Costache C., Bogdan A.T.

SY13.P.03 **Comparative ultrastructural studies of the alterations to mouse lung parenchyma during *Trichinella spiralis* or *Toxocara canis* infection**
Dabrowska J., Walski M., Dybicz M., Doligalska M.

SY13.P.04 **Effect of *Trichinella spiralis* antigens on its worm stages and infection phases**
Shaheen M.S., Galal L.A., Farag H.

SY13.P.05 **A novel microsatellite within lsrDNA Expansion Segment V of *Trichinella britovi* and *Trichinella native***
Masny A., Jagiello A., Pluciennicak G., Golab E.

SY13.P.06 **Regulation of the intestinal immune response by the hyphophysis during *Trichinella spiralis* infection in the golden hamster**
Hernández-Cervantes R., Quintanar-Stephano A., Moreno-Méndoza N., López-Griego L., López-Salazar V., Hernández-Bello R., César Carrero J., Morales-Montor J.

SY13.P.07 **Preliminary studies on intra-specific variability of Polish, Slovak And Czech *Trichinella* isolates by ISSR-PCR**
Goździk K., Reiterová K., Moskwa B., Čobádiová A., Hurníková Z., Šnábel V., Caba W.

SY13.P.08 **Clinical features of human trichinellosis and biological characteristics of *Trichinella* isolates**
Vutova K., Petkova S., Chipeva R., Velev V., Sabit Z., Pozio E., Marucci G., Goceva A., Ticholova M.

SY13.P.09 **TS Card Pork - useful but neglected test for the detection of *Trichinella* antibodies in swine**
Sofronic-Milosavljevic L.J., Chiurciu V., Djordjevic M., Petrovic M., Patrascu I.V.

Immunopathology of parasitic infections (SY15.P)

Poster presentations:

SY15.P.01 **The effect of *Anisakis simplex* larval products on murine dendritic cells**
Cuéllar C., Zamora V., Fernández-Fígares V., González J., Rodero M., Daschner A., Mendez S.

SY15.P.02 **Cytokine response of mice to heavy metal intoxication and *Ascaris suum* infection**
Dvoroznakova E., Jalcova M.

SY15.P.03 **Effect of heavy metals on *Ascaris suum* infection and macrophage activity in mice**
Dvoroznakova E., Jalcova M.

SY15.P.04 **Glucan immunomodulator can protect the host to migration of *Ascaris suum* larvae**
Dvoroznakova E.

SY15.P.05 **Serotonin and neuropeptide immunoreactivities in metacercariae of some trematodes**
Terenina N., Gustafsson M., Tolstenkov O., Movsesyan S.

SY15.P.06 **In vitro cytokine response of THP-1 cell line treated with selected *Ancylostoma ceylanicum* secreted proteins (ASPs)**
Siwinska A.M., Dlugosz E., Baska P., Wedrychowicz H.

SY15.P.07 **Cell and molecular aspects of immune response in peritoneal cavity of mice with *Mesocestoides vogae* infection**
Vendeľová E., Hrčková G., Velebný S.

SY15.P.08 **Localisation of *Toxocara canis* larvae and excretory/secretory antigens and apoptotic cells in hosts tissues during the acute and chronic phase of experimental larval toxocariasis**
Hrčková G., Velebný S., Vendeľová E.

SY15.P.09 **Molecular cloning and immunomodulatory effect of serine proteases from *Hypoderma diana***
Wasyl K., Zawistowska-Deniziak A., Baska P., Wisniewski M., Wedrychowicz H.

SY15.P.10 **Lymphoproliferative response, pre and post-infection, in BALB/c mice immunized with the recombinant chimeric protein L25a-HSP70M1 of *Leishmania braziliensis***
Rodríguez Borges V.M., González García A.C., Bourgon Rodríguez M.G., Pou Barreto C., Martínez E., Valladares B.

16:00 - 16:15 Coffee break** - At venues

Session 2 **Chairman: *Assist. Prof. Dr. Olimpia Iacob*.** Secretary: *Dr. Gianluca D'Amico*

16:15 - 18:00 **Global Network for Geospatial Health - GNOSISGIS; The first European symposium on parasites and geospatial health; The VI Symposium on geospatial health (SY16.P)**

Poster presentations:

SY16.P.01 **A Bayesian kriging model with covariates to estimate the probability of parasitic infection**
Catelan D., Musella V., Rinaldi L., Cringoli G., Biggeri A.

Detailed Program

Virtual microscopy of parasites (SY18.P)

Poster presentations:

SY18.P.01 **Light and electron microscopy observations of embryogenesis and egg development in the human liver fluke, *Opisthorchis viverrini* (Platyhelminthes, Digenea)**
Khampoosa P., Jones M.K., Lovas E.M., Srisawangwong T., Laha T., Piratae S., Thammasiri C., Suwannatrai A., Sripanidkulchai B., Eursitthichai V., Tesana S.

SY18.P.02 **Light and scanning electron microscopic observations on *Grillotia erinaceus* (Van Beneden, 1858) (Cestoda: Trypanorhyncha) plerocercoids in the Black Sea whiting, *Merlangius merlangus* L, 1758**
Özer A., Öztürk T., Kornyushin V., Kornyychuk Y., Yurakhno V.,

Parasites of insects with economic importance (SY22.P)

Poster presentations:

SY22.P.01 **Study of entomopathogenic nematodes from genera *Steinernema* and *Heterorhabditis* in Ukraine**
Kharchenko V., Sigareva D., Galagan T., Olenenko V.

SY22.P.02 **Prevalence of *Nosema apis* and *Nosema ceranae* in Slovak Republic**
Staroň M., Königová A., Molnár L., Toporčák J., Mudroňová D., Kuzyšinová K.

Allergy and zoonoses (SY24.P)

Poster presentations:

SY24.P.01 **Cytokine oroduction in gastro-allergic anisakiosis and associated chronic urticarial**
Cuéllar C., Fernández-Fígares V., Rodero M., Valls A., de Frutos C., Daschner A.

SY24.P.02 **Dust mite allergens: a major risk factor in development of allergies in Dubai**
Sultan D.M., Khalil M.M.

Drug resistance in parasites (SY26.P)

Poster presentations:

SY26.P.01 **Assessment of the effect of new ethyl and methyl carbamates on *Rhipicephalus microplus* resistant to conventional acaricides**
Pérez G. I.E., Prado-Ochoa M.G., Cuenca-Verde C., Lara R. M., Abrego R. H., Velázquez S. A.M., Angeles A. E., Muñoz-Guzmán M.A., Alba-Hurtado F.

SY26.P.02 **Flea and tick resistance in dogs and cats to Parakill product (fipronil)**
Chiurciu V., Cernea M., Cernea L.C., Chiurciu C., Voiu F., Ognean L.

SY26.P.03 **Is larval development test reliable enough to detect anthelmintic resistance in the field?**
Várady M., Dolinská M., Königová A.

SY26.P.04 **The field survey of ivermectin resistance in sheep parasite in the Slovak Republic**
Dolinská M., Várady M., Königová A.

SY26.P.05 **Evaluation of Cydectin Pour-on against lice on naturally infested cattle when treated at the start of the housing period**
Geurden T., Courouble F., Bartram D.J.

SY26.P.06 **Evaluation of the efficacy of Moxidectin plus Triclabendazole Pour-on against lice on cattle**
Geurden T., Van Brussel L., Rugg D., Bartram D.

SY26.P.07 **Evaluation of the period of persistent efficacy of Moxidectin plus Triclabendazole Pour-on solution against *Ostertagia ostertagi* and *Dictyocaulus viviparus* in cattle**
Geurden T., Rugg D., Van Brussel L., Smothers C.D., Bartram D.J.

SY26.P.08 **Insecticide susceptibility of wild-caught sand fly populations collected from certain districts of Aegean Region**
Karakus M., Gocmen B., Balcioglu C., Özbel Y.

SY26.P.09 **Efficacy of gastro-resistant caps of ronidazole against feline *Tritrichomonas foetus* infection**
Desquilbet L., Grellet A., Boogaerts C., Dore V., Antony M., Remilien C., Polack B., Perrot S.

SY26.P.10 **Allozyme profiles of *Haemonchus contortus* resistant and susceptible to anthelmintics, with an indication of dipeptidases linked with resistance**
Šnábel V., Várady M., Dolinská M., Wolstenholme A.

Goats and zoonoses (under the frame of cost action CAPARA –FA0805) (SS01.P)

Poster presentations:

SS01.P.01 **Use of the monoclonal antibody mm3 for detection of coproantigens of *Fasciola hepatica* in vaccinated and non-vaccinated goats**
Buffoni Perazzo L., Toalombo P., Martínez Moreno F.J., Zafra R., Pérez J., Martínez Ubeira F., Martínez Moreno A.

SS01.P.02 **Study of the local immune response at the early stage of *Fasciola hepatica* infection in goats immunised with Cathepsin L1 (Fhcl1)**
Buffoni Perazzo L., Zafra R., Pérez J., Martínez Moreno F.J.,Martínez Moreno A.

SS01.P.03 **Oral administration of *Curcubita moschata* seeds as a therapeutic alternative in goats' gastrointestinal nematodes control**
Carvalho C.D., Guimarães A.O., Madi R.R., Jeraldo V.L.S., Guiquet Leal D.A., Nardi A.R.M., Oliveira C.M.B., Melo C.M., Allegretti S.M.

SS01.P.04 **Intestinal parasites in goats in Serbia**
Katic-Radivojevic S., Borozan S., Dimitrijevic B., Nikolic A., Klun I., Djokic V., Djurkovic- Djakovic O.

SS01.P.05 **Phenotypic analysis of eggs of *Fasciola hepatica* recovered from creole goats, in Western Argentina**
Fantozzi C., Sidoti L., Neira G., Mercado C., Cuervo P., Mera y Sierra R.L.

SS01.P.06 ***Fasciola hepatica* infection and association with gastrointestinal parasites in creole goats, in plateau and andean regions of Western Argentina**
Cuervo P., Sidoti L., Fantozzi C., Neira G., Gerbeno L., Di Cataldo S., Mera y Sierra R.L.

St. Michael's Church

19:30 - 20:30 **Organ Concert***

Art Museum

20:00 - 21:30 **Cultural Dinner***

Detailed Program

27.07.2012, FRIDAY

DAY 3

Life Sciences Institute (27.07)

08:30 - 18:00 **Registration and secretariat**

Aula Magna Mihail Şerban (27.07)

09:00 - 11:00 ***Toxoplasma* and toxoplasmosis (SY05/1)**

Organiser: *Prof. Dr. Monica Junie (Cluj-Napoca, Romania)*
Chairmen: *Prof. Dr. Herve Pelloux, Prof. Dr. Monica Junie*. Secretary: *Dr. Petrică Ciobanca*

Keynote presentations:

09:00-09:30 / ***Toxoplasma* strains and human toxoplasmosis**
Prof. Dr. Marie-Laure Dardé (Limoges, France)
Authors: Dardé M.L., French Network for Toxoplasma strains, ToxoBS

09:30-10:00 / **Toxoplasmosis in immunocompromised patients: what are the key points in 2012?**
Prof. Dr. Herve Pelloux (Grenoble, France)

Oral presentations:

10:00-10:15 / **Seroprevalence of *Toxoplasma gondii* and *Neospora caninum* in dogs from the Mediterranean Island of Corsica**
Colin de Verdière J., Alliot A., Perret C., Thomas M., Aubert D., Villena I., Lacour S.A., Boireau P., Richomme C., Blaga R.

10:15-10:30 / **Prevalence of *Toxoplasma gondii* and *Encephalitozoon cuniculi* antibodies in domestic rabbits in the Czech Republic and Slovak Republic**
Neumayerova H., Jurankova J., Jeklova E., Kudlackova H., Faldyna M., Kovarcik K., Koudela B.

10:30-10:45 / **Endemic *Toxoplasma gondii* genotype causes fatal infections in animal hosts in Europe**
Jokelainen P.

10:45-11:00 / **Using magnetic capture and Real-Time PCR for detection of *Toxoplasma gondii* in tissue samples of experimentally infected goats and pigs**
Juránková J., Opsteegh M., van der Giessen J., Neumayerová H., Frencová A., Basso W., Deplazes P., Baláž V., Koudela B.

11:00 - 11:15 Coffee break** - At venues

11:15 - 13:00 ***Toxoplasma* and toxoplasmosis (SY05/2)**

Chairmen: *Prof. Dr. Marie-Laure Dardé, Dr. Carmen Costache*. Secretary: *Dr. Petrică Ciobanca*

Keynote presentations:

11:15-11:45 / **Clinical presentation of congenital toxoplasmosis: our experience and review of literature**
Prof. Dr. Monica Junie (Cluj-Napoca, Romania)
Authors: Junie L.M., Coroiu Z., Costache C.

11:45-12:15/ **Updates in *Toxoplasma gondii* infection in pregnancy and neonates**
Dr. Carmen Costache (Cluj-Napoca, Romania)

Oral presentations:

12:15-12:30 / **Evidence and partial characterization of a metalloprotease from *Toxoplasma gondii***
Bouleau A.P., Bellon G., Buache E., Escotte-Binet S., Hornebeck W., Garnotel R., Aubert D., Villena I.

12:15-12:30 / **Identification of parasite proteins interacting with the Transcription Factor UHRF1 in *Toxoplasma gondii* infected cells**
Sabou M., Brunet J., Kanjo G., Pfaff A.W., Candolfi E.

12:15-12:30 / **Spatial genetic structure of type II *Toxoplasma gondii* strains involved in human congenital toxoplasmosis in France**
Ajzenberg D., Collinet F., Aubert D., Villena I., Dardé M.L., Devillard S., ToxoBS network group

13:00 - 14:00 Lunch** - USAMVCN

14:00 - 16:00 ***Trichinella* and trichinellosis (SY13/2)**

Organisers: *Dr. Edoardo Pozio (Rome, Italy), Assist. Prof. Dr. Călin Gherman (Cluj-Napoca, Romania)*
Chairmen: *Prof. Dr. Pascal Boireau*, *Prof. Dr. Ioan Liviu Mitrea*. Secretary: *Dr. Miruna Oltean*

Keynote presentations:

14:00-14:30 / **The future of the *Trichinella* sp. control in the European Union**
Dr. Edoardo Pozio (Rome, Italy)

14:30-15:00 / ***Trichinella* in wild carnivores in Romania**
Assist. Prof. Dr. Călin Gherman (Cluj-Napoca, Romania)
Authors: Gherman C., Oltean M.

Oral presentations:

15:00-15:15 / **The role of birds of prey in the transmission of *Trichinella pseudospiralis***
Hurníková Z., Ågren E., Chovancová B., Molnár L., Komorová P., Forsman J., Letková V.

15:15-15:30 / **Cysts calcification of *Trichinella spiralis* in farm swine (*Sus scrofa domestica*)**
Cironeanu I.

15:30-15:45 / **Occurrence of *Trichinella* spp. in the sylvatic cycle in Germany**
Noeckler K., Mayer-Scholl A., Reckinger S.

15:45-16:00 / **The role of dogs in the circulation of *Trichinella* in the French Mediterranean Island of Corsica**
Lacour S.A., Richomme C., Zanella G., Vallee I., Grasset-Chevillot A., Heckmann A., Macé P., Casabianca F., Boireau P.

16:00 - 16:15 Coffee break** - At venues

16:15 - 18:00 ***Trichinella* and trichinellosis (SY13/3)**

Chairmen: *Dr. Edoardo Pozio, Assist. Prof. Radu Blaga*. Secretary: *Dr. Miruna Oltean*

Keynote presentation:

16:15-16:45 / **Stage specific genes and antigens in *Trichinella* genus**
Prof. Dr. Pascal Boireau (Maisons-Alfort, France)
Authors: Boireau P., Mingyuan L.

16:45-17:15 / **Trichinellosis in Romania: overview on the past, present situation and future perspectives**
Prof. Dr. Ioan Liviu Mitrea (Bucharest, Romania)

Oral presentations:

17:15-17:30 / **Impact-update of human trichinellosis -a retrospective epidemiological study in Braşov County - Romania during 1998-2012, for risk management in food safety and ecosanogenesis**
Costache D., Costache C., Bogdan A.T.

17:30-17:45 / **Production and characterization of monoclonal antibodies against a serine protease from newborn larvae stage of *Trichinella spiralis***
Yang Y., Lacour S.A., Laine-Prade V., Versille N., Liu M., Boireau P., Vallee I.
17:45-18:00 / **Seroepidemiological investigations on *Trichinella* spp. antibodies in cats from Romania**
Oltean M., Mircean V., Györke A., Paştiu O., Gherman C.M., Cozma V.

Navy Blue Amphitheatre (27.07)

09:00 - 11:00 **Global Network for Geospatial Health - GNOSISGIS; The first European symposium on parasites and geospatial health; The VI Symposium on geospatial health (SY16/1)**
Organisers: ***Prof. Dr. Thomas Krogsgaard Kristensen (Frederiksberg, Denmark), Prof. Dr. Laura Rinaldi (Naples, Italy)***
Chairmen: ***Prof. Dr. Thomas Krogsgaard Kristensen*****,** ***Prof. Dr. Robert Bergquist*****.** Secretary: *Dr. Cristian Domșa*

Keynote presentations:
09:00-09:30 / **One health and geospatial tools in parasitology**
Prof. Dr. Laura Rinaldi (Naples, Italy)
Authors: Rinaldi L., Musella V., Cringoli G.
09:30-10:00 / **Ecological niche models and the distribution and abundance of hookworms in Bolivia**
Prof. Dr. John B. Malone (Baton Rouge - LA, USA)
Authors: Malone J.B., Mudenda N., Nieto P., Vounatsou P., McCarroll J.C.

Oral presentations:
10:00-10:15 / **Sampling strategies for veterinary parasitological surveillance**
Biggeri A., Catelan D., Musella V., Rinaldi L., Cringoli G.
10:15-10:30 / **A new Bayesian Kriging model to estimate the probability of parasitic infections in the Campania Region (Italy)**
Musella V., Catelan D., Rinaldi L., Biggeri A., Morgoglione M.E., Cringoli G.
10:30-10:45 / **Spatial analysis of *Toxoplasma gondii* infection in goats in Serbia**
Djokić V.
10:45-11:00 / **Spatial distribution of soil and animal contamination by *Toxoplasma gondii* in a rural area**
Gotteland C., Forin-Wiart M.A., Poulle M.L., Charbonnel N., Gilot-Fromont E., Villena I.

11:00 - 11:15 Coffee break** - At venues

11:15 - 13:00 **Global Network for Geospatial Health - GNOSISGIS; The first European symposium on parasites and geospatial health; The VI Symposium on geospatial health (SY16/2)**
Chairmen: ***Prof. Dr. Laura Rinaldi, Prof. Dr. John B. Malone***. Secretary: *Dr. Cristian Domșa*

Keynote presentations:
11:15-11:45 / **Zoonotic parasitic diseases in Europe: climate and global change effects**
Prof. Dr. Santiago Mas-Coma (Valencia, Spain)
11:45-12:15 / **The use of spatial statistics tools and the maximum entropy method for study and forecasting of infectious animal diseases spread**
Prof. Dr. Fedor Korrennoy (Vladimir, Russia)
Authors: Korennoy F.I., Malone J.B, Mores C.N., Gulenkin V.M.
12:15-12:45 / **VecMap: A one-stop-shop for vector mapping**
Dr. Guy Hendrickx (Avia-GIS, Zoersel, Belgium)
Authors: Hendrickx G., Wint W., Bastier S., Ducheyn E.

Oral presentations:
12:45-13:00 / **Increased visual approaches in science communication**
Bergquist N.R.

13:00 - 14:00 Lunch** - USAMVCN

14:00 - 14:30 **Global Network for Geospatial Health - GNOSISGIS; The first European symposium on parasites and geospatial health; The VI Symposium on geospatial health (SY16/3)**
Chairmen: ***Prof. Dr. Laura Rinaldi, Prof. Dr. Fedor Korrennoy***. Secretary: *Dr. Cristian Domșa*

Oral presentations:
14:00-14:15 / **Shortcomings in our work to describe aspects of geospatial health issues**
Kristensen T.K.
14:15-14:30 / **Modelling questing *Ixodes ricinus*: spatial distribution in Romania**
Domșa C., Mihalca A.D.

14:30 - 16:00 **Virtual microscopy of parasites (SY18)**
Organiser: ***Prof. Emer. Dr. Ewert Linder (Stockholm, Sweden)***
Chairmen: ***Prof. Emer. Dr. Ewert Linder*****,** ***Dr. Carmen Costache*****.** Secretary: *Dr. Cristian Domșa*

Keynote presentations:
14:30-15:00 / **Parasite diagnostics in a flat world**
***Prof. Emer. Dr. Ewert Linder* (Stockholm, Sweden)**
Authors: Linder E., Lundin J.
15:00-15:30 / **Human giardiasis report in Romania: the principle of snowball!**
Dr. Carmen Costache (Cluj-Napoca, Romania)
Authors: Costache C., Colosi I., Anca L.
15:30-16:00 / Discussion

16:00 - 16:15 Coffee break** - At venues

Green Amphitheatre (27.07)

09:00 - 11:00 **Parasites of wildlife (SY20/1)**
Organisers: ***Prof. Dr. David Modrý (Brno, Czech Republic), Dr. Andrei Daniel Mihalca (Cluj-Napoca, Romania)***
Chairmen: ***Prof. Dr. Albert Marinculic*****,** ***Dr. Andrei Daniel Mihalca*****.** Secretary: *Dr. Anamaria Balea*

Keynote presentations:
09:00-09:30 / **Parasitic infections of African great apes: how much do we share with our nearest extant relatives?**
Prof. Dr. David Modrý (Brno, Czech Republic)
09:30-10:00 / **Studying parasites in marine mammals (sea lions and northern fur seals): the Romanian experience**
Dr. Mariana Ioniță (Bucharest, Romania)

Detailed Program

Oral presentations:
10:00-10:15 / **Comparative study of *Trichinella* spp. infestation in wild and domestic fauna in the Hunedoara County**
Pârău C.N.
10:15-10:30 / ***Trichinella* (Nematoda, Trichinellidae) of wild animals in Ukraine**
Didyk J.M.
10:30-10:45 / **Nutria and muskrat as intermediate hosts of *Echinococcus multilocularis* in a new endemic area, of the west part of France**
Boué F., Umhang G., Boucher J.M., Guedon G., Richomme C.
10:45-11:00 / ***Toxoplasma gondii* prevalence in Israeli crows and Griffon vultures**
Salant H., Hamburger J., King R., Baneth G.

11:00 - 11:15 Coffee break** - At venues

11:15 - 13:00 **Parasites of wildlife (SY20/2)**
Chairmen: *Prof. Dr. David Modrý*, *Assist. Prof. Dr. Călin Gherman*. Secretary: *Dr. Anamaria Balea*

Keynote presentations:
11:15-11:45 / **Reindeer parasitology**
Prof. Dr. Antti Oksanen (Oulu, Finland)
11:45-12:15 / **Arthropod parasites of endangered vertebrates: threatened or threatening?**
Dr. Andrei Daniel Mihalca (Cluj-Napoca, Romania)

Oral presentations:
12:15-12:30 / **Trypanosome polyparasitism and the decline of the critically endangered Australian potoroid, the Brush-Tailed Bettong, *Bettongia penicillata* (Gray, 1837)**
Thompson C., Botero Gomez L.A., Smith A., Wayne A., Thompson R.C.A.
12:30-12:45 / **Co-infection and genetic diversity of tick-borne pathogens in roe deer in Poland**
Welc-Falęciak R., Werszko J., Cydzik K., Bajer A., Michalik J., Behnke J.M.
12:45-13:00 / **Anisakidae infection in fish of the Aegean Sea**
Chaligiannis I., Lalle M., Pozio E., Sotiraki S.

13:00 - 14:00 Lunch** - USAMVCN

14:00 - 16:00 **Parasites of wildlife (SY20/3)**
Chairmen: ***Prof. Dr. Antti Oksanen, Dr. Mariana Ioniță (Bucharest, Romania)*.** Secretary: *Dr. Anamaria Balea*

Keynote presentations:
14:00-14:30 / **Birds and feather lice: co-speciation or evolutionary arms-race**
Attila D. Sándor (Cluj-Napoca, Romania)
Oral presentations:
14:30-14:45 / **Cryptosporidiosis in overwintering European hedgehogs (*Erinaceus europaeus*)**
Hofmannová L., Hauptman K., Hulcová K., Kváč M.
14:45-15:00 / **Prevalence of *Neospora caninum* and *Toxoplasma gondii* infection by PCR in red foxes (*Vulpes vulpes*) from Romania**
Șuteu O., Paștiu A., Györke A., Gherman C.M., Mihalca A.D., Cozma V.
15:00-15:15 / **Microhabitat selection and host specificity of the digenean *Pholeter gastrophilus* (Heterophyidae) in two cetacean species**
Fraija N., Aznar F.J., Raga J.A., Fernández M.
15:15-15:30 / **Molecular prevalence and genetic diversity of *Borrelia burgdorferi* sensu lato in wild canids and felids from Romania**
Dumitrache M.O., Kálmar Z., Sándor A.D., Paștiu A.I., Mircean V., Gavrea R.R., Oltean M., Gherman C.M., Mihalca A.D., Cozma V.
15:30-15:45 / **Role of wild birds as host of hard-ticks in Romania**
Mărcuțan I.D., Sándor A.D., Dumitrache M.O., Gherman C.M., D'Amico G., Mihalca A.D.
15:45-16:00 / **The parasites of European bison, *Bison bonasus* (Linnaeus, 1758) - the review**
Karbowiak G., Cabaj W., Demiaszkiewicz A.W., Wita I., Moskwa B., Werszko J., Pyziel A.M., Bien J., Gozdzik K., Lachowicz J.

16:00 - 16:15 Coffee break** - At venues

Blue Amphitheatre (27.07)

09:00 - 11:00 **Leishmaniases and their vectors: present and future (SY03/1)**
Chairmen: *Prof. Dr. Jérome Depaquit, Assist. Prof. Dr. Viorica Mircean*. Secretary: *Dr. Mirabela Dumitrache*

Keynote presentation:
09:00-09:30 / **Chemotherapy of leishmaniasis: present and future challenges**
Prof. Dr. Simon L. Croft (London, UK)

Oral presentations:
09:30-09:45 / **First detection of *Leishmania major*-like in naturally infected *Sergentomyia minuta* in Portugal**
Maia C., Cortes S., Dionísio L., Cristóvão J., Neto L., Afonso M.O., Campino L.
09:45-10:00 / **Proteomic approach for comparison of in vitro cultured Trypanosomatids: *Crithidia luciliae* and *Leishmania infantum***
Martinković F., Vučinić S., Horvatić A.
10:00-10:15 / **Molecular titration of *Leishmania* parasites by cloned kinetoplast sequence in Real Time PCR**
Reale S., Migliazzo A., Lupo T., Scopelliti D., De Maria C., La Monica T., Vitale F.
10:15-10:30 / **Phlebotomine sandflies in the Malagasy subregion (Madagascar, Archipelagos of Comoros and Seychelles): settlement and Endemism**
Randrianambinintsoa F.J., Leger N., Robert V., Depaquit. J.
10:30-10:45 / **Determination of species and intra species genetic variations of *Leishmania* parasite by ITS1 Real Time PCR Assay**
Töz S.O., Vardarl T., Çulha G., Zeyrek F., Ertabaklar H., Özbel Y., Alkan M.Z., Gündüz C.
10:45-11:00 / **In vitro effect of lisoquinoline alkoloids of *Berberis* herb on promastigotes of *Leishmania major* and tachyzoites of *Toxoplasma gondii***
Abasian L., Talebi Meymand F., Shirbazou S.

11:00 - 11:15 Coffee break** - At venues

11:15 - 13:00 **Leishmaniases and their vectors: present and future (SY03/2)**
Chairmen: *Prof. Dr. Simon L. Croft, Prof. Dr. Gad Baneth*. Secretary: *Dr. Mirabela Dumitrache*

Keynote presentation:
11:15-11:45 / **Phlebotomine species concept: a large intraspecific molecular variability or different cryptic species involved**
Prof. Dr. Jérome Depaquit (Reims, France)

Oral presentations:
11:45-12:00 / **Visceral leishmanisis in Boyer Ahmad District, South of Iran**
Moshfe A., Mohebali M., Sarkari B.
12:00-12:15 / **Do we really know *Phlebotomus* (*Larroussius*) *perfiliewi*?**
Akhoundi M., Depaquit J., Bounamous A., Leger N.

12:15-12:30 / **Naturally acquired and laboratory induced resistance to allopurinol in canine *Leishmania infantum* isolates from Israel**
Yasur Landau D., Jaffe C.L., David L., Baneth G.
12:30-13:00 / **Biorad presentation: The QX100TMDroplet DigitalTM PCR system - A Breakthrough in Quantitative PCR**
Livescu A. (Biorad)

13:00 - 14:00 Lunch** - USAMVCN

14:00 - 16:00 **Taeniasis/cysticercosis: identification and control strategies (SY09)**
Organisers: ***Prof. Dr. Ana Flisser (Mexico City, Mexico), Prof. Dr. Ioan Liviu Mitre (Bucharest, Romania)***
Chairmen: ***Prof. Dr. Ana Flisser, Prof. Dr. Keeseon Eom.*** Secretary: *Dr. Diana Onac*

Keynote presentations:
14:00-14:30 / **Strategies for control of *Taenia solium* in Mexico**
Prof. Dr. Ana Flisser (Mexico City, Mexico)
14:30-15:00 / **Molecular identification of human *Taenia* tapeworms**
Prof. Dr. Keeseon Eom (Cheongju, South Korea)
Authors: Eom K.S. Jeon H.H.
15:00-15:30 / **TSOL18 vaccine against swine cysticercosis**
Prof. Dr. Marshall Lightowlers (Melbourne, Australia)

Oral presentations:
15:30-15:45 / **Massive larval growth allows some cestodes to skip growth in the definitive host**
Benesh D.
15:45-16:00 / **Optimality, microevolutionary, and macroevolutionary perspectives on the life cycle of *Schistocephalus solidus***
Benesh D.

16:00 - 16:15 Coffee break** - At venues

Biodiversity Centre (27.07)

09:00 - 11:00 **IV K.E. Mott Symposium on schistosomiasis and foodborne trematodiases / B. Foodborne trematodiases (SY07/4)**
Organisers: ***Prof. Dr. Santiago Mas-Coma (Valencia, Spain), Dr. David Rollinson (London, UK)***
Chairman: ***Dr. Tatyana Guzeeva*****.** Secretary: *Dr. Tatiana Băguț*

Keynote presentations:
09:00-09:30 / **Human fascioliasis: control strategies in the present worldwide emergence situation**
Prof. Dr. Santiago Mas-Coma (Valencia, Spain)
09:30-10:00 / **Phenotyping of pure and hybrid fasciolids in Africa and Asia**
Prof. Dr. Maria Adela Valero (Valencia, Spain)

Oral presentations:
10:00-10:15 / **Human fascioliasis in Russia (2006-2011)**
Guzeeva M., Guzeeva T., Kartashev V., Mas-Coma S.
10:15-10:30 / **Human fascioliasis in Argentina: a multidisciplinary analysis**
Mera y Sierra R.L., Agramunt V.H., Cuervo P., Mas-Coma S.
10:30-11:00 / Discussion

11:00 - 11:15 Coffee break** - At venues

11:15 - 13:00 **IV K.E. Mott Symposium on schistosomiasis and foodborne trematodiases / B. Foodborne trematodiases (SY07/5)**
Chairman: ***Prof. Dr. Jong-Yil Chai*****.** Secretary: *Dr. Tatiana Băguț*

Keynote presentations:
11:15-11:45 / **DNA assessment of fossarine vectors of fascioliasis: differences between the Americas and Europe, with emphasis on *Lymnaea schirazensis***
Prof. Dr. M. Dolores Bargues (Valencia, Spain)
11:45-12:15 / **Emerging opisthorchiasis in the European Union**
Dr. Edoardo Pozio (Rome, Italy)

Oral presentations:
12:15-12:30 / **Determination of snails within the genus *Radix* and their role in *Fascioloides magna* life cycle**
Leontovyc R., Hunová K., Horak P.
12:30-12:45 / **Interactions between *Alaria alata* mesocercariae and the paratenic hosts**
Portier J., Lacour S.A., Grasset A., Heckmann A., Dumarest M., Gibout O., Ferté H., Vallée I.
12:45-13:00 / Discussion

13:00 - 14:00 Lunch** - USAMVCN

14:00 - 16:00 **IV K.E. Mott Symposium on schistosomiasis and foodborne trematodiases / B. Foodborne trematodiases (SY07/6)**
Chairman: ***Prof. Dr. Santiago Mas-Coma*****.** Secretary: *Dr. Tatiana Băguț*

Keynote presentations:
14:00-14:30 / **Human trematodiases in the Far East of Asia**
Prof. Dr. Jong-Yil Chai (Seoul, South Korea)
14:30-15:00 / **Human trematodiases in India: present situation**
Prof. Dr. Veena Tandon (Shillong, India)

Oral presentations:
15:00-15:15 / ***Fasciola hepatica* transmitted by *Lymnaea neotropica* in Argentina**
Mera y Sierra R.L., Artigas P., Cuervo P., Deis E., Sidoti L., Mas-Coma S., Bargues M.D.
15:15-15:30 / **A new baseline for human and animal fascioliasis transmission in Chile**
Agramunt V.H., Artigas P., Mera y Sierra R.L., Bargues M.D., Mas-Coma S.
15:30-16:00 / Discussion

16:00 - 16:15 Coffee break** - At venues

Detailed Program

A3 Amphitheatre (27.07)

09:00 - 11:00 **Update on dirofilariasis knowledge (SY12/1)**

Organiser: ***Prof. Dr. Fernando Simón (Salamanca, Spain)***
Chairmen: ***Prof. Dr. Vladimir Kartashev, Prof. Dr. Laura Helen Kramer*****.** Secretary: *Zsuzsa Kalmar*

Keynote presentations:

09:00-09:30 / **Proteomic analyses of *Dirofilaria* species**
Prof. Dr. Fernando Simón (Salamanca, Spain)
Authors: Simón F., González-Miguel J., Morchón R., Mellado I., Siles-Lucas M.

09:30-10:00 / **The history of *Wolbachia* endosymbiosis in filarial worms**
Prof. Dr. Claudio Genchi (Milano, Italy)

Oral presentations:

10:00-10:15 / **Dirofilariosis in Slovakia - results of the first full-area monitoring**
Miterpáková M., Hurníková Z., Iglódyová A.

10:15-10:30 / **Prevalence of *Dirofilaria immitis* and *Dirofilaria repens* in 310 stray dogs in Bulgaria**
Kostadinov M.

10:30-10:45 / **Human dirofilariasis: emergent diseases in Romania?**
Cretu C.M., Staniceanu F, Voinea L, Mihăilescu P, Pop M, Malis S, Popa L.G., Stanescu D., Coroiu Z., Gottstein B.

10:45-11:00 / **Dirofilariasis - case file**
Drăghici S., Jarca A.

11:00 - 11:15 Coffee break** - At venues

11:15 - 13:00 **Update on dirofilariasis knowledge (SY12/2)**

Chairmen: ***Prof. Dr. Fernando Simón, Prof. Dr. Claudio Genchi*****.** Secretary: *Zsuzsa Kalmar*

Keynote presentations:

11:15-11:45 / **Dirofilariosis in Russia - past and present**
Prof. Dr. Vladimir Kartashev (Rostov-on-Don, Russia)
Authors: Kartashev V., Ambalov Y., Ermakov A., Kartashov S., Klyuchnikov A., Guzeeva M., Guzeeva T., Afonin A., Bastrikov N., Morchón R., González-Miguel J., Simón F.

11:45-12:15 / ***Wolbachia* in *Dirofilaria***
Prof. Dr. Laura Helen Kramer (Parma, Italy)

Oral presentations:

12:15-13:00 / Discussion

13:00 - 14:00 Lunch** - USAMVCN

A4 Amphitheatre (27.07)

13:00 - 14:00 Lunch** - USAMVCN

14:00 - 16:00 **Goats and zoonoses (under the frame of cost action CAPARA –FA0805) (SS01/1)**

Organiser: ***Dr. Smaragda Sotiraki (Thessaloniki, Greece)***
Chairmen: ***Prof. Dr. Olgica Djurkovic-Djakovic, Dr. Smaragda Sotiraki*****.** Secretary: *Nikos Tzanidakis*

Keynote presentations:

14:00-14:30 / **From goats to men: some selected zoonoses of public health importance**
Prof. Dr. Jean Dupouy-Camet (Paris, France)

14:30-15:00 / **Toxoplasmosis in goats: a risk for human infection?**
Prof. Dr. Olgica Djurkovic-Djakovic (Belgrade, Serbia)

15:00-15:30 / ***Cryptosporidium*, *Giardia* and goats**
Dr. Thomas Geurden (Ghent, Belgium)

Oral presentations:

15:30-15:45 / **Cystic echinococcosis infection dynamics in livestock in Greece**
Challigianis I., Maillard S., Gottstein B., Sotiraki S.

15:45-16:00 / **High excretion of *Cryptosporidium ubiquitum* in adult goats around parturition**
Paraud C., Pors I., Rieux A., Brunet S.

16:00 - 16:15 Coffee break** - At venues

16:15 - 18:00 **Goats and zoonoses (under the frame of cost action CAPARA –FA0805) (SS01/2)**

Chairmen: ***Prof. Dr. Olgica Djurkovic-Djakovic, Dr. Smaragda Sotiraki*****.** Secretary: *Nikos Tzanidakis*

Keynote presentations:

16:15-16:45 / **Tick infestation and tick-borne infections of goats focusing on their zoonotic importance**
Prof. Dr. Robert Farkas (Budapest, Hungary)

16:45-17:15 / **Neglected helminth zoonoses and goats**
Prof. Dr. Laura Rinaldi (Naples, Italy)
Authors: Rinaldi L., Bosco A., Santaniello M., Guariglia I., Cappelli G., Cringoli G.

17:15-17:45 / ***Echinococcus granulosus* sensu lato on ovine and caprine hosts**
Dr. Andriano Casulli (Rome, Italy)

Oral presentations:

17:45-18:00 / **Epidemiological study in *Toxoplasma gondii* and *Neospora caninum* infections in small ruminants from Romania**
Cozma V., Paştiu A., Gavrea R.

MV Hall (27.07)

Session 1 **Chairman:** ***Prof. Dr. Sorin Morariu*****.** Secretary: *Dr. Ioana Matei*

16:15 - 18:00 **Giardiasis: molecular mechanisms, transmission and control (SY01.P)**

Poster presentations:

SY01.P.01 **Propose of a standard detection method for recovering *Giardia* spp. cysts from soil samples**
Oliveira C.M.B., Guiguet Leal D.A., Nardi A.R.M., Branco N., Allegretti S.M., Franco R.M.B.

SY01.P.02 **Environmental distribution of *Cryptosporidium* and *Giardia duodenalis* in estuarine and shellfish harvesting areas from Brazil**
Guiguet Leal D.A., Durigan M., Souza D.S.M., Allegretti S.M., Nardi A.R.M., Oliveira C.M.B., Barardi C.R.M., Franco R.M.B.

SY01.P.03 **Clinical and epidemiological survey of giardiosis in the clinical hospital of infectious diseases between 2006 and 2010**
Jarca A., Jarca C., Drăghici S.

SY01.P.04 ***Giardia lamblia*: correlation between sub-assemblages and symptoms**
Neagoe I., Palade A., Damian M., Steriu D.I., Lazar L., Toderan A., Nica M., Ceausu E.

Parasites of fish and other aquatic organisms (SY21.P)

Poster presentations:

SY21.P.01 **Molecular characterization of *Anisakis* larvae from marine fish caught off Sicily**
Costa A., Sciortino S., Pisano P., Martuscelli L., Reale S., Di Noto A.M.

SY21.P.02 **Anisakid infections in 8 species of lantern fish (Myctophidae) from the Western Mediterranean**
Mateu P., Fraija N., Gil de Sola L., Raga J.A., Fernández M., Aznar F.J.

SY21.P.03 **Disentangling the taxonomy and geography of *Lepeophtheirus* species of littoral fish species from South-Eastern Pacific Coast**
González M.T., Lopez Z., Irribarren P.

SY21.P.04 **First record of genus *Macvicaria* (Digenea: Opecoelidae) in fish from Chilean coast**
Henríquez V., López Z., Irribarren P., González M.T.

SY21.P.05 **First report of adult of genus *Neobothriocephalus* (Cestoda: Bothriocephalidea) parasitizing *Sebastes oculatus* in the coast of Northern Chile**
López Z., Irribarren P., Henríquez V., González M.T.

SY21.P.06 **Parasites of fish in Kazakhstan Irtysh River area**
Zhumabekova B.K., Tarassovskaya N.E., Syzdykova G.K.

SY21.P.07 **Study of the species composition of fish parasites of Sevan Lake**
Hovhannisyan R., Rukhkyan M.

SY21.P.08 **Molecular and morphological evidence for a cryptic species of the *Rhabdias bufonis* (Hartwich, 1972) s.l. species complex (Nematoda: Rhabdiasidae) from the green frogs of *Rana esculenta* species complex in Italy, and genetic differentiation from its congeners in frogs and toads**
Cipriani P., Mattiucci S., Paoletti M., Santoro M., Nascetti G.

SY21.P.09 **Gastric helminths in the swordfish *Xiphias gladius* collected off the coast of Central-South of Chile**
Muñoz G., García N., Verónica V.

SY21.P.10 **Eumetazoan parasite communities of labrisomid fish from central Chile**
Muñoz G., Castro R.

SY21.P.11 **Metacercarial infections of freshwater fishes at Pasak Cholasid Reservior, Thailand**
Veeravechsukij N., Krailas D., Namchote S., Boonmekam D., Jaruprasit S., Muengdee S.

SY21.P.12 **Accumulation of diverse clones of the digenean *Proctoeces cf. lintoni* in the gastropods *Fissurella* spp. in Northern Chile**
Oliva M.E., Duran C., Cárdenas L., Valdivia I.M.

SY21.P.13 **The most frequent parasitic fish fauna of young carp in the cyprinid fish ponds in Serbia**
Radosavljevic V., Jeremic S., Milicevic V., Zutic J., Veljovic L.

SY21.P.14 **Phenotypic plasticity in haptoral structures of *Ligophorus cephali* (Monogenea: Dactylogyridae) on the gills of *Mugil cephalus* (Teleostei: Mugilidae) from the Albufera Lake, Spain: a geometric morphometric approach**
Rodríguez González A., Balbuena J.A.

SY21.P.15 **First steps in the search of vaccine candidates against fish ectoparasites: *Sparicotyle chrysophrii* and *Lepeophtheirus salmonis***
Agusti C., Wiik-Nielsen C.R., Sánchez-García N., Montero F.E., Raga J.A., Grove S.

SY21.P.16 **Species of *Oswaldocruzia travassos*, 1917 (Nematoda: Molineidae) parasitizing amphibians from the territory of Ukraine**
Svitin R.S.

SY21.P.17 **Metazoan parasite fauna of vimba (*Vimba vimba* L, 1758), collected from fish lakes in lower Kizilirmak Delta, Turkey**
Öztürk T., Özer A., Yılmaz D., Çam A.

SY21.P.18 **Parasıte fauna of rudd, *Scardinius erythropthalmus* L., 1758, collected from lower Kızılırmak Delta (Samsun) in Turkey**
Özer A., Öztürk T., Yılmaz D., Çam A.

Epidemiology of zoonoses (SY23.P)

Poster presentations:

SY23.P.01 **Detection of *Toxocara canis* DNA with different primers using Phire® Animal Tissue Direct PCR Kit**
Pérez Luna I., Muñoz-Guzmán M.A., Sánchez-Mendoza A.E., Cuenca-Verde C., Montiel-Sosa F., Alba-Hurtado F.

SY23.P.02 **PCR-RFLP and sequencing of ITS region for the genotyping of *Enterocytozoon bieneusi* isolates from Tunisian HIV-patients**
Chabchoub N., Abdelmalek R., Kanoun F., Breton J., Thellier M., Bouratbine A., Aoun K.

SY23.P.03 **Diagnosis of latent *Pneumocystis jiroveci* in sputum samples of patients under chemotherapy via nested PCR**
Rezavand B., Mahmoodzadeh A., Izadi M., Sadraei J., Riazipour M., Bagheri O.

SY23.P.04 **Infection rates of helminthesis at the catchment area of Pasak Cholasid Dam under the royal project, Lopburi Province and its related areas, Thailand**
Bootjinda T., Wongsaroj T., Namchote S., Janecharut T., Krailas D.

SY23.P.05 **Molecular diagnosis of a case of gastric anisakiasis associated to *Anisakis pegreffii* (Nematoda: Anisakidae)**
Costa A., Meucci C., Cipolletta L., Fraulo P., Di Noto A.M., Paoletti M., Mattiucci S.

SY23.P.06 **Lethal *Pneumocystis jiroveci* pneumonia 24 Years after kidney transplantation: a case study**
Hosseini M.J., Rezavand B.

SY23.P.07 **Comparison of microscopy, culture and DNA based methods to detect *Blastocystis* sp. in fecal samples**
Jalili A., Hajarzadeh R.

SY23.P.08 **Prevalence and clinical manifestation of nematodosis in Children in Slovakia**
Königová A., Kinčeková J., Hrčková G., Velebný S., Várady M., Dolinská M., Molnár L., Kuchta M.

SY23.P.09 **Identification of bindings partners of CagA *Helicobacter pylori* virulence factor**
Lancrajan I.M., Horge M., Puscas I., Ardelean D.I.

SY23.P.10 **Unusual cryptosporidiosis cases in Sweden – *Cryptosporidium viatoris* and *Cryptosporidium* Chipmunk Genotype I**
Lebbad M., Beser J., Insulander M., Karlsson L., Mattsson J.G, Silverlås C.

SY23.P.11 **Microsporidia in immunocompetent and immunodeficient patients in Poland**
Bednarska M., Bajer A., Jankowska I., Czubkowski P., Wolska B., Pawelas A., Wielopolska M., Graczyk T.K.

SY23.P.12 **Occurrence of intestinal parasites among refugee seekers from humenné refugee camp in Slovakia**
Jalili N., Blažeková M., Hupková H.

SY23.P.13 ***Cryptosporidium* species, Gp60 subgenotypes and clinical manifestations in AIDS patients from Romania**
Neagoe I., Palade A., Damian M., Steriu D.I., Toderan A., Nica M., Duiculescu D., Ceausu E.

SY23.P.14 **Geographical distribution of *Ascaris lumbricoides* and *Trichuris trichiura* in Serbia**
Nikolic A., Klun I., Djokic V., Bobic V., Vujanic M., Živkovic T., Djurkovic-Djakovic O.

USAMVCN (27.07)

19:00 - 22:00 **Transylvanian Outdoor Dinner***

Detailed Program

28.07.2012, SATURDAY

DAY 4

Life Sciences Institute (28.07)

09:00 - 14:00 **Registration** - Life Sciences Institute, USAMVCN

Aula Magna Mihail Şerban (28.07)

09:00 - 11:00 **Epidemiology of zoonoses (SY23/2)**

Organiser: ***Prof. Dr. Ioan Stelian Bocşan (Cluj-Napoca, Romania)***
Chairmen: ***Prof. Dr. Donato Traversa*****,** ***Assist. Prof. Dr. Irina Brumboiu*****.** Secretary: *Dr. Tatiana Băguț*

Keynote presentations:
09:00-09:30 / **Parasitic infections: epidemiological considerations on parasite - host relationships in human medicine**
Prof. Dr. Ioan Stelian Bocşan (Cluj-Napoca, Romania)

Oral presentations:
09:30-09:45 / **Cryptosporidiosis: an Irish perspective**
De Waal T., Mirashemi M., Zintl A., Lucy F.
09:45-10:00 / **Zoonotic transmission of *Cryptosporidium meleagridis* on a Swedish farm**
Silverlås C., Insulanderb M., Lebbad M., Mattsson J.
10:00-10:15 / **Is it giardiasis or is it irritable bowel syndrome?**
Dumitrașcu D.L., Grad S.
10:15-10:30 / **Prevalence of intestinal parasitic infections among 1-10 year-old children in Northwest of Iran**
Ahady M.T., Asadi L., Salehi B., Sharghi A.
10:30-10:45 / ***Toxoplasma gondii* infection in wild boars from north-west of Romania**
Paştiu A., Györke A., Balea A., Onac D., Oltean M., Cozma V.
10:45-11:00 / **Romvac - short presentation**
Chiurciu V.

11:00 - 11:15 Coffee break** - At venues

11:15 - 13:00 **Epidemiology of zoonoses (SY23/3)**

Chairmen: ***Dr. Smaragda Sotiraki*****,** ***Prof. Dr. Ioan Stelian Bocşan*****.** Secretary: *Dr. Tatiana Băguț*

Keynote presentations:
11:15-11:45 / **Pet intestinal worms: a continuing threat in a changing parasitic world**
Prof. Dr. Donato Traversa (Teramo, Italy)
11:45-12:15 / **Is *Alaria alata* a harmful pathogen or just a cause of significant economic loss?**
Prof. Dr. Albert Marinculic (Zagreb, Croatia)

Oral presentations:
12:15-12:30 / **Opportunistic intestinal parasites and malnutrition in Madagascar: how to design studies?**
Roux G., Gosinary F., Raherinampinaina G., Randremanana R., Holianjovony J., Soloniando S., Hariniaina E., Robinson A., Jambou R.
12:30-12:45 / **Prevalence of *Toxoplasma gondii* in wild cervids from Romania**
Balea A., Paştiu A., Györke A., Oltean M., Onac D., Cozma V.
12:45-13:00 / **Giardiasis in infantile population from Cluj county**
Anca L., Colosi I., Junie M., Sicoe F., Costache C.

13:00 - 14:00 Lunch** - USAMVCN

14:00 - 16:00 **Closing Ceremony (PS02)**

Chairmen: ***Prof. Dr. Santiago Mas-Coma, Prof. Dr. Jean Dupouy-Camet, Prof. Dr. Doina Codreanu-Bălcescu, Prof. Dr. Vasile Cozma***

14:00-14:15 / **Presentation of the 13th ICOPA, August 2014 in Mexico City, Mexico**
Prof. Dr. Ana Flisser (Mexico City, Mexico)
14:15-14:30 / **Presentation of the 24th WAAVP Conference, August 2013 in Perth, Australia**
Prof. Dr. Andrew Thompson (Murdoch, Australia)
14:30-14:45 / **Presentation of ESCCAP Toxocara 2012, October 2012 in Budapest, Hungary**
Prof. Dr. Claudio Genchi (Milano, Italy) and Prof. Dr. Laura Helen Kramer (Parma, Italy)
14:45-15:00 / **EFP Young Scientist Awards (Research in basic Parasitology; Research in applied Parasitology)**
Prof. Dr. Jean Dupouy-Camet (Paris, France)
15:00-15:15 / **Best Photograph in Parasitology 2012**
Dr. Andrei Daniel Mihalca (Cluj-Napoca, Romania)
15:15-15:30 / **Bid for EMOP XII**
15:30-15:35 / **EMOP Flag Transfer to theEMOP XII Organisers**
Prof. Dr. Doina Codreanu-Bălcescu
15:35-15:45 / **Good bye message**
Prof. Dr. Santiago Mas-Coma (Valencia, Spain)

Navy Blue Amphitheatre (28.07)

09:00 - 11:00 **Parasites of fish and other aquatic organisms (SY21/1)**

Organiser: ***Prof. Dr. Simonetta Mattiucci (Rome, Italy)***
Chairmen: ***Prof. Dr. Tor Andreas Bakke, Prof. Dr. Liviu Miron*****.** Secretary: *Dr. Maria Bindea*

Keynote presentations:
09:00-09:30 / **Integrating genetic, morphological and ecological data for the characterization of cryptic species of anisakid nematodes: implications for their epidemiology, for the human and the ecosystem health**
Prof. Dr. Simonetta Mattiucci (Rome, Italy)
09:30-10:00 / **The importance of parasites in trout farming**
Prof. Dr. Liviu Miron (Iaşi, Romania)

Oral presentations:
10:00-10:15 / **Upstream-downstream gradient in infection levels by fish parasites: a common river pattern?**
Blasco-Costa I., Martin A., Poulin R.
10:15-10:30 / **Parasites of fish from Lakes Naivasha and Turkana, Rift Valley, Kenya**
Otachi E.O., Magana A.M., Jirsa F., Frank-Fellner C.
10:30-10:45 / **Parasite fauna of common carp, *Cyprinus carpio* L. 1758 in a natural conservation area in Samsun, Turkey and its relation with host size and season**
Öztürk T., Özer A., Çam A., Yılmaz D.
10:45-11:00 / **Parasite fauna of the Black Sea Whiting, *Merlangius merlangus* L. 1758, and its dynamics in relation with some host factors**
Özer A., Kornyychuk Y., Öztürk T., Yurakhno V., Kornyushin V.

Detailed Program

11:00 - 11:15 Coffee break** - At venues

11:15 - 13:00 **Parasites of fish and other aquatic organisms (SY21/2)**

Chairmen: ***Prof. Dr. Simonetta Mattiucci. Assist. Prof. Dr. Călin Gherman***. Secretary: *Dr. Maria Bindea*

Keynote presentations:

11:15-11:45 / **Recent data on human diphyllobothriosis**
Prof. Dr. Jean Dupouy-Camet (Paris, France)
Authors: Dupouy-Camet J., Haydar M., Yera H.

Oral presentations:

11:45-12:00 / **The *Gyrodactylus* species complex infecting holarctic *Salvelinus* spp.**
Hahn C., Robertsen G., Bachmann L., Bakke T.A.

12:00-12:15 / **Museomics for ectoparasites eecovered from historical fish collections - lessons from *Gyrodactylus***
Hahn C., Bakke T.A., Harris P.D., Bachmann L.

12:15-12:30 / **Infestation of gobiid fishes by monogeneans in the Vistula River Basin, Poland**
Rubtsova N.

12:30-12:45 / **Parasites of non-native gobiids fish in the Włocławek Reservoir on the Lower Vistula River: first study in Poland**
Mierzejewska K., Kvach Y., Stańczak K., Kakareko T., Hliwa P., Martyniak A.

12:45-13:00 / **The occurrence and parasitization of the invasive Ponto-Caspian gobiids in the Vistula River Basin, Poland**
Kvach Y., Kornyychuk Y., Rubtsova N., Yurakhno V., Mierzejewska K., Grabowska J., Ovcharenko M.

13:00 - 14:00 Lunch** - USAMVCN

Green Amphitheatre (28.07)

09:00 - 11:00 **Giardiasis: molecular mechanisms, transmission and control (SY01)**

Organiser: ***Dra. Guadalupe Ortega-Pierres (Mexico City, Mexico)***
Chairmen: ***Prof. Dr. Staffan Svärd*****,** ***Prof. Dr. Sonia Drăghici.*** Secretary: *Dr. Adriana Jarca*

Keynote presentations:

09:00-09:30 / **Understanding giardiasis at the molecular level**
Prof. Dr. Staffan Svärd (Uppsala, Sweden)

09:30-10:00 / ***Giardia*-polyparasitism and zoonotic transmission: some observations based on recent studies in Laos and Vietnam**
Prof. Dr. Andrew Thompson (Murdoch, Australia)
Authors: Thompson A., Conlan J., Thi Phong Lan N., Elliot A., Pallant L.

10:00-10:30 / **Current and new drugs against *Giardia* and giardiasis**
Dra. Guadalupe Ortega-Pierres (Mexico City, Mexico)
Authors: Ortega-Pierres G., Argüello- García R.

Oral presentations:

10:30-10:45 / **Fatty acid-CoA ligase (ACL) as a novel drug target in *Giardia duodenalis***
Ortega-Pierres G., Fengguang G., Zhang H., Fonseca-Liñán R., Bazán-Tejeda L., Argüello-García R., Zhu G.

10:45-11:00 / **Prevalence of giardiosis in children societies and risk factors**
Jarca A., Jarca C., Cozma V.

11:00 - 11:15 Coffee break** - At venues

13:00 - 14:00 Lunch** - USAMVCN

Blue Amphitheatre (28.07)

09:00 - 11:00 **Current dynamics of human parasitic diseases, 2006-2011: is Romania different? (SY27)**

Organiser: ***Assist. Prof. Dr. Lidia E. Lazăr (Bucharest, Romania)***
Chairmen: ***Assist. Prof. Dr. Lidia Lazăr*****,** ***Dr. Suzana Cilievici.*** Secretary: *Dr. Petrică Ciobanca*

Keynote presentations:

09:00-09:30 / **Romanian County-map design of human parasitic diseases: findings from the clinic-focused specialty practice**
Dr. Suzana Cilievici (Bucharest, Romania)
Authors: Lazăr L.E., Cilievici S.E., Constantin C.M., Codreanu R.R., Popa L.G., Neagoe I.M.

10:30-10:00 / **Detect, prevent and promote parasitic infections alert in Romanian migrant**
Dr. Ionela Neagoe (Bucharest, Romania)
Authors: Lazăr L.E., Neagoe I.M., Tovarnac V., Cilievici S.E., Codreanu R.R., Steriu D.

10:00-10:30 / **Medical parasitology - a neglected discipline: towards expanded curricula inside global international health concept**
Assist. Prof. Dr. Lidia Lazăr (Bucharest, Romania)
Authors: Lazăr L.E., Cilievici S.E., Codreanu R.R., Neagoe I.M.

10:30-11:00 **/ Strongyloidasis - an intestinal helmintiasis with polymorphic clinical picture**
Dr. Suzana Cilievici (Bucharest, Romania)
Authors: Cilievici S.E., Lazăr, L.E. Codreanu R.R., Constantin C.M.

11:00 - 11:15 Coffee break** - At venues

13:00 - 14:00 Lunch** - USAMVCN

Biodiversity Centre (28.07)

09:00 - 11:00 **EFP - General Assembly**

11:00 - 11:15 Coffee break** - At venues

11:15 - 13:15 **Editing parasitology (WS01)**

Organisers: ***Prof. Dr. Vasile Cozma (Cluj-Napoca, Romania), Prof. Dr. Bertrand Losson (Liege, Belgium)***
Chairmen: ***Prof. Dr. Vasile Cozma*****,** ***Prof. Dr. Mohammad Bagher Rokni.*** Secretary: *Dr. Oana Paștiu*

11:15-11:30 / **Publishing in Veterinary Parasitology: aims and scope of the journal**
Prof. Dr. Claudio Genchi (Milano, Italy)

11:30-12:00 / **History and development of The Korean Journal of Parasitology**
Prof. Dr. Jong-Yil Chai (Seoul, South Korea)

12:00-12:15 / **Editing parasitology in Asia**
Prof. Dr. Mohammad Bagher Rokni (Tehran, Iran)

12:15-12:30 / **Parasitology journals in Romania: past, present and future**
Prof. Dr. Vasile Cozma (Cluj-Napoca, Romania)

12:30-12:45 / **Current problems faced by editors of parasitological journals**
Prof. Dr. Zdzisław Piotr Świderski (Warsaw, Poland)
Authors: Świderski Z.P., Gibson D.I.

12:45-13:00 / **Main actually trends in parasitic protists research; some editing concerns in Romania**
Prof. Dr. Doina Codreanu-Bălcescu

13:00-13:15 / **Publishing strategies in impacted journals**
Victor Velter

Detailed Program

13:00 - 14:00 Lunch** - USAMVCN

Social program (28.07)

16:30 - 19:00 **Cluj-Napoca City Tour*****

Grand Hotel Napoca (28.07)

20:00 - 23:00 **Gala Dinner***

29.07.2012, SUNDAY

DAY 5

08:00 - 20:00 Trip***

* - Cost is included in the registration fee
** - Cost is included in the price of the accommodation. Participants who did not book the hotel thorugh the EMOP registration system have to buy the lunch vouchers at the registration desk.
*** - Only for those who booked one. Offers may still be valid for new bookings. Please contact office@wens.ro

Abbreviations: SY - Symposium; PS - Plenary session; WS - Workshop; RT - Round Table; SS - Satellite Symposium

Social Program

Tuesday, 24 July 2012

Official Reception (Grand Hotel Napoca)

Grand Hotel Napoca 4* is located in the heart of Cluj-Napoca, at 20 minutes to the airport. The New Grand Hotel Napoca is the biggest Conference Center in Transylvania.
Address: Octavian Goga Street, Cluj-Napoca; www.hotelnapoca.ro

Wednesday, 25 July 2012

Chamber music concert (Auditorium Maximum) & Merial Welcome Event (Casa Universitarilor – Piramida Restaurant)
Address: Emmanuel de Martonne Street, No. 1.

Guests: conductor Gergely Balint, The Anatholis Vocal Group and Miniorchestra Classis
Gergely Balint graduated Gheorghe Dima Music Academy of Cluj Napoca with Master's degree, performing violonist at the “Transylvania” State Philharmonic Orchestra with an impressive portfolio.
The Anatholis Vocal Group
The components of the group are the following: Sandor Simonfi-bass, Nistor Bogdan-baritone, Gelu Moldovan-tenor, Florin Pop-tenor, accompanied on piano by Kristian Kosa. They have all graduated The "Gh. Dima" Academy of Music and they are all soloists in the Cluj National Opera House with two or three representations weekly on this stage.
Miniorchestra Classis
This ensemble was created by two young graduates of „Gh. Dima” Music Academy from Cluj-Napoca, violinist Gergely Balint and pianist Kosa Krisztian. Also playing café-concerts and cocktail-music they have decided to diversify their activity, so they play not only in concert halls but also in high-profile restaurants and clubs, providing these with quality music and entertainment.

Social Program

Thuersday, 26 July 2012

Organ Concert (St. Michael Church)

The Church of Saint Michael is a Gothic-style Roman Catholic church in Cluj-Napoca. It is the second largest church (after the Black Church of Braşov) in the geographical region of Transylvania, Romania. The tower is the highest one in Transylvania. The first related document dates from 1349; the construction was completed between 1442-1447 and the tower that stands today was erected in 1862.

Address: Unirii Square, Cluj-Napoca.

Guest: Erich Türk

Erich Türk (born in 1972) studied organ at the *„Gh. Dima" Music Academy* in Cluj with Ursula Philippi and at the *University Of Music And Performing Arts* in Vienna with Michael Radulescu. He also studied the harpsichord with Ilton Wjuniski and Gordon Murray. He participated at several master-classes for organ, harpsichord and basso continuo in Portugal, France, Germany, Switzerland and Moscow. Between 1995 and 1999 he had been organist and choir conductor of the Evangelical Church in Mediaş. Since 1995 he is teaching organ, harpsichord, figured bass and chamber music at the "Gh. Dima" Music Academy of Cluj-Napoca. As soloist as well as a member of the *Baroque Ensemble "Transylvania"* and other chamber music ensembles he is diligently performing throughout most European countries. He made radio, TV and CD recordings, and with the Baroque Ensemble "Transylvania" he realized a documentary DVD on Transylvanian music. At the international *"J.S. Bach" Organ Contest* in Bruges 2000 he has been awarded the 2nd prize and the public´s prize. In April 2004 he obtained his PhD degree for a dissertation written on the stylistics of organ building.

Cultural Dinner (Art Museum)

The Art Museum of Cluj-Napoca has an extraordinary valued heritage of Romanian and European art paintings, graphics and decorative art from the 15th to the 20th centuries. It is a baroque building of the 18th century, built between 1774 and 1775, considered the most representative for the baroque style of Transylvania.

Address: Banffy Palace, Unirii Square, No. 30, Cluj-Napoca; www.macluj.ro.

Guest: Transilvania Brass – instrumental group

The excellent association between different breathing instruments made of yellow cooper – trumpet, horn, trombone and tube – gets a special musical timber, characteristic for american jazz-groups. This group is compound by Grigore Ciobanu – trumpet, Marius Beteag – trumpet, Lucian Marcu – horn, Cornelius Man – trombone, Ionuț Mandi – tuba, Alexandru Popovici – percussion. The repertory contains famous musical songs which bring the "new" on the Romanian stage. The first appearance of the band dates from 2010 on the stage of University Cultural Hall being a real success.

XIth European Multicolloquium Of Parasitology

Cluj-Napoca – Romania
July 25th-29th, 2012

Keynote presentations

ANNIVERSARY OF 100 YEARS FROM PROF. DR. ERNEST M. UNGUREANU'S BIRTH 1912-2012

Tarcoveanu E.

University of Medicine and Pharmacy "Gr. T. Popa", Surgery Clinic, Hospital "Saint Spiridon" Iaşi, Romania.
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Abstract
An outstanding specialist and teacher, school founder and guide, modeling characters, a kind human being to all who knew him, Professor Ernest Ungureanu was born on 5 July 1912, in the small village of Bistricioara in the Neamț district, where he spent the first years of his life. His parents later moved to another village, Văculeşti, Dorohoi, in the neighboring district of Botoşani, where he completed the fourth and fifth forms in a single year (1923). After three years of study at Laurian Upper Secondary School, in the city of Botoşani, he moved to "Gr. Ghica Voievod" Upper Secondary School in the town of Dorohoi, where he passed his Baccalaureate.
During the autumn of 1932 he went to the Iasi School of Natural Sciences, (1932-1936), where he studied and was a disciple of such extraordinary professors as Paul Bujor, Ioan Borcea, Constantin Motaş and a few others. Ever since the end of the first year, the biologist Vasile Radu (who at the time was head of the research division of the Compared Anatomy Department), allowed him access to the department's laboratory and introduced him to the techniques of research in histology and cytology; he published his first scientific article while he was his mentor's research assistant. At the end of the third year, Professor Paul Bujor recommended him to Professor Leon Ballif from the Iaşi Medical School, who intended to initiate cytology research regarding the glands of internal secretion. Thus, the student Ernest Ungureanu founded a small cytology laboratory, as part of the Socola Hospital in Iaşi; he engaged in the systematic study of the dynamics of thyroid secretions, the adrenal glands and the ovaries under the influence of the hypophysis hormone; Ernest Ungureanu published a part of the results of his researches in collaboration with Professor Leon Ballif and Dr. Gheracovici.
During the same period, as a student, he met Prof. Dr. Constantin I. Parhon, who often came from Bucharest to Iaşi to visit the Socola Hospital and who took a special interest in the results of the research Ernest Ungureanu did in his modest cytology laboratory.
In 1936, Ernest M. Ungureanu defended his graduation thesis with the title "Regeneration", receiving the *Magna cum laudae* degree.
In 1937, Professor Mihai Ciucă granted the young graduate Ernest Ungureanu with the position of entomologist at the Iaşi Institute of Public Hygiene. This was the beginning of a life-long friendship and scientific collaboration between the two. This was the beginning of a life-long friendship between the two. So he had the opportunity to expand his researches to doing field work in the area of malaria epidemiology, which he continued in the Medical Entomology Laboratory hat was founded at Osoi, next to the city of Iaşi. Small as they were, these laboratories acquired a great scientific importance through the researches conducted there by Ernest Ungureanu within the Malaria Eradication Programme of the League of Nations, from Geneva, a programme which was led by Prof. Mihai Ciucă, who acted as secretary of the Geneva International Commission for Hygiene. This programme included collaboration with the Rockefeller Malaria Unit of the Socola Hospital in Iaşi and the Malaria Therapy Unit in Horton-Epson, Great Britain. In these laboratories, Ernest Ungureanu worked till 1941.
In 1946 he defended his doctoral thesis in Natural Sciences with the topic: "Research on Anophelism in relation with Malaria transmission in North-Eastern Romania," for which he was awarded the Magna cum Laude PhD degree.
In 1938, the Malaria Commission of the League of Nations awarded him an internship to study malaria and mosquitoes from Great Britain, where he worked in the Malaria Therapy Unit of Horton-Epson, near London.
In 1946, the young researcher Ernest Ungureanu continued his studies of mosquitoes from the River Thames and from others locations in the world, studying at Horton-Epson, London School of Tropical Medicine, London University, Cambridge University, British Museum, 5 (Entomological Section).

On the basis of his researches, Ernest Ungureanu, working with talent, scholarly devotion and competence, Ernest Ungureanu managed to solve some problems concerning the systematic study of Anopheles as transmitter of malaria in Europe and particularly the morphological character of *Anopheles maculipennis* females, which had been looked for many years by scientists having a great experience in this field.

We must mention that these discoveries made by Ernest Ungureanu were presented by himself during the Meetings of the Royal Entomological Society in London.

The results of his researches were published in volumes which received a very good appreciation from scientists and, being valuable, these results have been published in the malariological books, representing a rich source of inspiration for national and international studies in this field.

At the Malaria Therapy Unit in Horton-Epson, he met the most reputed specialists of the time. During 1946 he worked at the Ross Institute of Tropical Medicine and Hygiene of London with the director of the institute, Prof. G. MacDonald, who appreciated him very much.

Besides, the young researcher Ernest Ungureanu continued his research at the Moltena Institute of Cambridge where he worked with Sir Richard Cristophers, who was impressed by his scientific talent and by the power of observation of his young collaborator and colleague. He worked steadily and he maintained a close scientific collaboration all the time with the world-famous malariologist P. G. Shute, head of the Horton Malaria Laboratory, which had the most advanced technique of the day.

From 1950 to 1955 he worked with Professor Mihai Ciucă, director of the Institute of Parasitology, Bucharest; the young professor Ernest Ungureanu became deputy director of this Institute, receiving a part of the managerial responsibilities of the new institution of research.

From 1948 to 1963 he was director of the Malaria and Parasitological Department of the Institute of Hygiene in Iaşi, whose general manager he had been in 1944, as well as in 1960-1961.

In 1948 he was appointed Professor at the Parasitological Department of the Iaşi Institute of Medicine and Pharmacy, where he worked until 2002 (with a break when he worked as International Expert of the World Health Organization, Geneva in the field of malaria eradication and tropical diseases).

In 1954 he became Doctor in Biological Sciences.

Thanks to an internship awarded by the World Health Organization, Ernest Ungureanu did research in the malaria field at the Institute of Public Health in Rome where he worked and where he collaborated with Professors Mesana and Bettini and at the Institute of Malaria Studies, where he worked with Professors G. MacDonald and Busvine. During the period he was active in the World Health Organization team, he worked in Rome on two more occasions.

Ernest Ungureanu studied the insecticides and the characteristics of the most widespread malaria carrier in Africa, *Anopheles gambiae*, perfecting a method of insect processing through conservation, freezing, fixing and drying them. This method, which he called **"New methods of dissecting carrier insects", was published in extenso in the World Health Organization Bulletin and was then requested by over 150 laboratories from Europe, Asia, America, Australia and Africa.**

According to his own words, in his professional training, Prof. Dr. Ernest Ungureanu was considerably influenced by the famous scientist Mihai Ciucă, as well as by Dr. Dumitru Cornelson, director of the Iaşi Institute of Public Health. In his turn, he formed many generations in his capacity as Professor at the "Gr. T. Popa" University of Medicine and Pharmacy, Iaşi, Medical Parasitological Department, from 1948 to 1976 and as Consultant Professor and PhD advisor till 2002.

He was nominated International Expert of the World Health Organization, Malaria Eradication Division, in Geneva. Professor Ernest Ungureanu worked as International Consultant Expert of the World Health Organization, Member of the International Experts Panel, Member of the Malaria Commission of the World Health Organization, Member of the Steering Committee of Malaria Field Research of the World Health Organization, Geneva.

All his researches in the field of clinical diagnosis, treatment and prevention of parasitic diseases materialized in over 350 scientific articles published, as well as lectures delivered in Romania and in countries on all continents.

Three of his most important papers regarding malaria were published in the Records of the Romanian Academy, in Archives of Microbiology and in Transaction of the Imperial Society of Entomology, London. These papers were invaluable for the instruction of health care workers and for the management of malaria control in Romania under the guidance of Mihai Ciucă, member of the Romanian Academy. For his contribution to the progress of science, Professor Ernest M. Ungureanu received the prestigious Victor Babeş Award in 1949.

He paid special attention to the research in the field of medical entomology, **discovering new species of mosquitoes in Romania and a species previously unknown to scientists, a species which ought to have received the name of Prof. Ernest Ungureanu.** Out of modesty, he refused to give his name to his discovery and he named it *Theobaldia glaphiloroptera* **Var. *Zottai*, thus dedicating his discovery to the memory of his Professor Gh. Zotta.**

In order to rationally prevent malaria carriers by means of modern insecticides, he also built original equipment, including a micro-pipette which he described in the official Bulletin of the World Health Organization and which Professor MacDonald referred to in his *Treatise on Malaria.*

Within the same field of entomology, he discovered the morphological characteristics of malaria carriers in Europe; the results of his researches had practical application and strongly contributed to the eradication of malaria in Romania. These successful efforts were appreciated and emphasized upon, being quoted and referred to in malariology treatises in many countries around the world.

In their turn, his studies on epidemiology and the eradication of helminthiasis in Eastern Romania (Moldavia) and the Danube Delta allowed for the first correct assessment of the importance of the issue of parasites in relation to their environment; the research dealt with the influence of environmental factors on the epidemiology of intestinal parasites in flat geographical areas and also in hilly and mountainous areas. He presented his conclusions to the World Health Organization Panel of Experts during the Rio de Janeiro World Conference in 1963 and these results were very well received.

His researches on the epidemiology of bothriocephalosis in the Danube Delta involved the study of the whole Danube Delta population and the treatment of the infected people, accompanied by sanitary educational recommendations which aimed at avoiding the spread of bothriocephalosis in the rest of Romania. **Prof. Dr. Doc. Ernest Ungureanu, through his remarkable research work, discovered three new parasitic diseases, unknown in Romania. These are: opistorchosis, gongylonemosis and ancylostomia caused by *Ancylostoma caninum*.**

Professor Ernest Ungureanu also conducted experiments regarding the antihelmintic activity of some natural substances, which enabled him to classify them into three categories: medicines activated by bile, medicines inactivated by bile and medicines uninfluenced by bile. Clinical observations made on bothriocephal infectious revealed the appearance of conditioned reflexes in the presence of parasites, which account for the diverse manifestations produced by the same species of parasite.

Due to his steady work, Professor Ernest M. Ungureanu came to be the author of twenty-two patented inventions and innovations, among which the preparation of a colouring substance for malaria and haematology, which right after the war facilitated the activity of haematology and malaria laboratories engaged in detecting malaria and recurrent fever and leucocitary formulae.

As International Expert of World Health Organisation, Malaria Division, he organized courses of epidemiology, malaria eradication and laboratory techniques in Manila (three courses), Kuala-Lumpur, Bangkok (two courses), Lagos (Nigeria), Tunis (two courses each), and Alger (three courses). All the courses were delivered by Professor Ungureanu both in French and English within the same session, this kind of presentation being very difficult and exhausting. All these programmes were watched and appreciated by physicians from different corners of the world: from the Far East, South-East Asia and Africa.

In order to improve the field work of the World Health Organisation, Professor Ernest Ungureanu proposed to the World Health Organisation, Malaria Division, the introduction of a new method, the guided study visits, replacing individual study visits that had had no results. Being a member of the Executive Board for Researches in the Malaria Field of the World Health Organization, Prof. Ernest Ungureanu approved research projects and study scholarships and made comments on the results of researches carried out by various Medical Institutions from all over the world.

As a member of the Malaria Commission of the World Health Organization, he evaluated the situation of preventive sanitary organizations and of the level of the malaria fight in Australia and Singapore, in order to certify the eradication of malaria; his recommendations were approved and highly appreciated by the General Assembly of the World Health Organization and by the Executive Board of the World Health Organization.

Prof. Dr. Ernest Ungureanu also evaluated the activity and the possibilities of training medical teachers and creating new curricula, also formulating proposals for improving these curricula, in different World Health Organization educational Centres such as those in Laknow (Pakistan), Dacca (Bangladesh), New-Delhi (India), Addis-Abeba (Ethiopia), Sennar (Sudan), Alexandria and Cairo (Egypt). Other important activities of Prof. Dr. Ernest Ungureanu in the World Health Organization regard the efforts of improving the activity of some Institutes concerning the research contracts with the World Health Organization: the Institute of Tropical Medicine (Hamburg); London School of Tropical Medicine and Hygiene, London; the Institute of Parasitology of Rome; the Institute of Medicine in Lyden (Holland); Alexandria, Egypt; Singapore.

In 1980, upon World Health Organization request, Professor Ernest Ungureanu evaluated the possibilities of research in the malaria field first at the National Institute of Microbiology and Parasitology of New-Delhi (India), and then in Dacca (Bangladesh), Kathmandu (Nepal), Burma Djakarta (Indonesia), Thailand and Colombo (Sri-Lanka). What he envisaged was the identification and selection of possibilities and forces to be used in the World Health Organization Education Centre subsequently created, on his proposal, in Kuala Lumpur (Malaysia).

As Expert of the Malaria Eradication Commission of the World Health Organization, Professor Ungureanu elaborated recommendations concerning the improvement of malaria fight in: the Philippines, Thailand, Malaysia, Bangladesh, India, Pakistan, Indonesia, Iran, Israel, Egypt, Sudan, Ethiopia, Tunis and Algeria. He also completed projects of malaria pre-eradication in the African region South of Sahara, Mauritius Islands and in Central American countries.

The conferences Professor Ungureanu presented in the World Health Organization regional offices in Washington, Manila, Brazzaville, Copenhagen, Alexandria, New-Delhi, Geneva met with real success. At the beginning and at the end of each mission on site, his scholarly comments and recommendations were turned into rigorous reports submitted to the general director of the World Health Organization and to regional offices.

His substantial scientific reports and the conferences on malaria eradication in Romania were presented to the Health Ministry of Beijing, the Academy of Sciences of Shanghai, The Faculty of Medicine of Canton, the Faculty of Medicine and the Health Ministry of Hanoi, Djakarta, Dacca, Phenian, as well as to International Congresses.

With respect to his teaching activity, apart from training many generations of students and specialist physicians, in Romania (at “Gr. T. Popa” University of Medicine and Pharmacy, The Institute of Hygiene, The “Ion Ionescu de la Brad” Agricultural University, all from Iaşi), as well as abroad, Professor Ungureanu organized courses and seminaries in Manila, Bangkok, Kuala-Lumpur, Alexandria, Adis-Abeba, Lome, Lagos, Tunis, Alger. We must mention that he delivered specialised courses for a large number of series of malariologists, epidemiologists and sanitary engineers from the Far East, Asia, Africa and America. He also examined the curricula and the research of the World Health Organization teaching centres and the research programmes of the malaria projects of W.H.O. all over the world, making specific recommendations with a view to improving their activity.

Apart from all these activities, we must mention that **Professor Dr. Ernest M. Ungureanu organized and directed the antimalaria campaign in Moldova and contributed to the organization and the guidance of the antimalaria campaign in Romania.**

He organised and directed the first Malariology School founded in Romania. The elaboration and the unfolding of courses and laboratory practice were performed under his strict supervision.

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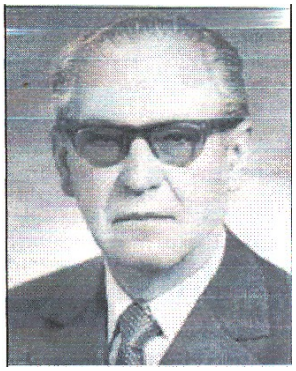
In appreciation of his special merits, Prof. Dr. Doc. Ernest M. Ungureanu was nominated as active member and honorary member of many Societies and Academies.

Here are some of his titles: Professor Emeritus of the University of Medicine and Pharmacy “Gr. T. Popa”, Iaşi, Honour Member of the New York Academy, Honour Member of the Academy of Medical Sciences, Honour Member of the Balcan Medical Union, Honour Member of the Academy of Scientists, Romania, President of the Society of spreading the Science and Culture, the county of Iaşi, Member in the editorial board of the Journal ”Angemants Parasitology” (Germany), etc.

Regarding the place of parasitology in the medical training, Professor Ernest Ungureanu emphasised the importance of a central place, since parasite diseases are widely spread and imitate the different symptoms of other diseases.
He underlined incessantly that "the study of parasitology should be carried out in an interdisciplinary manner. Parasitology ought to have sufficient teachers and must receive in universitary curricula a sufficient number of course hours and laboratory practice, the necessary amount of time in order to achieve a thorough training of future doctors and specialists."
The academic, didactic and managerial activity carried out by the eminent scientist Professor Ernest Ungureanu, not only in Romania, but on all continents, in order to fight against malaria, represents a brilliant example appreciated even today at an international level, an example of dedication and fruitful work, performed with abnegation and talent, sometimes under unfavourable circumstances, facing the risks and perils of the equatorial jungle or of other regions of the Earth.
He is one of the people who honoured their country in the sense given by the famous French scientist Louis Pasteur: "Science has no country, but the scientist must have one, he should offer his country the prestige that his works acquire throughout the world". Professor Dr. Doc. Ernest M. Ungureanu entirely corresponded to this statement, belonging to the elite of Romanian and international science.



TRIBUTE TO
PROFESSOR ALEXANDRU NICULESCU, PHD IN SCIENCE,
DOCTOR HONORIS CAUSA
REMEMBER 1916- 2011

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Now, in 2011 – the International Year of Veterinary Medicine, we have a duty of honour to remember our distinguished teacher, Professor Alexandru Niculescu, PhD in Science, that extraordinary man, who would have turned 95 and still constitutes a living example among the great professors that the Veterinary Science has had so far.
Professor Alexandru Niculescu, PhD in Science, D.H.C., was born on February 5th 1916, in Bucharest, in a modest family, with parents who died at a rather young age, leaving their children orphans, children who were brought up by the relatives.
Endowed with a native intelligence, the child, then, the adolescent, adapted to the hardships of life, becoming a brilliant student, which facilitated his entrance, in 1936, at the Veterinary Medicine Academy, among the first students; since then, except for the years of WW II, when he was on the front, he had never left the Academy, being an example of dedication, vitality and intelligence until 2004, when he died.
Only 18 months before turning 90, he passed away, but only physically, from the earthly life, our Docent Professor DHC, Professor Alexandru Niculescu, an extraordinary man, creator of a new school, a scholar in the field of Veterinary Science, who will remain as a reference personality for our profession, which he served with dedication for almost 70 years, 62 of which merely in the discipline that he created, under the name of Parasitology, Pathology and Clinical of the Parasitic Diseases. Professor Alexandru Niculescu organized this discipline on modern bases, on scientific principles with a large practical application, being, in the same time, for 30 years, the Head of Department, for 6 years, the Dean of the Veterinary Medicine Academy, a Scientific Director and Pro-rector of the University of Agronomical Science and Veterinary Medicine Bucharest.
Professor Alexandru Niculescu, PhD in Science, DHC, followed all steps in the teachers' hierarchy, respectively of University Preparatory (1944), Assistant Professor (1945), University Lecturer (1947), Associate Professor (1951), Professor (1963), and held leadership positions, like: Head of Department (1958-1981), Dean of the Veterinary Medicine Academy (1952-1957), Pro-rector (1957-1963), Director of Studies at the University (1963-1967); since 1963 he had been a PhD Supervisor Professor, preparing numerous researchers and Professors in the field of Parasitology, the former and current Professors of the Veterinary Medicine Academies in Romania.
If the names of his fore Professors – Ioan Ciurea and Gh. Dinulescu are linked with the beginnings of the organization and introducing Parasitology in the Academy, Professor Alexandru Niculescu's name is linked with the clinic disciplines studied in the 4th, 5th, 6th years of University, structuring, in the same time, the curricula of the discipline, according to the practical requirements and modern evolution of knowledge in the field, thus the presentation of diseases is made after a natural scheme: definition, importance in Zooeconomics and Zoonotic, Epidemiology, Morpho-clinical peculiarities, correlations in the pathogen activity of parasites, diagnosis and differential diagnosis, specific treatment, prevention and control measures. The objectivity and rightness of these orientations had been verified and demonstrated in the following years through the request and participation of teachers in solving some major problems in the country, like: sheep scabies, poultry coccidiosis, gastrointestinal and pulmonary helmintosis in herbivores and swine. Professor Alexandru Niculescu, PhD in Science, DHC, led himself the actions of controlling these diseases and he formulated, together with his collaborators, new medicines, like: ENTOMOXAN, COCCIDIZIN, GALISAN, FLAGICID, TRICOFITIN A and B, FILIXON, a.o., for which he received inventor patents.

His scientific and publishing activity, acknowledged in our country and abroad, is impressive, having over 300 scientific works communicated and 12 books and specialized treaties; we mention that his Parasitology Treaty reached the 7th edition in 1998.
Professor Alexandru Niculescu, PhD in Science, DHC, worshipped his mentors, (PhD Ioan Ciurea and PhD Gh. Dinulescu), but, in the same time, he formed a new school of Professors: E. Simionescu, I. Suteu, I. Cosoroaba, T. Lungu, C. Milla, A. Purcherea, N. Dlcan and others; he tutored the scientific activity of 28 PhD, who received their scientific title of Doctor in Veterinary Medicine.
Since ancient times, it has been well known, that around the Master, gathered people who wanted to drink from the science and wisdom of the one who was opening the roads to perfection, towards molding a concept. Today, we, the people who have understood the mission of the school creator, bow our heads as a tribute to the 95 years since the birth of Professor Alexandru Niculescu.
Every soul that received his scientific, competent, modern guidance, cannot be but proud to have developed some works that brought them among the famous parasitologysts, works which, without the master's endorsement, without a thorough study of every scientific statement, of every experiment, could not have fulfilled their desideratum.
Professor Alexandru Niculescu wished to create from us people who should step forward, the novel to be back to top, that there should be no equal under the aspect of genuineness; grand is the masterpiece to which he did not hesitate and did not spare any effort, putting in balance and looking in the specialized literature the possible correspondence, always highlighting the particular degree of contribution of each of us.
We cannot but remember the emotion of our first courses, the way in which the exposure of the lectures was always tied with our power of understanding. The teacher knew how to make his lecture looked for, listened to with pleasure and interest. Then, the teacher knew how, from the stack of subjects, to find for each the problem which should have produced the first emotions in developing a diploma thesis and after that, the scientist had always found the issues, the methodology, the material and the method through which the enthusiasm of the researches could come out and complete, in a doctoral thesis, the accumulations of years of study.
It is proper to remind that Professor Alexandru Niculescu, PhD in Science, DHC, was one of the grand masters among the past and present Professors of the Veterinary Medicine Academy, being endowed with an exceptional oratory talent, captivating, making his classes extremely attractive, despite the complexity and numerous scientific names of the etiological parasitic agents.
Gifted with a divine grace, with oratory qualities as rarely can be seen, he managed to make the presence of his students in classes a real pleasure; he considered the Academy a creative institution, a real source of intellectual, which he instilled in the veterinarians, since their students' days, to participate to the scientific circles.
Professor Alexandru Niculescu used to say that Veterinary Medicine is firstly a science and it must be applied with Art and judgement.
I met him with a smile and a carney, sometimes with implied, with a part of humour between two almost rhythmical movements of his hand that sat his glasses, all of these made for us to build him in our souls as a pedestal on which to put our role model in life and profession. Life in not only a scientific or spiritual success, it also has many bumps and hardships, but the wisdom of speech and its story, as only his oratorical talent, supported by the depth of his accumulations in so many years of teaching, made it possible for each of us, who sat around him, to feel the impulse to follow the path to the shore. Joy and happiness are the major attribute of the young soul, together with the mimic expression of satisfaction which I had always found in our Professor, for there is no joy without a tear, for even the emotion of joy and happiness bring tears which are shining like the morning dew.
Professor Alexandru Niculescu's endless devotion to the Academy and subject are also proved by the fact that, after 1995, when the Academy resumed its activity in its place, he was indeed an active presence for restoring the scientific and didactic activities on the level of his requirements and, together with his former collaborators, PhD Anca Purcherea, had a decisive role in forming the present team of Professors in the Discipline, who were his PhD.
For his professional, scientific and military merits he received numerous military and scientific orders and distinctions, being a Docent Professor in Science, an honour member of The General Association of the Veterinary Doctors in Romania, a laureate of the "Gh. Ionescu Brăila" Prize, of the Veterinary Science Society, a foreign member of „Societó de Parazitologi Comparie", DHC of UASMV Cluj-Napoca; military honours: Crucea Serviciu Credincios Medical cl. II-a, Ordinul Meritul Militar cl. II-a, Ordinul Comemorarea celui de al II-lea Război Mondial – 1995.

Professor Alexandru Niculescu, PhD in Science, DHC, was a good, civilized, sensitive man, a humanist, full of affection and comprehension, having the only wish to do good, he had an immense love for people; he was wise, tolerant, but brave, open to people, a distinguished personality, emblematical for the Academy, he demonstrated a noble modesty, always finding a place in his peers' hearts; he was discreet and a stability factor, manifesting availability without restrictions; he had a special humor, a subtle one, chasing sadness away, being a convinced optimist, he cultivated value, kindness and tolerance, using kindness as a way to reconcile, proving an infinite kindness and understanding, he never refused anybody, having satisfaction when helping people, he was always admired for his good heart.

For me, the emotion of joy is greater as I was often asking myself: "how could the son of an honest peasant, with a healthy mind and love for his country and people find in a new world the support of a man with such a great soul, who guided his steps in the chosen profession, so closely related in feelings with his origin…". I answered myself in time, knowing the qualities of the man of humanity and science to whom we pay tribute today.

NEGLECTED TROPICAL DISEASES: ACTION ROADMAP FOR THE PRESENT DECADE

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TRENDS IN PARASITIC DISEASES: PAST, PRESENT AND FUTURE

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THE DYNAMIC OF TICK-BORNE PATHOGEN TRANSMISSION

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Introduction

Ixodid ticks transmit through their bite a broad range of protozoan, viral and bacterial pathogens to animals and humans (Heyman et al., 2010).
Lyme borreliosis in humans and babesiosis as well as anaplasmosis are ones of the most important tick-borne diseases.
In Europe, the reported increase in incidence of tick-borne diseases appears largely due to changes in human behavior (recreation and travel with pets), ecology (changes in ecosystem management with consequent increased wildlife host abundance) and climate related changes (increased tick survival and abundance) (Beugnet and Marie, 2009; Gray et al., 2009).
A combination of factors mentioned above have, for instance, resulted in autochthonous cases of canine babesiosis in the Netherlands (Matjila et al., 2005), Germany, Poland and very recently also in Norway (Oines et al., 2010), showing that the vector for *Babesia canis*, e.g., *Dermacentor* ticks are expanding their distributional range further into North-Western Europe. Another example also recently reported in Germany concerns the increased incidence of canine tick-borne diseases in veterinary practices due to increased import and recreational travel abroad with pets. Finally, it has been reported that *Ixodes ricinus* ticks, the vector of Lyme borreliosis, *Anaplasma phagocytophilum* and TBEV reach higher altitudes in the mountains, higher than in former years and spread north in Sweden, Finland Germany and west in Austria (Suss, 2010).
It is thus predictable that tick borne diseases will be an increasing burden in Europe in the near future and an effort has to be conducted to improve prevention plans to avoid tick bites and/or to identify new drugs that will block pathogen transmission as early as possible after tick bites.

How to study dynamics of pathogen transmission by ticks?

To study the biology of ticks and the dynamics of pathogen transmission, it is indispensable to be able to maintain tick colonies under laboratory conditions and to have efficient techniques to feed them. Moreover, compared to other haematophagous arthopods, feeding ixodid ticks is a slow and complex process lasting several days to weeks before repletion and detachment. Host attachment is preceded by a behavioural sequence that depends on the presence of an appropriate array of chemical and physical stimuli. These characteristics mean that in ticks, unlike mosquitoes, the transmission of pathogens is not immediate and time required varies from one pathogen to another.
In vivo studies: The use of natural hosts for tick feeding and methods of direct infection on animals is the method of choice to obtain conditions that are closed to physiological state. However, acquisition, housing, and handling of animal hosts can be complicated, expensive and sometimes even impossible. Moreover, maintaining the natural host of a specific tick-borne pathogen is impossible in laboratory, particularly for wildlife. The most commonly used model of tick infection directly on animal concerns infections by pathogens infecting cattle as *Babesia divergens* transmitted by *I. ricinus* (Joyner et al., 1963; Donnelly and Peirce,1975; Lewis and Young, 1980), *Anaplasma marginale* by *D. andersoni* (Kocan et al., 1986), *Theileria parva* by *Rhipicephalus appendiculatus* (Bailey, 1960; Musyoki et al., 2004), or *T. mutans* and *Cowdria ruminantium* transmitted by *A. variegatum* (Young et al., 1996). Finally, laboratory mice have also been used for studying *Bartonella birtlesii* transmission by *I. ricinus* (Reis et al., 2011a), *Borrelia burgdorferi* by *Ixodes scapularis* (Burkot et al., 2001) or *Anaplasma phagocytophilum* (Massung et al., 2004). However, given the technical difficulties inherent to in vivo feeding, *in vitro* systems have developed in recent years.
In vitro feeding techniques: Ticks can be infected in vitro using, with more or less success: direct injection through the cuticule, infection by capillary feeding via the mouthparts and digestive tract, or by membrane feeding techniques. Membrane feeding techniques mimic the natural conditions of tick infection more closely than other methods because pathogens are mixed in blood and are absorbed throughout the blood meal via the digestive tract. Membrane feeding permits a direct assessment of pathogen concentration in the blood sample ingested by the ticks. Repeated assays with large tick numbers are also possible with this system. Finally, membrane-feeding techniques may allow one to evaluate precisely the dynamics of pathogen transmission, as illustrated by our work with *Babesia* EU1 and *Bartonella henselae* (Bonnet et al., 2007; Cotte et al., 2008; Bonnet et al., 2009).

How long does it take for a tick to transmit a pathogen?

The duration of attachment required for ticks to transmit a pathogen has been studied in different models and it is now well known that this duration varies according to the bacterial, protozoan, or viral nature of the pathogen concerned

In this presentation we will use 3 examples that illustrate the differences in dynamic transmission depending on the nature of the pathogens: a virus, the TBE, considered as a rapid transmitted agent, a bacteria, *Borrelia burgdorferii*, the agent of Lyme disease and a parasite, *Babesia* EU1, a newly described zoonotic agent. We will document the molecular mechanisms, when known, that explain the dynamics of pathogens mentioned and we will evaluate the impact of this knowledge on how to fight against these pathogens.

Conclusions

The early events after tick attachment which lead to the actual transmission of pathogens are crucial and need to be studied in further detail in order to optimize the blocking of pathogen transmission. In particular, effort has to be made to develop control strategies of several pathogens transmitted by the same vector ticks.

References

Bailey K.P. 1960. Note on the rearing of *Rhipicephalus appendiculatus* and their infection with *Theileria parva* for experimental transmission. Bull. Epizoot. Dis. Afr. 8:33-43.

Beugnet F., Marie J.L. 2009. Emerging arthropod-borne diseases of companion animals in Europe. Vet. Parasitol. 163(4):298-305.

Bonnet S., Jouglin M., L'Hostis M., Chauvin A. 2007. *Babesia* sp. EU1 from roe deer and transmission within *Ixodes ricinus*. Emerg. Infect. Dis. 13(8):1208-10.

Bonnet S., Brisseau N., Hermouet A., Jouglin M., Chauvin A. 2009. Experimental *in vitro* transmission of *Babesia* sp. (EU1) by *Ixodes ricinus*. Vet. Res. 40(3):21.

Burkot T.R., Happ C.M., Dolan M.C., Maupin G.O. 2001. Infection of *Ixodes scapularis* (Acari: Ixodidae) with *Borrelia burgdorferi* using a new artificial feeding technique. J. Med. Entomol. 38(2):167-71.

Cotte V., Bonnet S., Le Rhun D., Le Naour E., Chauvin A., Boulouis H.J., Lecuelle B., Lilin T., Vayssie Taussat M. 2008. Transmission of *Bartonella henselae* by *Ixodes ricinus*. Emerg. Infect. Dis. 14(7):1074-80.

Donnelly J., Peirce M.A. 1975. Experiments on the transmission of *Babesia divergens* to cattle by the tick *Ixodes ricinus*. Int. J. Parasitol. 5(3):363-7.

Gray J.S., Dautel H., Estrada-Pena A., Kahl O., Lindgren E. 2009. Effects of climate change on ticks and tick-borne diseases in Europe. Interdiscip. Perspect. Infect. Dis. 2009:593232.

Heyman P., Cochez C., Hofhuis A., van der Giessen J., Sprong H., Porter S.R., Losson B., Saegerman C., Donoso-Mantke O., Niedrig M., Papa A. 2010. A clear and present danger: tick-borne diseases in Europe. Expert Rev. Anti. Infect. Ther. 8(1):33-50.

Joyner L.P., Davies S.F., Kendall S.B. 1963. The experimental transmission of *Babesia divergens* by *Ixodes ricinus*. Exp. Parasitol. 14:367-73.

Kocan K.M., Wickwire K.B., Hair J.A., Ewing S.A., Barron S.J. 1986. Percutaneous infection of nymphal *Dermacentor andersoni* with *Anaplasma marginale*. Am. J. Vet. Res. 47(8):1662-4.

Lewis D., Young E.R. 1980. The transmission of a human strain of *Babesia divergens* by Ixodes ricinus ticks. J. Parasitol. 66(2):359-60.

Massung R.F., Priestley R.A., Levin M.L. 2004. Transmission route efficacy and kinetics of *Anaplasma phagocytophilum* infection in white-footed mouse, *Peromyscus leucopus*. Vector Borne Zoonotic Dis. 4(4):310-8.

Matjila T.P., Nijhof A.M., Taoufik A., Houwers D., Teske E., Penzhorn B.L., de Lange T., Jongejan F. 2005. Autochthonous canine babesiosis in The Netherlands. Vet. Parasitol. 15;131(1-2):23-9.

Musyoki J.M., Osir E.O., Kiara H.K., Kokwaro E.D. 2004. Comparative studies on the infectivity of *Theileria parva* in ticks fed *in vitro* and those fed on cattle. Exp. Appl. Acarol. 32(1-2):51-67.

Oines O., Storli K., Brun-Hansen H. 2010. First case of babesiosis caused by *Babesia canis* in a dog from Norway. Vet. Parasitol. 4;171(3-4):350-3.

Reis C., Cote M., Le Rhun D., Lecuelle B., Levin M.L., Vayssier-Taussat M., et al. Vector competence of the tick *Ixodes ricinus* for transmission of *Bartonella birtlesii*. PLoS Negl. Trop. Dis. 5(5):e1186.

Suss J. 2011. Tick-borne encephalitis 2010: epidemiology, risk areas, and virus strains in Europe and Asia-an overview. Ticks Tick Borne Dis. 2(1):2-15.

Young A.S., Waladde S.M., Morzaria S.P. 1996. Artificial feeding systems for ixodid ticks as a tool for study of pathogen transmission. Ann. NY Acad. Sci. 791:211-8.

COMMON TICK-BORNE RICKETTSIOSES FOR HUMANS IN EUROPE

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Summary
In European countries, ticks are considered as the main vector of human infectious diseases. Ticks transmit a number of different pathogens, including bacteria, viruses, and protozoa. Tick-borne rickettsioses are caused by obligate intracellular bacteria belonging to the spotted fever group (SFG) of the genus *Rickettsia* within the family Rickettsiaceae in the order *Rickettsiales*. The prevalence of rickettsial diseases has significantly increased in recent years through tick bites in European countries because of modification of human behavior (with outdoor activities and travels) and the changes in the environment, still in debate the hypothesis of highly aggressiveness of the tick due to global warming. Several rickettsioses are caused by bacteria initially isolated from ticks and subsequently considered pathogenic after description of first cases in human. It is necessary to consider the *Rickettsia* found in arthropods capable of biting humans as potential human pathogens. The rickettsial field underwent a beneficial progression because of technological advances in molecular methods particularly PCR, whole-genome sequencing, and improvement of culture conditions. Now in European countries, 10 pathogenic for human rickettsiae can be detected in ticks in nature, some rickettsiae are isolated from or detected only in ticks, and there are also several imported rickettsioses. Here, I present the most usually encounter tick-borne rickettsioses in Europe. I focus on the epidemiological and clinical aspects of as well as the emergence and reemergence of these diseases. Increasing public education about tick-borne diseases and avoidance of tick bites is a good prevention. European clinicians aware of the clinical signs of tick-transmitted diseases in patients with unexplained febrile illnesses, and a careful travel and tick exposure history. Empiric antimicrobial therapy (most often with doxycycline) is appropriate in most cases of significant clinical illness in which tick-borne infections are suspected by epidemiological and clinical manifestations, and may be lifesaving.

A NEW APPROACH IN TICK CONTROL IN DOGS

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For several years, dogs are being more exposed to ticks. The reasons are multifactorial but include the fact that 1 – biotopes are more favorable to the tick species infesting pets and humans and 2 – that social changes have increased the risk of contact with ticks in the wild. The increase of tick infestations is directly related to the increased number of tick borne diseases in dogs and in humans, therefore a better tick control is needed.
The discovery of a synergistic effect between two acaricide molecules: fipronil and amitraz was identified as a way to improve the tick control on dogs.
The first step in developing this product was to demonstrate in vitro the synergistic effect between both molecules against ticks. This has been done by in vitro mortality tests and in vitro motility tests.
The killing tests proved three mode of action of the combination.
In a second step, a spot on formulation for dogs was developed. Dose determinations studies were conducted to determine the amitraz concentration to be added to the known fipronil concentration. Then dose confirmations were conducted to study the detachment effect on existing tick infestation, the prevention of attachment on new infestations, the speed of kill on various tick species, the duration of efficacy, but also the efficacy on fleas.
Other properties like water and shampoo resistance were included.
The combination is the first one to kill ticks between 6 to 24 hours during more than a month, and to avoid tick attachment and blood meal. Therefore, specific experimental designs have also studied the prevention of disease transmission. The new combination is able to prevent the development of canine babesiosis, canine monocytic ehrlichiosis, granulocytic anaplasmosis and Lyme borreliosis for 4 weeks.

IMMUNOLOGICAL ASPECTS IN *TRYPANOSOMA CRUZI* INFECTION

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The intracellular protozoan parasite *Trypanosoma cruzi* causes one of the most important diseases worldwide, Chagas' disease, which is a health threat for an estimated 10 million people, living mostly in Latin America (Telleria and Tibayrnec, 2010). More than 25 million people are at risk of the disease. It is estimated that in 2008, Chagas disease killed more than 10000 people. Their importance derives not only from their mortality and morbidity but also from the lack of available therapies. Despite intensive research, the etiology of Chagas' disease, both in humans and in experimental animal models of the disease, is not clearly understood. Cardiopathy is the prevalent pathology associated to this infection. Although the acute and chronic phases of the disease share some similar pathological findings, it is still unclear whether similar pathogenic mechanisms operate. Moreover, it is plausible that the pathology of the acute phase may affect the final outcome of the chronic phase. However, the underlying cellular and molecular mechanisms either mediated by the parasite or by the immune response against it are poorly understood. To date, many pathogenic mechanisms have been described to explain how cardiac pathology develops. They can be mediated directly by the parasite or caused by an inflammatory/immune/autoimmune mechanism or a combination of these (Girones and Fresno, 2003; Girones et al., 2005; Marin-Neto et al., 2007).

We have analyzed the pathogenic mechanism underling *T. cruzi* infection, specially the contribution of autoimmunity and/or the direct infection by this parasite. During *T. cruzi* infection autoreactive T cells recognizing an autoantigen Cha and the SAPA parasite antigen by molecular mimicry are induced. The adoptive transfer of Cha-SAPA autoreactive T cell clones produces similar cardiac alterations than infection, suggesting that autoimmune mechanisms trigger by the parasite are involved in pathogenesis (Girones et al., 2001; Girones et al., 2007).

On the other hand, we have analyzed the immune systemic response as well as the local response in the heart of infected animals. We have documented cardiac infiltration by arginase I expressing immature myeloid cells, and various lymphocytes subsets, Th1, Th2, Th17 and Tregs. We reported the existence of a population of infiltrating myeloid derived suppressor cells (MDSC) expressing arginase I and iNOS in the hearts of mice during the acute phase of Chagas disease where they have the potential to suppress T lymphocytes present in the infiltrate (Cuervo et al., 2008; 2011). We found that the heart-infiltrating myeloid CD11b+ cells included granulocytic Ly6G+ and monocytic Ly6G- subpopulations, the monocytic myeloid cell subset, but not the granulocytic subset, expressed arginase I and iNOS activity and was able to inhibit T cell proliferation *in vitro* in an NO-dependent manner closely related to the so-called MDSCs. The presence of MDSCs correlates with depletion of L-arginine from plasma that can have a systemic suppressor effect on T cell function extending to other inflamed organs and tissues. MDSC iNOS activity may be required for efficient control of parasite load in the heart that leads to survival of infected mice, but iNOS in combination with arginase I also could be detrimental for the host when iNOS/arginase I expression is persistent through the acute phase of infection causing plasma L-arginine depletion. Our results indicate that this latter effect predominates since MDSC levels are related with more susceptible host phenotypes and notably, supplementation of infected mice with L-arginine diminished parasite load and increased survival. This may have consequences in chronic cardiac Chagas disease.

We also performed a comparative study of the CD4+ T cell subsets involved in the control of parasite burden after infection in the acute and chronic phases of infection, both in susceptible and non-susceptible mice. Notably, infection increased the number of natural Treg in the thymus of non-susceptible mice. In addition, heart CD4+ T cell infiltration and expression of Th1 cytokines was higher in infected non-susceptible mice and was associated with the presence of Treg cells. On the contrary, heart parasite burden was higher in susceptible mice that showed much lower heart CD4+ T cell infiltration characterized by the presence of Th17 cells. Finally, increase in IL-6 expression in heart of susceptible mice was associated with high mortality, and conduced to greater heart parasite persistence and inflammation in the chronic phase. Our results suggest that a combined Th1/Treg response is protective during the acute phase of infection, and consequently, mice develop an almost

asymptomatic chronic phase. Contrarily, a combined Th1/MDSCs/Th17 response results in high mortality, and a chronic phase characterized by high parasite burden and inflammation.
Different phylogenetic lineages have been described in *T. cruzi* (Zingales et al., 2009). We found that those lineages can be distinguished by the expression of glycoproteins and present different capacity to infect host cells that is associated to variable degrees of pathogenicity. We also analyzed the humoral and cellular responses and immunopathogenesis during *in vivo* infections with different strains, to address if there is any link between the genetic lineages of *T. cruzi* and the clinical outcome of Chagas' disease. Our results indicate great differences in tissue tropism, immune responses and pathogenesis among them.
In summary, the understanding of those immunopathogenetic mechanisms will contribute to the development of better therapies or vaccines to Chagas' disease.

References

Cuervo H., Guerrero N.A., Carbajosa S., Beschin A., De Baetselier P., Gironès N., Fresno M. 2011. Myeloid-derived supressor cells infiltrate the heart in acute *Trypanosoma cruzi* infection. J. Immunol. 187:2656-2665.

Cuervo H., Pineda M.A., Aoki M.P., Gea S., Fresno M., Girones N. 2008. Inducible nitric oxide synthase and arginase expression in heart tissue during acute *Trypanosoma cruzi* infection in mice: arginase I is expressed in infiltrating CD68+ macrophages. J. Infect. Dis. 197:1772-1782.

Girones N., Carrasco-Marin E., Cuervo H., Guerrero N.A., Sanoja C., John S., Flores R., Fernandez-Prieto L., Chico-Calero I., Salgado H., Carrion J., Fresno M. 2007. Role of *Trypanosoma cruzi* autoreactive T cells in the generation of cardiac pathology. Ann. NY Acad. Sci. 1107:434-444.

Girones N., Cuervo H., Fresno M. 2005. *Trypanosoma cruzi*-induced molecular mimicry and Chagas' disease. Curr. Top. Microbiol. Immunol. 296:89-123.

Girones N., Fresno M. 2003. Etiology of Chagas disease myocarditis: autoimmunity, parasite persistence, or both? Trends Parasitol. 19:19-22.

Girones N., Rodriguez C.I., Carrasco-Marin E., Hernaez R.F., de Rego J.L., Fresno M. 2001. Dominant T- and B-cell epitopes in an autoantigen linked to Chagas' disease. J. Clin. Invest. 107:985-993.

Marin-Neto J.A., Cunha-Neto E., Maciel B.C., Simoes M.V. 2007. Pathogenesis of chronic Chagas heart disease. Circulation 115:1109-1123.

Telleria J., Tibayrenc M. 2010. American Trypanosomiasis. Chagas Disease: One hundred years of research. Elsevier.

Zingales B., Andrade S.G., Briones M.R., Campbell D.A., Chiari E., Fernandes O., Guhl F., Lages-Silva E., Macedo A.M., Machado C.R., Miles M.A., Romanha A.J,. Sturm N.R., Tibayrenc M., Schijman A.G. 2009. A new consensus for *Trypanosoma cruzi* intraspecific nomenclature: second revision meeting recommends TcI to TcVI. Mem. Inst. Oswaldo Cruz 104:1051-1054.

HOST-PARASITE INTERACTION IN EXPERIMENTAL FASCIOLIASIS

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Fascioliasis is considered an important human disease caused by two liver fluke species: *Fasciola hepatica* and *Fasciola gigantica* (Fasciolidae). Several geographic areas have been described as endemic for the disease in humans, including hypoendemic, mesoendemic and hyperendemic areas, with prevalences and intensities ranging from low to very high (Mas-Coma et al., 2009). The last stage of the disease in humans encompasses an obstructive or chronic phase which may develop after months to years of infection, including bacterobilia, lithiasis and mild to moderate anaemia, especially in heavy infections. Therefore, the experimental fascioliasis/Wistar rat model is a useful tool when analyzing the host-parasite interaction. Thus, this model is used in pathological research in the advanced chronic period because the rat's resistance level, susceptibility and pathology mimic chronic disease in humans (Valero et al., 2003, 2006a; 2006b; Gironès et al., 2007). In this sense, a parasite isolate and lymnaeid snail vectors from a human fascioliasis hyperendemic area of *F. hepatica* were used for standard experimental procedures in the Wistar rat model. The immune response, bacterobilia and anemia were assessed employing this model.

The immune response in advanced chronic fascioliasis was analysed in the experimental rat model at 20 weeks post-infection (p.i.). Cytokine quantification in infected rat serum revealed basal levels. The predominant immunoglobulin (Ig) isotype was IgG1. Flow cytometry analysis of T cell (CD3+, CD4+, and CD8a+), B cell (CD45R+), and macrophage (CD11b+) populations in spleens showed no significant differences between infected and control rats. Mononuclear cell proliferation in the spleen in response to T and B mitogens was strongly inhibited in infected versus control rats. During early chronic infection, there is a predominance of a Th2 response, which decreases in advanced chronic infection characterized by a persistent immune suppression.

Bacterobilia in advanced chronic fascioliasis was analysed in the model at 200, 300 and 400 days p.i. The same procedure was applied in control rats. Liver tests were determined using stored serum samples. Bacteriological bile culture revealed viable bacteria (*Escherichia coli*, 45%, *Enterococcus faecalis*, 45% and *Klebsiella pneumoniae*, 10%). The presence of bacterobilia was associated with liver serum enzymes, including aspartate aminotransferase (AST or SGOT), alanine aminotransferase (ALT or SGPT), alkaline phosphatase (AP) and total bilirubin levels. Multivariate analysis suggested an association between bacterobilia and the following factors: duration of parasitic infection and intensity of parasitic infection. The results supported the impression that the obstruction caused by advanced chronic fascioliasis in the rat may be related to biliary sepsis.

Anaemia is a global health problem in low – and middle – income countries due to its negative effects on physical development, intellectual performance, work capacity and pregnancy (De Maeyer et al., 1989). Anaemia due to fascioliasis was traditionally thought to be related to a direct loss of whole blood caused either through ingestion of blood by the flukes or haemorrhage induce by them (Chen and Mott, 1990; Behm and Sangster, 1999). The relationship between liver fluke infection and anaemia in human fascioliasis endemic areas has not been demonstrated, given that numerous nutritional deficiencies and infectious diseases coexist in liver fluke infected subjects and that both are related to an increased risk of anaemia when present (Curtale et al., 2005; 2007).

Anaemia in advanced chronic fascioliasis was analysed in the model. Haematological parameters were analysed at 20 and 60 weeks p.i. Not only was the presence of blood in faeces, but also the cytokine and antibody response as well as the relative splenic weight assessed. Pigment stones and bile specimens were collected. Serum IgG1, IgG2a and IgE were determined in rat serum samples. Cytokine levels were correlated with haematological parameters. Screening for gastrointestinal bleeding was carried out. All microbiological analyses of the bile samples from the control rats were negative, but bacterobilia associated with liver-fluke infection was detected in 53.8% of rats with *F. hepatica* at 60 weeks p.i (*Escherichia coli* and *Enterococcus faecalis*). Brown pigment stones were simultaneously located with liver flukes in the common bile ducts in some infected rats. The weight of the non-infected controls was significantly higher when compared to the parasitized rats. The % of splenic weight was significantly higher in the parasitized rats when compared to the non-infected controls. Occult blood loss was not found in any control rat, while blood presence in faeces was

encountered in infected rats independent of the intensity of infection. In the 3 samples analysed from each rat, blood emission was detected in all samples in some rats, while it was intermittent in others. Anti-red blood cell (RBC) antibodies in rat serum samples were not detected in the experiment at 20 weeks p.i. (0.0%). In the experiment at 60 weeks p.i., anti-RBC antibodies were detected in serum in three rats (13.6%), and they also presented lower haemoglobine levels. The comparison of TNF-α, IL-10 e IL-4 levels in the specimens with anaemia versus the specimens without anaemia showed that TNF-α increased only in the two rats without anaemia, but IL-4, IL-10 and IL-1β levels increased in the rats with anaemia. Elevated TNF-α, IL-10, and IL-1β levels were detected in rats with serum iron deficiency. Specific IgG1, IgG2a and IgE responses were induced in infected rats with respect to non-infected control rats. A predominance of the IgG1 versus the IgG2a subclass was observed in serum samples at 20 and 60 weeks p.i. Significant negative correlations were obtained between eosinofilia and the amount of RBC. Significant negative correlations were obtained between eggs per gram of faeces, parasite body area or fluke burden versus haemoglobine.

There is disagreement on whether haematophagia occurs, and if it does, whether it is sufficient to produce the anaemic symptoms observed in the host with fascioliasis. It has been estimated that blood is lost at the rate of 0.2-0.5 ml per day per fluke. Despite the fact that protein components of plasma and blood cells are reabsorbed after digestion in the intestine, considerable amounts of iron are lost, which are not reabsorbed. Under these circumstances, the rate of erythropoiesis is increased but is limited in the later stages of the infection by the availability of dietary iron and protein, which depends on the quality of the diet and intensity of anorexia (Behm and Sangster, 1999). The results of our study on the presence of blood in faeces show that blood loss occurs at 20 and 60 weeks p.i. Although this phenomenon is not present in all anaemic-parasitized rats, it does not appear to be the only cause of liver-fluke related anaemia. The samples taken at 20 weeks p.i. show the presence of blood in faeces causing the loss of iron. This blood loss is chronic and remains until 60 weeks p.i., possibly resulting in iron deficiency anaemia. Erythrocyte production requires iron. Ferropenic erythropoiesis leads to the development of microcytosis and hypochromia, a consequence of the decrease of the synthesis of haemoglobin through the lack of iron.

The results show that the type of anaemia in fascioliasis may be considered a biomarker of the chronicity period of the disease, changing from normocytic to macrocytic in the early chronic period (20 weeks p.i.) and to microcytic in the advanced chronic period (60 weeks p.i.). Likewise, changing from normochromic in the early chronic period to hypochromic in the advanced chronic period. Human fascioliasis has been associated with normocytic hypochromic anaemia and to a lesser extent with macrocytic and microcytic hypochromic anaemia, with significant increases of reticulocyte counts (Valero et al., 2008). The extrapolation of our results to human fascioliasis could explain the different types of anaemia previously described.

The negative correlation between the haematological parameters versus fluke burden, eggs per gram of faeces and total parasite body size has been demonstrated. In human populations, haemoglobine levels appear related to epg levels of this parasite (Curtale et al., 2005). In hyperendemic zones, the majority of adult human subjects should be in the chronic phase and may frequently have heavy fluke burdens due to the high contamination risk (Mas-Coma et al., 1999). Consequently, a high risk of anaemia may be expected in human subjects inhabiting areas where fascioliasis is highly endemic and where high egg outputs detected in humans suggest that liver fluke burdens may also be very high. Multivariate analysis suggested an association between anaemia and the following factors: fluke burden, eggs per gram of faeces, body area of parasite, presence of blood in faeces, IgG1 and eosinophil levels, and % of splenic weight. Of all variables analysed, fluke burden is the one which presents the highest anaemia risk, even exceeding the variable presence of blood in faeces. The development of anaemia appears to be complex and may involve multiple mechanisms. However, to the mechanisms that until now explain fascioliasis-related anaemia (compensatory increase in erythrocyte production and a continuous drain on iron stores resulting from the parasites' blood-sucking activities) the following causes ought to be added: haemolysis of red blood cells, the general effects of inflammation on erythropoiesis, concomitant parasitic and bacterial infections and pre-morbid nutritional abnormalities. The results of the rodent model lead to the assumption that a high risk of anaemia in subjects with a heavy parasitic burden in human hyperendemic areas of fascioliasis is to be expected.

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References

Behm C.A., Sangster N.C. 1999. Pathology, pathophysiology and clinical aspects. In: Dalton, J.P. (Ed.), Fasciolosis. CAB International Publishing, Wallingford, Oxon, UK, pp. 185-224.

Chen M.G., Mott K.E., 1990. Progress in assessment of morbidity due to *Fasciola hepatica* infection: a review of recent literature. Trop. Dis. Bull. 87:1-38.

Curtale F., Hassanein Y.A., Barduagni P., Yousef M.M.,Wakeel A.E., Hallaj Z., Mas-Coma S. 2007. Human fascioliasis infection: gender differences within schoolage children from endemic areas of the Nile Delta, Egypt. Trans. R. Soc. Trop. Med. Hyg. 101:155-160.

Curtale F., Hassanein Y.A., Savioli L., 2005. Control of human fascioliasis by selective chemotherapy: design, cost and effect of the first public health, school-based intervention implemented in endemic areas of the Nile Delta, Egypt. Trans. R. Soc. Trop. Med. Hyg. 99: 599-609.

De Maeyer E.M., Dallman P., Gurney J.M., Mhallberg L., Sood S.K., Srikantia S.G., 1989. Preventing and Controlling Iron Deficiency Anemia Though Primary Health Care: A Guide for Health Administrators and Programme Managers. WHO, Geneva.

Gironès N., Valero M.A., García-Bodelón M.A., Chico-Calero I., Punzón C., Fresno M., Mas-Coma S. 2007. Immune suppression in advanced chronic fascioliasis: an experimental study in a rat model. J. Infect. Dis. 195:1504-1512.

Mas-Coma S., Bargues M.D., Esteban J.G., 1999. Human fasciolosis. In: Dalton, J.P. (Ed.), Fasciolosis. CAB International Publishing, Wallingford, Oxon, UK, pp. 411-434.

Mas-Coma S., Valero M.A., Bargues M.D. 2009. *Fasciola*, lymnaeids and human fascioliasis, with a global overview on disease transmission, epidemiology, evolutionary genetics, molecular epidemiology and control. Adv. Parasitol. 69:41-146.

Valero M.A., Santana M., Morales M., Hernandez J.L., Mas-Coma S. 2003. Risk of gallstone disease in advanced chronic phase of fascioliasis: an experimental study in a rat model. J. Infect. Dis.188:787-793.

Valero M.A., Navarro M., Garcia-Bodelon M.A., Marcilla A., Morales M., Hernandez J.L., Mengual P., Mas-Coma S. 2006a. High risk of bacterobilia in advanced experimental chronic fasciolosis. Acta Trop.100:17-23.

Valero M.A., De Renzi M., Panova M., García-Bodelón M.A., Periago M.V., Ordoñez D., Mas-Coma S. 2006b. Crowding effect on adult growth, pre-patent period and egg shedding of *Fasciola hepatica*. Parasitology 33:453-463.

Valero M.A., Gironès N., García-Bodelón M.A., Periago M.V., Chico-Calero I., Khoubbane M., Fresno M., Mas-Coma S. 2008. Anaemia in advanced chronic fasciolosis. Acta Trop. 108:35-43.

ANTHELMINTIC RESISTANCE IN NEMATODES INFECTING LIVESTOCK: HOW TO WIN THE BATTLES AND PROLONG THE WAR

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The development/selection of anthelmintic-resistant populations of gastrointestinal nematode parasites infecting livestock has been a consequence of sustained, mass treatment strategies which confer a selective advantage on individuals able to survive drug exposure (Sutherland and Scott, 2009).

Historically, resistance has been detected in the field only a few years after the commercial release of each class of anthelmintic. Resistance to the benzimidazoles (BZ), imithidiazole derivatives (I/D) and the macrocyclic lactones (ML) is now commonplace in many regions (Kaplan, 2004). Also, while the issue was initially much more prevalent in small ruminants, a significant increase in reports of resistance in nematodes infecting cattle suggests it is also becoming a more significant problem in this livestock species (Sutherland and Leathwick, 2011).

Time will tell whether the more recent entries to the sheep anthelmintic market suffer a similar fate. Time will also tell whether the research community and other interested parties have learned from past lessons, and have introduced practices which delay the development of detectable resistance while achieving acceptable levels of animal productivity.

In order to develop and test management strategies which delay the development of resistance, it is desirable to identify the underlying drivers which increase the risk of selecting for resistant populations. Some of these discussed below.

Risk factors

Frequency of treatment/persistence of exposure

There is a belief held by some that more frequent use of drenches will result in increased anthelmintic resistance. However, while it is obvious that a complete absence of drenching will not select for resistance (assuming that 'resistance' genes are not already present in a population and do not confer other selective advantages), there is no direct correlation between the number of drenches administered and the levels of resistance (Leathwick et al., 1995). The explanation of this is largely due to management and epidemiological factors. For example, a single drench may confer significant selection pressure in a system in which there is little or no pasture contamination with susceptible genotypes (Besier et al., 2001) (the concept of refugia is discussed in greater detail below).

Considering the effect of drenching frequency as a factor in selecting for anthelmintic resistance becomes more complicated as many of the treatments on the market have varying periods of persistent activity; this is particularly true in cattle, where the market is dominated by pour-on and injectable products, normally containing a single active ML, which have longer periods of activity than e.g., transient oral products such as ivermectin (Williams et al., 1999; Vercruysse et al., 2000). With persistent compounds, there is an opportunity for an extended period in which susceptible genotypes are screened out, under conditions in which resistant genotypes can establish and produce progeny (Sutherland et al., 1999; 2000).

Persistent compounds, at least in New Zealand, have been considered to be an effective way of controlling parasite establishment for extended periods of time. As such, they have been used to prevent pasture larval contamination – otherwise known as the provision of "safe pasture" (Familton et al., 1995).

Drenching for "safe pasture"

The idea of "safe pasture" i.e. contaminated by few or no parasite larvae is an attractive proposition for livestock producers due to the obvious benefits for productivity (Familton et al., 1995). Managing grazing and pastures to provide this is possible, but normally has inherent costs in areas such as increased management input and reduced stocking density. An alternative approach is to use anthelmintics (which is not without cost in terms of labour and product).

Unfortunately, attempts to remove all parasites will almost certainly result in the selection of resistant populations. This is because suppressive chemical treatment has the potential to allow resistant worms to survive and produce progeny while removing the contribution of susceptible worms to future generations.

The contribution of the ewe to pasture contamination during the post-parturient relaxation of immunity (Boag and Thomas, 1971), in which parasite egg production increases and may result in larval availability to lambs in the spring, has also led to the promotion of drench use (often persistent drenches) in lactating ewes to 'break the cycle' (Familton et al., 1995). However, if ewes are considered as a source of pasture contamination, then surely exposing these worms will provide a strong selective advantage for resistant parasites. This was investigated in a large-scale field trial (Leathwick et al., 2006) which established in a long-term empirical field experiment that administering drench capsules to lactating ewes significantly increased the level of anthelmintic resistance.

Use of 'ineffective' drenches

It is obvious that worms will be killed by a drench if they are susceptible to treatment – assuming they have come into contact with sufficient of the product; it can also be assumed that, *in vivo*, worms which are resistant to a given drench are able to survive.

However, this is dependant to an extent on factors such as the genetics of resistance to, and the pharmacokinetic profiles of, specific products. For example, persistent drenches with declining plasma profiles, such as moxidectin, may allow the establishment of worms which are heterozygous for resistance for a period before those which are fully susceptible (this assumes homozygous-resistant worms were already able to establish) (Sutherland et al., 2003). Given that a proportion of the progeny of the resulting population of homo- and heterozygous-resistant worms will be homozygous, such a product will, in theory, provide a selective advantage for the resistant allele.

It is reasonable to assume, therefore, that drenches with the greatest efficacy against 'resistant' worms – whether homozygous or heterozygous, will delay the development of resistance (Leathwick, 2012).

Management strategies which work

Do not drench for safe pasture – utilise refugia

Maintaining a refuge of susceptible worms on pasture is relatively simple in theory but can be difficult to put into practice.

Livestock farmers, given the choice, would presumably minimise or remove pasture larval contamination. However, there is empirical evidence that ensuring an adequate *refugia* of susceptible genotypes not only delays the development of resistance (Van Wyk, 2001), but does not significantly impact on animal productivity (Leathwick et al., 2008).

While the concept of *refugia* has widespread support, the practicalities are more complex, and a number of studies have examined how to leave some animals sundrenched (Greer et al., 2009; Kenyon et al., 2009) or use older animals as a source of unselected parasites (Waghorn et al., 2008).

When considering the concept of utilising *refugia*, it should also be noted that individual worm species have unique epidemiological characteristics, such as seasonal variability in relative abundance *(*Vlassoff et al*.,* 2001*).* Such factors may have a significant effect on the impact of *refugia*. As an example, resistant *Haemonchus contortus* genes will not be diluted or replaced susceptible genes from *Trichostronglyus colubriformis*.

Know your resistance status and use a drench which works

Given that killing resistant parasites is an effective method of delaying the development of resistance, and that drenches are administered to either remove worms or prevent their establishment, it goes without saying producers should ensure they are using products which do this effectively. However, at least in New Zealand, the majority of farmers do not carry out regular checks of drench efficacy, if at all.

There is compelling evidence, both from modelling and empirical studies that the best way of increasing efficacy against resistant parasites (as well as preventing the development of resistance) is to use drenches containing at least two active families (Leathwick, 2012). Combination chemotherapy is widely used in Australasia, which has been partly in response to increasing levels of resistance i.e. to increase the chances of at least one of the actives being effective. However, it is now generally accepted that using combinations before significant resistance has arisen to one or more of the constituent actives can delay or prevent resistance developing (Leathwick et al., 2012).

In situations in which significant levels of resistance already exist to one of the two actives in a double-combination, then obviously the advantages of using the combination will be reduced (Leathwick et al., 2012). However, there may still be additive effects on activity which support the use of the combination. It is also unlikely that all parasite species present are resistant to the same active – as such, the combination will continue to delay or prevent the development of resistance in these species (Bartram et al., 2012).

Combine refugia and combination drenches

While combination drenches are a valuable tool in delaying resistance, the advantage they provide is reduced if there is not an adequate refuge of susceptible genotypes on a property (Leathwick et al., 2012). In this situation, most or all of the worms are exposed to treatment and are thus 'available for selection'. An example of this is a finishing farm, where young stock are purchased from elsewhere and drenched as they are moved on to the property.

Thus, in order for combination therapy to be most effective, farmers must develop a strategy to manage their worm populations in *refugia*.

Managing resistance in large ruminants

While there are broad similarities in the risk factors and management strategies between small (sheep and goats) and large ruminants (cattle and deer), there are also some obvious differences (Sutherland and Leathwick, 2011). The most striking is the range of drench products available and how they are applied.

In small ruminants, at least in some countries, there are now two new actives commercially available for use in sheep. Monepantel (Zolvix, Novartis Animal Health) (Kaminsky et al., 2008) was released in New Zealand in 2009 and derquantel (in combination with abamectin) (Startect, Pfizer Animal Health) (Little et al., 2010) in the same country in 2010. Sheep farmers thus have two products available to which there is presumably no resistance, and have the opportunity to use these strategically to not only minimise the selection for resistance, but also as a tactic to extend the effective lives of the previously available products (Leathwick and Hosking, 2009).

The other obvious difference is the routes of administration of drenches to large ruminants. Primarily for ease of application and safety, most drenches are given either as injections or topically (pour-ons).

Issues with variability in delivery of product with pour-ons have been known for some time, and a number of studies have demonstrated that animals lick the product, either from their own backs or from their neighbours (Laffont et al., 2001). This can result in some animals receiving either very high or very low doses of drug. Following on from the variability in drug delivery is variability in efficacy, something which has been observed on a number of occasions (Bisset et al., 1990; Vermunt et al., 1996).

Recent work in New Zealand (Leathwick, personal communication) has compared the efficacy of the same active (moxidectin) against naturally-infected cattle when given either as an oral, pour-on or injectable. As expected, efficacy was significantly higher with the oral than with the pour-on. Unexpectedly, however, the efficacy of the injectable product was no better than the pour-on, despite the fact that these products are known to attain much higher levels in serum than pour-ons (Hennessy and Alvineria, 2002). In each case, this reduced efficacy (which in the absence of the oral drench would have been considered as a diagnosis of resistance) was against the small intestinal parasite *Cooperia oncophora.* This last paragraph indicates that a) while significant advances have been made in resistance management, that there is still much work to be done and b) that worm control and resistance is more complex than it seems, and involves various active families, routes of administration and target species which reside in different target organs.

References

Bartram D.J., Leathwick D.M., Taylor M.A., Geurden T., Maeder S.J. 2012. The role of combination anthelmintic formulations in the sustainable control of sheep nematodes. Vet. Parasitol. 186:151-158.

Besier R.B., Palmer D.G., Woodgate R.G. 2001. New recommendations for sustainable strategic drenching. Proc. Aust. Sheep. Vet. Soc. 32-36.

Bisset S.A., Brunsdon R.V., Forbes S. 1990. Efficacy of a topical formulation of ivermectin against naturally acquired gastro-intestinal nematodes in weaner cattle. NZ. Vet. J. 38:4-6.

Boag B., Thomas R.J. 1971. Epidemiological studies on gastro-intestinal nematode parasites of sheep. Infection patterns on clean and autumn-contaminated pasture. Res. Vet. Sci. 12:132-139.

Familton A.S., McAnulty R.W., Thompson K.F., Sedcole J.R. 1995. The effect of anthelmintic treatment of ewes during pregnancy. Proc. NZ Soc. Anim. Prod. 55:211-213.

Greer A.W., Kenyon F., Bartley D.J., Jackson E.B., Gordon Y., Donnan A.A., McBean D.W., Jackson F. 2009. Development and field evaluation of a decision support model for anthelmintic treatments as part of a targeted selective treatment (TST) regime in lambs. Vet. Parasitol. 164:12-20.

Henessy D.R., Alvinerie M.R. 2002. Pharmacokinetics of the macrocyclic lactones: Conventional wisdom and new paradigms. *In:* Macrocyclic lactones in antiparasitic therapy (Vercruysse, J., Rew, R.S., eds) CABI Publishing.

Kaminsky R., Ducray P., Jung M., Clover R., Rufener L., Bouvier J., Weber S.S., Wenger A., Wieland-Berghausen S., Goebel T., Gauvry N., Pautrat F., Skripsky T., Froelich O., Komoin-Oka C., Westlund B., Sluder A., Maser P. 2008. A new class of anthelmintics effective against drug-resistant nematodes. Nature 452:176-180.

Kaplan R.M. 2004. Drug resistance in nematodes of veterinary importance: A status report. Trends in Parasitol. 20:477-481.

Kenyon F., Greer A.W., Coles G.C., Cringoli G., Papadopolous E., Cabaret J., Berrag B., Varady M., Van Wyk J.A., Thomas E., Vercruysse J., Jackson F. 2009. The role of targeted selective treatments in the development of refugia-based approaches to the control of gastrointestinal nematodes of small ruminants. Vet. Parasitol. 164:3-11.

Lassont C.M., Alvinerie M., Bousquet-Meilou A., Toutain P.L. 2001. Licking behaviour and environmental contamination arising from pour-on ivermectin for cattle. Int. J. Parasitol. 31:1687-1692.

Leathwick D.M. 2012. Modelling the benefits of a new class of anthelmintic in combination. Vet. Parasitol. 186: 93-100.

Leathwick D.M., Hosking B.C. 2009. Managing anthelmintic resistance: Modelling strategic use of a new anthelmintic class to slow the development of resistance to existing classes. NZ. Vet. J. 57:203-207.

Leathwick D.M., Miller C.M., Atkinson D.S., Haack N.A., Alexander R.A., Oliver A.M., Waghorn T S., Potter J.F., Sutherland I.A. 2006. Drenching adult ewes: Implications of anthelmintic treatments pre- and post-lambing on the development of anthelmintic resistance. NZ Vet. J. 54:297-304.

Leathwick D.M., Miller C.M., Atkinson D.S., Haack N.A., Waghorn T.S., Oliver A.M. 2008. Managing anthelmintic resistance: Untreated adult ewes as a source of unselected parasites, and their role in reducing parasite populations. NZ. Vet. J. 56:184-195.

Leathwick, D. M., Vlassoff, A. and Barlow, N. D. 1995. A model for nematodiasis in New Zealand lambs: The effect of drenching regime and grazing management on the development of anthelmintic resistance. Int. J. Parasitol. 25:1479-1490.

Leathwick D.M., Waghorn T.S., Miller C.M., Candy P.M, Oliver A.M.B. 2012. Managing anthelmintic resistance - Use of a combination anthelmintic and leaving some lambs untreated to slow the development of resistance to ivermectin. Vet. Parasitol. 187:285-294.

Little P.R., Hodge A., Watson T.G., Seed J.A., Maeder S.J. 2010. Field efficacy and safety of an oral formulation of the novel combination anthelmintic, derquantel-abamectin, in sheep in New Zealand. NZ. Vet. J. 58:121-129.

Sutherland I.A., Brown A.E., Leathwick D.M. 2000. Selection for drug-resistant nematodes during and following extended exposure to anthelmintic. Parasitology 121:217-226.

Sutherland I.A., Brown A.E., Leathwick D.M., Bisset S.A. 2003. Resistance to prophylactic treatment with macrocyclic lactone anthelmintics in *Teladorsagia circumcincta*. Vet. Parasitol. 115:301-309.

Sutherland I.A., Leathwick D.M. 2011. Anthelmintic resistance in nematode parasites of cattle: A global issue? Trends in Parasitol. 27:176-181.

Sutherland I.A., Leathwick D.M., Brown A.E. 1999. Moxidectin: persistence and efficacy against drug-resistant *Ostertagia circumcincta*. J. Vet. Pharmacol. Therap. 22:2-5.

Sutherland I.A., Scott I. 2009. Chapter 6: Anthelmintic Resistance. *In:* Gastrointestinal nematodes in sheep and cattle, pp 117-131, Wiley-Blackwell, London.

Van Wyk J.A. 2001. Refugia – Overlooked as perhaps the most potent factor concerning the development of anthelmintic resistance. Onderst. J. Vet. Res. 68:55-67.

Vercruysse J., Dorny P., Claerebout E., Demeulenaere D., Smets K., Agneesens J. 2000. Evaluation of the persistent efficacy of doramectin and ivermectin injectable against *Ostertagia ostertagi* and *Cooperia oncophora* in cattle. Vet. Parasitol. 89:63-69.

Vermunt J.J., West D.M., Pomroy W. E. 1996. Inefficacy of moxidectin and doramectin against ivermectin-resistant *Cooperia* spp. of cattle in New Zealand. NZ. Vet. J. 44:188-193.

Vlassoff A., Leathwick D.M., Heath A.C.G. 2001. The epidemiology of nematode infections of sheep. NZ. Vet. J. 49:213-221.

Williams J.C., Loyacano A.F., Derosa A., Gurie J., Clymer B C., Guerino F. 1999. A comparison of persistent anthelmintic efficacy of topical formulations of doramectin, ivermectin, eprinomectin and moxidectin against naturally acquired nematode infections of beef calves. Vet. Parasitol. 85:277-288.

ACTUAL ASPECTS IN THE EVALUATION OF ETIOLOGICAL THERAPIES

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In the clinical practice, improving the quality and safety of medical interventions are continuous preoccupations. The decision for the most adequate treatment is an important part of actions for realisation of those goals. On the odder side, the resources for healthcare are limited and the clinical decisions, must be carefully analysed and re-exanimate to choose the most effective alternative for patient. Many possibilities to solution these problems are offered by the results of treatments assessment studies and the use of the clinical decision analysis modelling.
Among the experimental studies, for treatments evaluation, the randomised controlled clinical trial is the most appropriate, mainly because of the quality to avoid errors. The intention-to-treat analysis, in a randomised trial, is a test for the clinical decision, in which many patients may not remain with the originally assigned treatment. Clinical decision analysis uses the probabilities of different medical events to guide the complex clinical decisions, in which there are considered simultaneously, multiple variables. This analytical tool, can select from different diagnostic and therapeutic options, that which is the most appropriate in a given clinical situation. In therapies, for having more closely information to individual patient, in the clinical trials, subgroups analyses are used.
When a randomized trial can't be realized, the evaluation or comparison between treatments will be made with observational studies. In those comparisons, the internal validity will be affected. Ensuring the validity of the therapeutic trials is an important condition in the intention to generalize the results.
Therapies evaluation and decision analysis are basic tools in medical practice for an efficient management of patient, treatment, clinical decisions and costs.

CERCARIAL DERMATITIS: CRUCIAL MOMENTS IN TRANSMISSION OF BIRD SCHISTOSOMES

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Cercarial dermatitis is a hypersensitive skin reaction (disease) caused by schistosome larvae - cercariae. Besides mammalian and human schistosomes (*Schistosoma* spp. and other genera), avian schistosomes are frequently reported as the causative agent. As cercariae develop in water snails, the disease is restricted to the aquatic environment.
Clinical manifestation of cercarial dermatitis depends on the host immunological status (Horák and Kolářová, 2005). Usually, dermatitis develops in sensitized people who already had a contact with the parasite. It is therefore generally accepted that skin reactions in the form of cercarial dermatitis may represent a protective tool to effectively kill the parasite in the skin. Under laboratory conditions, experimental animals repeatedly exposed to schistosome cercariae exhibit Th2 polarization of the immune response (Kouřilová et al., 2004). However, if exposed for the first time to the agent, they do not always mount an effective immune reaction, and schistosome larvae may escape and migrate to different host organs (Horák and Kolářová, 2001). This can be a risky event: schistosomula of visceral species may occur in the lungs, whereas those of nasal species are attracted by the nervous tissue (Horák et al., 2008). In the latter case, schistosomula of *Trichobilharzia regenti* migrate to the spinal cord and brain of experimental birds and mice, and cause damage to the tissue, followed in some cases by neuromotor disorders. Only in birds, the migrating worms reach nasal mucosa where they mature, mate and lay eggs.
In some areas of the world cercarial dermatitis is regarded as an (re)emerging disease, because it appeared newly (no reports in the past) or the number of human cases (water reservoirs with schistosomes) increased; this applies to e.g., Chile, the UK, the United States, Norway and Denmark (Horák and Kolářová, 2011). Global climate changes and eutrophication may contribute to dissemination of schistosomes by birds and snails, growth of snail populations, accelerated development of parasites, increased contact of people with the causative agent, etc.
As already mentioned, bird schistosomes require water snails for their larval development. The prevalence of infections in snails is usually low (below 1%), but in some locations/under certain circumstances it may reach up to 50%. These differences are, at least partly, influenced by the local spectrum of snail species and their susceptibility to infection. Snail-schistosome interactions leading to the development of schistosome cercariae represent a complicated matter: the parasite must avoid snail immune response, the snail host must provide a proper physiological/nutritional environment for schistosome asexual multiplication and development, the infection by schistosomes must be dominant over other trematode species that have attacked the same snail host, etc. (Horák and Kolářová, 2005; 2011). As only a few snail species are studied as laboratory models, our knowledge of factors playing a role in species-specific snail susceptibility/resistance may represent limits of understanding the diversity of schistosome transmission modes.
Although not always apparent from a routine helminthological examination of birds (many papers published on helminthofauna of birds refer to intestinal helminths only), these definitive hosts are frequently parasitized by schistosomes (Kolářová et al., 2010), and anseriform birds seem to play a dominant role. In addition, there are reports on schistosomes in passeriform birds. In some localities, the prevalence of schistosomiasis in birds may reach up to 90-100% (e.g., New Zealand, the United States) (Horák and Kolářová, 2011). The intensity of transmission to birds is poorly studied, but we know from experimental infections that at least two ways of infection exist: percutaneous (penetration of the bird skin) and peroral (penetration of the bird mucosa); of course that the percutaneous way represents the dominant mode of infection. Although there is limited data about natural infections, bird schistosomes seem to be pathogenic in some cases, and their development under natural conditions may differ from that in experimental birds (Skírnisson et al., 2012); this phenomenon might reflect host physiological suitability.

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References

Horák P., Kolářová L. 2001. Bird schistosomes - do they die in mammalian skin? Trends Parasitol. 17:66-69.

Horák P., Kolářová L. 2005. Molluscan and vertebrate immune responses to bird schistosomes. Parasite Immunol. 27:247-255.

Horák P., Kolářová L. 2011. Snails, waterfowl and cercarial dermatitis. Freshwater Biol. 56:779-790.

Horák P., Mikeš L., Rudolfová J., Kolářová L. 2008. Penetration of *Trichobilharzia* cercariae into mammals: dangerous or negligible event? Parasite 15:299-303.

Kolářová L., Horák P., Skírnisson K. 2010. Methodical approaches in the identification of areas with a potential risk of infection by bird schistosomes causing cercarial dermatitis. J. Helminthol. 84:327-335.

Kouřilová P., Hogg K.G., Kolářová L., Mountford A.P. 2004. Cercarial dermatitis caused by bird schistosomes comprises both immediate and late phase cutaneous hypersensitivity reactions. J. Immunol. 172:3766-3774.

Skírnisson K., Kolářová L., Horák P., Ferté H., Jouet D. 2012. Morphological features of the nasal blood fluke *Trichobilharzia regenti* (Schistosomatidae, Digenea) from naturally infected hosts. Parasitol. Res. 110:1881-1892.

DIAGNOSIS *OF ACANTHAMOEBA* KERATITIS

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Early and sensitive diagnosis and on time treatment may prevent the poor outcome of *Acanthamoeba* keratitis. The infection can be misdiagnosed particularly at the onset of the disease due to a lack of specific clinical signs. *In vivo* confocal microscopy, when performed by an experienced operator, could be helpful to suggest the diagnosis of *Acanthamoeba* keratitis. But, a biological diagnosis, performed on corneal samples, is until required to confirm the infection. Direct examination and culture presented low sensitivity. Culture lacks sensitivity, in particular if the patients have been treated by antibiotics with potential amoebicidal activity. PCR has shown usefulness for the confirmation of AK allowing faster analysis and better sensitivity. Between the PCR assays described, the JDP primers are genus-specific and allow sequencing of the amplimers for further identification of most *Acanthamoeba* genotypes. Real-time TaqMan® PCR assays have recently been described for the detection and quantification of *Acanthamoeba*. According to the assays, the spectrum of *Acanthamoeba* genotypes is different. Because the genome of *Acanthamoeba* is highly variable between the isolates, the combination of primer sets is recommended to increase the detection rates of PCR. Nevertheless, false-negative PCR results are possible. In fact, the quality of corneal scrapings may lead to reduce number of *Acanthamoeba* in the samples and PCR inhibitors, including topical solutions, could be present. The use of commercial kits for the DNA extraction improves partially the sensitivity of the PCR assay by eliminating the inhibitors. Controls should be added in assays performed on clinical samples to verify their quality and the absence of inhibitors.

THE USE OF MODERN GENETICAL TOOLS FOR BREEDING PARASITE RESISTANT HONEYBEES

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Classical breeding has produced several honeybee populations that are resistant to selected diseases in the past decades. The availability of the complete honeybee genome has opened a new era in honeybee genetics. DNA tools allow for the identification of genes that are important for colony health. The function of immune genes in the honeybee has been unraveled for several infections. The use of haploid drones in genetic studies further facilitates progress in the identification of genes that are relevant to colony health. Marker assisted selection can greatly accelerate the propagation of desirable stock. The wealth of genetic tools available, establish the honeybee as a model system for genetic research. Nevertheless, in spite of all these novel tools and innovative approaches, honeybee breeding will remain to be a difficult, labor intensive and time consuming task.

POLLINATOR DECLINE – POSSIBLE CAUSES AND INTERACTIONS IN BUMBLEBEES

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Recent studies on naturally occurring and agriculturally used pollinating insects (e.g., honey bees and bumblebees) revealed strong decline in abundance and diversity possibly due to parasite infections. Several groups of parasites (e.g., nematodes, protozoa, microsporidia, bacteria and viruses) might trigger the observed pollinator colony decline all over the world.
Host-parasite interactions are characterised by intensive arms race on both sites. Parasites might increase their virulence in order to exploit hosts efficiently which might be counteracted by activation of the hosts immune system. Insect hosts attack parasites by means of their innate immune system. Parasite related factors (e.g, carbohydrates, lipids or peptides) switch on the innate immune system by up- or down-regulation of immune related pathways (Toll, Imd, JNK and JAK/STAT). These result in an anti-parasite response via melanization, proteasome-dependent degradation, apoptosis and expression of antimicrobial peptides (AMPs).
Here, I want to summarize diversity of bumblebee parasites, their potential role in pollinator decline and host-parasite interaction on the molecular level using the buff-tailed bumblebee *Bombus terrestris*.

ALLERGY AND ZOONOSES

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The parasitic zoonoses

Zoonoses in general, but particularly parasitic ones, have a big impact on human health and changing patterns of disease burdens occur with disease emergence. The increase in human population along with socioeconomic changes lead to migrating populations which move to new geographical regions with a different ecology and to the modification of animal husbandry practices; these changes are responsible for the disease emergence and the increase of disease burden. More than a half of emerging infectious diseases are zoonoses (Torgenson and McPherson, 2011).

Relation between allergy and zoonoses

According to the hygiene hypothesis (Strachan, 1989) a higher exposition to a rural environment as well as to helminth burden, as it occurs in low income countries, would prevent the development of allergic diseases which conversely have dramatically increased in industrialised Countries where such exposition has almost disappeared.

Allergy and Protozoa infections

Toxoplasmosis

This disease is estimated for a burden of 2-8 x 10^6 DALYs (disability adjusted life years) at global level (Torgenson and McPherson, 2011), however, infection especially in industrialised countries seems to underlie a progressive decline, according to seroprevalence data (Hofhuis et al., 2010; Pinto et al., 2011).

Animal stable contact during early life and raw milk consumption predicted *T. gondii* seropositivity in young adulthood (Radon et al., 2004). The lower concentration of proteolytic enzymes in the digestive tract of infants is responsible for a higher risk of transmission of *T. gondii* infection via raw farm milk (Tenter et al., 2000).

The association between *T. gondii* seropositivity and atopy in a study carried out in was not strong, however, the highest risk factor for developing atopy in rural subjects was represented by regular contact with farm animals. It has to clarify, possibly in studies involving a large number of individuals, whether *T. gondii* infection represent an intermediate factor in the association between farm contact and atopy (Radon et al., 2004).

In a study performed in the Baltic region no difference was observed in seroprevalence for toxoplasmosis between atopic and non atopic individuals (Janson et al., 2007).

In a study carried out in Brazil, a negative association between atopy and toxoplasmosis was demonstrated for the first time, focusing on the antibody and cytokine responses suggesting that the immunomodulation induced by the parasite may play a protective role in the development of allergic diseases (Fernandes et al., 2010).

Allergy and helminth infections

Helminths, particularly those localized at intestinal level, represent a major public health problem at global level, with an estimation of over 2 billions of infected individuals (Supali et al., 2010).

Helminth infections are characterised usually by a persistent Th2 polarization, with eosinophilia and increased total IgE levels, and for this reason they represent ideal models to study allergy and other Th2-mediated pathological conditions (Bruschi, 2012).

It is now well established that chronic helminth infections protect against allergic diseases through active regulatory processes (Smits et al., 2010).

Echinococcosis

This disease is estimated for a burden of 2-5 x 107 DALYs (disability adjusted life years) at global level (Torgenson and McPherson, 2011).

It is a neglected infectious disease caused by the larva stage of *Echinococcus granulosus* or *E. multilocularis*, in most of cases. It still constitutes a major public health problem not only in developing countries. The infection is characterised by a fine interaction between host response which is polarised in a Th2 direction and immune response evasion strategy of the parasite (Siracusano et al., 2012). One of the most important complications of the disease is represented by the rupture of tissue cysts with the following occurrence of type I hypersensitivity reactions such as urticaria, asthma up to

anaphylaxis, especially in hydatidosis patients (Vuitton, 2004). Fatal reactions represent a negligible risk of all kinds of percutaneous treatment (Neumayr et al., 2012). To our knowledge, no study was carried out aiming to relate atopy to this disease, from an epidemiological point of view.

Taeniasis/cysticercosis

This disease is estimated for a burden of 2-5 x 10^6 DALYs at global level (Torgenson and McPherson, 2011). What already stated for echinococcosis is also valid for this zoonosis.

Trichinellosis

It is a widely spread zoonotic infection caused by the parasitic nematode *Trichinella* spp. in mammals (included man), birds as well as in reptiles. Even in this infection the immune response is skewed to a Th2 type (Bruschi and Chiumiento, 2012).

Data are accumulating which show that infection with this helminth may affect the evolution of experimental models of human diseases such as diabetes, multiple sclerosis but also respiratory allergy or inflammation. These latter processes were ameliorated by infection, in fact the levels of macrophages and eosinophils in the bronchial alveolar lavage fluid in infected animals exposed to allergen were reduced compared to uninfected animal group and airway hyper-responsiveness completely disappeared. Furthermore, infection reduced the IL-5 levels, while stimulating those of IL-10 and TGFβ suggesting the activation of T regulatory cells which in fact resulted increased in lung draining lymph nodes (Park et al., 2011).

Experimental trichinellosis was used also to study the immune mechanisms of anaphylaxis in response to parasitic antigens. In infected IgE deficient mice intravenous injection of *Trichinella* antigen caused a fatal anaphylactic shock in 100% of animals suggesting that this reaction can be also mediated by other isotypes such as, for example IgG1. Mice deficient for this isotype underwent fatal response, as well (Bruschi et al., 1999).

Studies in experimental trichinellosis, used as a model of Th2 polarization, allowed to show *in vivo* the downregulating effect of the *Helicobacter pylori* neutrophil activating protein on the Th2 responses (Del Prete et al., 2008; Chiumiento et al., 2011).

Toxocarosis

This is a worldwide diffused zoonosis caused by *Toxocara canis* or *Toxocara cati*, i.e. two roundworms of dogs and cats, respectively which can also infect humans, occasionally. The migration of larvae through the lungs may result in respiratory distress such as wheezing, coughs, mucous production and hyper-reactivity of the airways. Epidemiological and experimental studies suggest that infection with this helminth exacerbates allergic manifestations, including asthma. In particular, mechanisms involved in such process are increased Th2 cytokine production (IL-5 and IL-4/IL-13) responsible for eosinophilia and increased total IgE levels, respectively, nevertheless the role of T regulatory cells remains to be elucidated (Pinelli and Aranzamendi, 2012).

In a study carried out in Italy, on more than 700 patients who resulted negative for allergy tests but suffering of chronic recurrent respiratory, eye, skin or gastrointestinal symptoms, about 31% resulted positive for *T. canis* IgG antibodies. Among them, about 25% suffered of asthma. Two thirds of patients underwent subsequent antihelminthic therapy with a complete remission of symptoms (Qualizza et al., 2011).

Anisakidosis

Anisakis simplex allergy was described for the first time in Japan about twenty years ago. After that, a lot of papers have been published on this issue. Patients with acute allergic symptoms after consumption of infected fish resulted positive for serum specific IgE, opening the way to consider the nematode as a potential food allergen.

During last recent years, a discussion among researchers has originated on the real possibility to underlie allergic symptomatology, after ingestion of dead larvae, i.e. whether or not infection with the parasite is essential for such manifestation (Dashner et al., 2012).

Concluding remarks

A better understanding of the fine mechanisms exploited by helminth parasites to modulate host immune response might be useful in the future to identify molecules capable to quench the allergic responses and hopefully to be tested as pharmaceutical drugs.

References

Bruschi F. 2012. Editorial. Endocr. Metab. Immune Disord. Drug Targets 12:2-3.

Bruschi F., Chiumiento L. 2011. Immunomodulation in trichinellosis: does *Trichinella* really escape the host immune system? Endocr. Metab. Immune Disord. Drug Targets 12:4-15.

Bruschi F., Pozio E., Watanabe N., Gomez-Morales M.A., Ito M., Huang Y., Binaghi R. 1999. Anaphylactic response to parasite antigens: IgE and IgG1 independently induce death in *Trichinella*-infected mice. Int. Arch. Allergy Appl. Immunol. 119:291-296.

Chiumiento L., Del Prete G., Codolo G., De Bernard M., Amedei A., Della Bella C., Piazza M., D'Elios S., Caponi L., D'Elios M.M., Bruschi F. 2011. Stimulation of TH1 response by *Helicobacter pylori* neutrophil activating protein decreases the protective role of IgE and eosinophils in experimental trichinellosis. Int. J. Immunopathol. Pharmacol. 24:895-903.

Daschner A., Cuéllar C. Rodero M. 2012. The *Anisakis* allergy debate: does an evolutionary approach help? Trends in Parasitol. 28:9-15.

Del Prete G., Chiumiento L., Amedei A., Piazza M., D'Elios M.M., Codolo G., de Bernard M., Masetti M., Bruschi F. 2008. Immunosuppression of TH2 responses in *Trichinella spiralis* infection by *Helicobacter pylori* neutrophil-activating protein. J.Allergy Clin. Immunol. 122: 908-913.

Fernandes J.F.C., Taketomi E.A., Mineo J.R., Miranda D.O., Alves R., Resende R.O., Ynoue L.H., Sung S.S.J., Silva D.A.O. 2010. Antibody and cytokine responses to house dust mite allergens and *Toxoplasma gondii* antigens in atopic and non-atopic Brazilian subjects. Clin. Immunol. 136:148-156.

Hofhuis A., van Pelt W., van Duynhoven Y.T., Nijhuis C.D., Mollema L., van der Klis F.R., Havelaar A.H., Kortbeek LM. 2010. Decreased prevalence and age-specific risk factors for *Toxoplasma gondii* IgG antibodies in The Netherlands between 1995/1996 and 2006/2007. Epidemiol. Infect. 24:1-9.

Janson C., Asbjornsdottir H., Birgisdottir A., Sigurjonsdottir R.B., Gunnbjörnsdottir M., Gislason D., Olafsson I., Cook E., Jögi R., Gislason T., Thjodleifsson B. 2007. The effect of infectious burden on the prevalence of atopy and respiratory allergies in Iceland, Estonia, and Sweden. J. Allergy Clin. Immunol. 120:673-679.

Neumayr A., Troia G., de Bernardis C., Tamarozzi F., Goblirsch S., Piccoli L., Hatz1 C., Filice C., Brunetti E. 2011. Justified Concern or Exaggerated Fear: The Risk of Anaphylaxis in Percutaneous Treatment of Cystic Echinococcosis. A Systematic Literature Review. PloS Negl. Trop. Dis. 5:e1-7.

Park H.K., Cho M.K., Choi S.H., Kim Y.S., Yu H.S. 2011. *Trichinella spiralis*: infection reduces airway allergic inflammation in mice. Exp. Parasitol. 127:539-544.

Pinelli E., Aranzamendi C. 2012. *Toxocara* infection and its association with allergic manifestations. Endocr. Metab. Immune Disord. Drug Targets 12:33-44.

Pinto B., Castagna B., Mattei R., Bruzzi R., Chiumiento L., Cristofani R., Buffolano W., Bruschi F. 2012. Seroprevalence for toxoplasmosis in individuals living in North West Tuscany: access to Toxo-test in central Italy. Eur. J. Clin. Microbiol. Infect. Dis. 31:1151-1156.

Qualizza R., Incorvaia C., Grande R., Makri E., Allegra L., 2011. Seroprevalence of IgG anti-*Toxocara* species antibodies in a population of patients with suspected allergy. Int. J. Gen. Med. 4:783-787.

Siracusano A., Delunardo F., Teggi A., Ortona E. 2012. Cystic Echinococcosis: Aspects of Immune Response, Immunopathogenesis and Immune Evasion from the Human Host. Endocr. Metab. Immune Disord. Drug Targets 12:16-23.

Smits H.H., Everts B., Hartgers F.C., Yazdanbakhsh M. 2010. Chronic Helminth Infections Protect Against Allergic Diseases by Active Regulatory Processes. Curr. Allergy Asthma Rep. 10:3-12.

Supali, T.; Verweij, J.J., Wiria, A.E.; Djuardi, Y.; Hamid, F.; Kaisar, M.M.M.; Wammes, L.J.; van Lieshout, L.; Luty, A.J.F.; Sartono, E. and Yazdanbakhsh, M. 2010. Polyparasitism and its impact on the immune system. Int. J. Parasitol. 40:1171-1176.

Tenter A.M., Heckeroth A.R., Weiss L.M. 2000. *Toxoplasma gondii*: from animals to humans. Int. J. Parasitol. 30:1217-1258.

Torgerson P.R., Macpherson C.N.L. 2011. The socioeconomic burden of parasitic zoonoses: global trends. Vet. Parasitol. 182:79-95.

Vuitton D.A. 2004. Echinococcosis and allergy. Clin. Rev. Allergy Immunol. 26:93-104.

ASCARIDS OF HUMAN IMPORTANCE: WHERE ARE WE NOW?

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In their guide to human helminths, Coombs and Crompton (1991) list ten species of ascarid that are known to infect humans. These range from the numerous *Ascaris lumbricoides* via the emerging and pathogenic *Baylisascaris procyonis* to the relatively obscure *Toxocara pteropodis*. The focus of my talk will be upon *Ascaris lumbricoides* and *Toxocara canis*. Both helminths are highly prevalent but remain neglected; in fact *A. lumbricoides* is now listed as one of 16 neglected tropical diseases that are described as poverty-promoting conditions (Hotez et al., 2006). Both *Ascaris* and *Toxocara* are receiving increasing attention, for a variety of reasons, one of which relates to the focus of this session.

A. lumbricoides is a remarkably infectious and persistent parasite (Crompton, 2001). An estimated 1472 million people (a quarter of the world's population) are infected and the level of morbidity, assessed as disability adjusted life years, is about 10.5 million (Holland, 2005). Chronic ascariasis is known to contribute to insidious morbidity, including growth retardation and effects on cognitive development, particularly in growing children (Hall et al., 2008). Additionally both *A. lumbricoides* and *Ascaris suum*, its counterpart in pigs, are known to cause liver and lung pathology as a consequence of their migration through these organs (Stephenson, 1987). Pulmonary symptoms can be pronounced and are described as Loeffler's syndrome (Loeffler, 1956). Acute ascariasis, associated with heavy worm burdens, can result in intestinal obstruction, other serious complications and in extreme cases, death (O'Lorcain and Holland, 2000). Large-scale deworming programmes are being rolled out worldwide and there is an urgent need to understand how such control may alter pattern and process in human geohelminth infection (Basanez et al., 2012).

Furthermore, the growing perception that infection with *Ascaris* and other geohelminths have an effect on the host immune response, with consequences for concurrent important infectious diseases, such as malaria (Kirwan et al., 2010), renewed interest in the consequences of early infection with worms in the context of the hygiene hypothesis (Yazdanbakhsh et al., 2002) and the modulatory consequences for development of allergies (Cooper et al., 2009), all greatly enhance the public heath significance of such parasites. Recently the draft genome of *A. suum* has been published and this effort will significantly enhance of our understanding of the molecular biology and immunobiology of ascarids and herald new approaches to their treatment and control (Jex et al., 2011).

Toxocara canis is a highly prevalent gastrointestinal nematode infection of dogs and other canids (Holland and Smith, 2006). Widespread environmental contamination of the environment, with eggs shed in host faeces, facilitates infection of so-called abnormal or paratenic hosts including mice and humans (Holland and Smith, 2006). In such hosts, larvae undergo a somatic migration through the tissues and organs of the body but fail to develop to maturity as adult worms in the intestine. The presence of migrating larvae in the tissues contributes to pathology that is dependent upon the intensity of infection and the location of the larvae (Smith, 1991). The implications of this paratenesis have far-reaching consequences for the biology of the parasite and extend from the behaviour of wild mice to the cerebral consequences in infected humans (Holland and Hamilton, 2006).

Although our understanding of the pathology in humans is incomplete, seroprevalence studies provide support for high levels of exposure in the human population (Smith and Noordin, 2006). Toxocariasis is now considered to be the most common human parasitic infection in the United States particularly among the impoverished. The infection is also highly prevalent in many developing countries and its global importance is likely to be significantly underestimated (Hotez and Wilkins, 2009). The main route of transmission for humans is still considered to be via the ingestion of embryonated eggs from soil or soil-contaminated hands or food. However, recent data has suggested a role for direct contact with dogs, carrying potentially infective eggs on their hair (Roddie et al., 2008).

Humans exhibit a number of well-recognised clinical entities including visceral larva migrans (VLM), ocular toxocariasis (OT) and covert toxocariasis (CT) (Smith et al., 2009). Larval involvement in the eye with consequent visual impairment remains the most devastating sequela of human infection (Good et al., 2004). However, cerebral or neurological toxocariasis is a much less-well understood phenomenon. Humans are known to carry *Toxocara* larvae in their brains (Hill et al., 1985) and

despite the small number of cases of cerebral toxocariasis being described historically; these are now on the increase due to enhanced awareness and improved diagnosis (see for example, Salvador et al., 2010). Epidemiological evidence of the impact of toxocariasis on cognitive deficits in humans is sorely lacking. Appropriate animal model systems can provide insights into the significance of cerebral toxocariasis (Hamilton et al., 2008; Liao et al., 2008).

To conclude, *Ascaris* and *Toxocara* as case studies of ascarids of human importance can highlight a range of fascinating and enigmatic aspects of the host-parasite relationship. It is hoped that such insights will encourage further investigation and greater investment in what undoubtedly remain classic, neglected diseases.

References

Coombs I., Crompton D.W.T. 1991. A guide to human helminths. Taylor and Francis, London, New York and Philadelphia, pp. 196.

Cooper P.J. 2009. Interactions between helminth parasites and allergy. Curr. Opin. Allergy Clin. Immunol. 9(1):29-37.

Crompton D.W.T. 2000. *Ascaris* and ascariasis. Adv. Parasitol. 48:285-375.

Good B., Holland C.V., Taylor M.R., Larragy J., Moriarty P., O'Regan, M. 2004. Ocular toxocariasis in schoolchildren. CID 39(2):173-8.

Hall A., Hewitt G., Tuffrey V., de Silva, N. 2008. A review and meta-analysis of the impact of intestinal worms on child growth and nutrition. Mat. Child Nut. 5:18-236.

Hamilton C.M., Brandes S., Holland C.V., Pinelli E. 2008. Cytokine expression in the brains of *Toxocara-canis* infected mice. Parasite Immunol. 30:181-185.

Hill I.R., Denham D.A., Scholtz, C.L. 1985. *Toxocara canis* larvae in the brain of a British child. Trans. Royal. Soc. Trop. Med. Hyg. 79(3):351-354.

Holland C.V. 2005) *Ascaris*, *Trichuris*, hookworm and *Enterobius*. In: Topley and Wilson's Parasitology, 10th edition. Hodder Arnold, UK. pp. 712-736.

Holland C.V., Hamilton C.M. 2006. The significance of cerebral toxocariasis. *In:* Holland, C.V., Smith, H.V. (Eds). *Toxocara the Enigmatic Parasite*. CABI Publishing Oxfordshire, UK, pp. 58-73.

Holland C.V., Smith H.V. (Eds). 2006. *Toxocara* the Enigmatic Parasite. CABI Publishing Oxfordshire, UK, pp. 301.

Hotez P., Molyneux D.H., Fenwick A., Ottesen E., Sachs S.E., Sachs J.D. 2006. Incorporating a rapid-impact package for neglected tropical diseases with programs for HIV/AIDS, Tuberculosis, and Malaria. PloS Med. 3:e102.

Hotez P., Wilkins P.P. 2009. Toxocariasis: America's most common neglected infection of poverty and a helminthiasis of global importance? PLoS Neg. Trop. Dis. 3(3):e400.

Jex A.R., Liu S., Li B., Young N.D., Hall R.S., Li Y., Yang L., Zeng N., Xu X., Xiong Z., Chen F., Wu X., Zhang G., Fang X., Kang Y., Anderson G.A., Harris T.W., Campbell B.E., Vlaminck J., Wang T., Cantacessi C., Schwartz E.M., Ranganathan S., Geldhof P., Nejsum P., Sternberg P.W., Wang J., Wang J., Gasser R.B. 2011. *Ascaris suum* draft genome. Nature 479:529–533.

Kirwan P., Jackson A.L., Asaolu S.O., Abiona T.C., Bruce M.C., Ranford-Cartwright L., O'Neill S.M., Holland C.V. 2010. Impact of repeated four-monthly anthelmintic treatment on *Plasmodium* infection in preschool children: a double-blind placebo-controlled randomized trial. BMC Infect. Dis. 10:277.

Liao C.-W., Fan C.-K., Kao T.-C., Ji D.-D., Su K.-E., Lin Y.-H., Cho W.-L. 2008. Brain injury-associated biomarkers of TGF-beta I, SI00B, GFAP, NF-L, tTG, AbetaPP, and tau were concomitantly enhanced and the UPS was impaired during acute brain injury caused by *Toxocara canis* in mice. BMC Infect. Dis. 8:84.

Loeffler W. 1932. Zur Differentialdiagnose der Lungen-infiltrierungen. II Ueber fluchtige Succedanininfiltrate (mit Eosinophilie). Beitrage zur Klinick der Tuberkulose 79:368-382.

O'Lorcain P., Holland C.V. 2000. The public health significance of *Ascaris lumbricoides*. Parasitol. 121:51-71.

Roddie G., Stafford P., Holland C.V., Wolfe A. 2008. Contamination of dog hair with the eggs of *Toxocara canis*. Vet. Parasitol. 152:85-93.

Salvador S., Ribeiro R., Winckler M.I., Ohlweiler L., Riesgo R. 2010. Pediatric neurotoxocariasis with concomitant cerebral, cerebellar, and peripheral nervous system involvement: case report and review of the literature. J. Pediat. 86(6):531-534.

Smith H.V. 1991. Immune evasion and immunopathology in *Toxocara canis* infection. In: Kennedy, M.W. (Ed). Parasitic nematodes – antigens, membranes and genes. Taylor and Francis London, UK, pp. 117-139.

Smith H.V., Holland C. V., Taylor M., Magnaval J-F., Schantz P., Maizels R. 2009. How common is human toxocariasis? Towards standardizing our knowledge. Trends Parasitol. 25(4):182-188.

Smith H.V., Noordin R. 2006. Diagnostic limitations and future trends in the serodiagnosis of toxocariasis. *In:* Holland C.V., Smith H.V. (Ed.). *Toxocara* the Enigmatic Parasite. CABI Publishing Oxfordshire, UK, pp. 89-112.

Stephenson L.S. 1987. Ascariasis. *In:* Stephenson, L.S. (Ed). The Impact of Helminth Infections on Human Nutrition. Taylor and Francis, London and New York, pp. 89-127.

Yazdanbakhsh M., Kremser P.G., van Ree R. 2002. Allergy, parasites and the hygiene hypothesis. Science 296:490-494.

IMMUNOREGULATION BY ZOONOTIC HELMINTHS AND ITS EFFECT ON ALLERGIC DISEASES

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Infections with helminth are characterized by a dominant T helper 2 (Th2) type of immune response. Th2 cells produce cytokines such as interleukin (IL)-4, IL-5 and IL-13 which mediate increased levels of circulating IgE and eosinophilia. Interestingly, a strong Th-2 type of immune response is also characteristic of allergic diseases. Nevertheless, infections with certain helminths have been found to be protective against allergies (Yazdanbakhsh et al., 2002). It has become clear in recent years that helminths induce in addition a regulatory network that dampens the protective Th2 responses immune response against helminths. This induced immunosuppresive network that include regulatory-T (Treg), alternatively activated macrophages and regulatory B cells, appears to be essential for parasite survival and its effect can be extended to other inflammatory diseases such as allergies (Maizels and Yazdanbakhsh, 2008; Taylor et al., 2012).
The association between helminth infections and allergy is not always the same. While certain helminth infections have been reported to protect against allergic diseases, other helminths can exacerbate this immunopathology (Pinelli and Aranzamendi, 2012). Here, the contrasting effects on allergic manifestations by two zoonotic helminths namely, *Toxocara canis* and *Trichinella spiralis* and the possible factors involved will be discussed.
In addition to a dominant type of Th2 immune response toxocariasis and allergic asthma share also clinical features such as wheezing, coughs, mucus hyper-secretion and bronchial hyper-reactivity. In order to study the effect of *Toxocara* infection on allergic manifestations we combined two murine models namely, the murine model for toxocariasis previously described (Pinelli et al., 2005; 2007) and a murine model for allergic airway inflammation. For this study we infected BALB/c mice with 500 embryonated *T. canis* eggs and exposed them to ovalbumin (OVA) sensitization followed by OVA-challenge. Results indicate that infection with *T. canis* in combination with OVA treatment led to exacerbation of pulmonary inflammation, eosinophilia, airway hyper-responsiveness, OVA specific and total IgE. In conclusion, a previous infection with *T. canis* leads to exacerbation of experimental allergic airway inflammation (Pinelli et al., 2008).
Also evidence from epidemiological studies suggests a positive association between *Toxocara* infections and allergic manifestations. However, findings on a lack of association between this parasitic infection and allergies have also been reported (reviewed in Aranzamendi et al., 2012).
Interestingly no study so far has reported in an inverse association between *Toxocara* infection and allergies. The hygiene hypothesis proposes that early childhood exposure to infectious agents such as virus, bacteria and parasites protect against developing allergic diseases. The effect of helminth infection on allergic diseases may vary depending on the helminth species. Studies with different helminths suggest that depending on the parasite species, infection can either protect or exacerbate allergies. Other factor that may influence the association between helminth infection and allergies is whether the host is a definitive or accidental host. In an accidental host the parasite does not usually develop to the adult stage and in case of tissue dwelling helminths the continuous migration of the larvae through different organs including the lungs, can cause more damage compare to a definitive host where migration is transitory. It is likely that there are differences between parasites of humans that have evolved with their host and have developed strategies to survive without causing much damage compared to parasites such as *Toxocara* spp. in the accidental host. The host genetics may also play an important role, such as gene polymorphisms which has been found to be associated with susceptibility to different helminth infections. Another factor may be sporadic versus chronic infections. In chronic infections helminths appear to drive an immunosuppressive response, whereas sporadic or transient infections may enhance allergic manifestations. Finally, the intensity of infection may also influence the association between helminth infections and allergic manifestations by which high parasite burden may induce a suppressive type of immune response compared to light infections (Cooper, 2009; Pinelli and Aranzamendi, 2012).
Humans are accidental hosts for *T. canis* and as described above infection with this helminth results in exacerbation of allergic asthma. To investigate the effect of infection of an accidental versus a

definitive host on allergic asthma, we carried out studies using *Trichinella spiralis*. This nematode can infect many different mammals including mice and humans in which the parasite completes its life cycle. The infected mammals are therefore definitive hosts for *T. spiralis*.
After ingestion of *T. spiralis* infected meat, the larvae are released in the stomach of the infected host which thereafter migrates to the small intestine where they mature into adult worms. Female worms release within one week after infection newborn larvae that rapidly disseminate and eventually enter skeletal muscle to remain for many years. We have carried out studies using the *T. spiralis* excretory/secretory products (TspES) and investigated its effect on the functionality of dendritic cells (DC) and T cell activation. We found that TspES suppress *in vitro* DC maturation induced by lipopolysaccharide (LPS) derived from different bacteria. Using different TLR agonists, we show that the suppressive effect of TspES on DC maturation is restricted to TLR4. These helminth products were also shown to interfere with the expression of several genes related to the TLR-mediated signal transduction pathways. Interestingly, we found that TspES suppress *in vitro* DC maturation induced by LPS from enterobacteria but not by *Neisseria meningitidis*. During migration from intestine to muscle, *T. spiralis* larvae may drag enterobacteria which could lead to sepsis. However, the number of trichinellosis patients with sepsis is scarce. The immunosuppresive effect against enterobacteria LPS by the secreted products from this helminth could be a strategy to prevent the induction of sepsis and ensuring their host as well as their own survival (Aranzamendi et al., 2012)
In order to investigate the effect of TspES on T cell activation we used splenocytes derived from OVA-TCR transgenic D011.10 that were incubated with TspES-pulsed DC+OVA. Results indicate that the presence of TspES resulted in expansion of CD4+CD25+ T cells that express high levels of FOXP3+. These Treg cells were shown to have suppressive activity and to produce TGF-β. Together these results indicate that *T. spiralis* secretion products can suppress DC maturation and induces expansion of functional Treg cells *in vitro*.
The immunosupressive effect of *T. spiralis in vitro* has been also observed *in vivo*. Several studies using experimental models have shown that *T. spiralis* can suppress different immunopathologies. Boles et al. (2000) have shown that *Trichinella* infection results in suppression of multiple sclerosis (MS) in the rat. These authors used this model to compare the anti-inflammatory effects of the intestinal and late migratory phases of *Trichinella* infection on development of myelin basic protein (MBP)-induced MS-like debilitation. Findings from this study indicate that the late migratory phase of infection which occurred during the peak of MBP-induced debilitation, significantly improved performance scores in mobility, coordination and strength. Gruden-Movsesijan et al. (2008) studied the dose-dependent effect of *Trichinella* infection on experimental autoimmune encephalomyelitis (EAE) in rats (Gruden-Movsesijan et al., 2008). Findings from this study indicate that established *Trichinella* infection resulted in amelioration of the clinical course of induced EAE in a dose-dependent manner. At present, we are investigating the effect of *T. spiralis* infection on experimental allergic asthma and the role of Tregs in this process.
The potential immunomodulatory effect of helminths has been exploited and used in clinical studies which have shown that administration of eggs from the pig nematode *Trichuris suis* reduces disease severity in patients with inflammatory bowel disease (IBD) (Summers et al., 2005). However, also treatment of mice with *Trichinella* antigen has been shown to significantly reduce severity experimental colitis (Motomura et al., 2009). These authors propose helminth antigen-based therapy for inflammatory bowel diseases (IBD) instead of infection with live parasites. Recently, we have shown that *Trichinella spiralis* soluble antigens induce significant suppression of symptoms in murine experimental autoimmune encephalomyelitis, which is a validated animal model for multiple sclerosis. These are relevant findings since it also show that infection with helminth parasites is not a prerequisite for suppression of inflammatory diseases (Kujik et al., 2012).
Identification and characterization of the molecules and the mechanisms involve in immunoregulation is essential in order to develop new tools for prevention and/or treatment of inflammatory disease.

References

Aranzamendi C., Fransen F., Langelaar M., Franssen F., van der Ley P., van Putten J.P., Rutten V., Pinelli E. 2012. *Trichinella spiralis*-secreted products modulate DC functionality and expand regulatory T cells *in vitro*. Parasite Immunol. 34:210-23.

Boles L.H., Montgomery J.M., Morris J., Mann M.A., Stewart G.L. 2000. Suppression of multiple sclerosis in the rat during infection with *Trichinella pseudospiralis*. J. Parasitol. 86:841-4.

Cooper P.J. 2009. Interactions between helminth parasites and allergy. Curr. Opin. Allergy Clin. Immunol. 9:29-37.

Gruden-Movsesijan A., Ilic G.L., Mostarica-Stojkovic M., Stosic-Grujicic S., Milic M., Sofronic-Milosavljevic L. 2008. *Trichinella spiralis*: modulation of experimental autoimmune encephalomyelitis in DA rats. Exp. Parasitol. 118:641-7.

Kuijk L.M., Klaver E.J., Kooij G., van der Pol S.M., Heijnen P., Bruijns S.C., Kringel H., Pinelli E., Kraal G., de Vries H.E., Dijkstra C.D., Bouma G., van Die I. 2012. Soluble helminth products suppress clinical signs in murine experimental autoimmune encephalomyelitis and differentially modulate human dendritic cell activation. Mol. Immunol. 51:210-8.

Maizels R.M., Yazdanbakhsh M. 2008. T-cell regulation in helminth parasite infections: implications for inflammatory diseases. Chem. Immunol. Allergy 94:112-123.

Motomura Y., Wang H., Deng Y., El-Sharkawy R.T., Verdu E.F., Khan W.I. 2009. Helminth antigen-based strategy to ameliorate inflammation in an experimental model of colitis. Clin. Exp. Immunol. 155:88-95.

Pinelli E., Aranzamendi C. 2012. *Toxocara* infection and its association with allergic manifestations. Endocr. Metab. Immune Disord. Drug Targets 12:33-44.

Pinelli E., Brandes S., Dormans J., Fonville M., Hamilton C.M., der Giessen J. 2007. *Toxocara canis*: effect of inoculum size on pulmonary pathology and cytokine expression in BALB/c mice. Exp. Parasitol. 115:76-82.

Pinelli E., Brandes S., Dormans J., Gremmer E., Van Loveren H. 2008. Infection with the roundworm *Toxocara canis* leads to exacerbation of experimental allergic airway inflammation. Clin. Exp. Allergy 38:649-658.

Pinelli E., Withagen C., Fonville M., Verlaan A., Dormans J., Van Loveren H., Nicoll G., Maizels R.M., van der Giessen J. 2005. Persistent airway hyper-responsiveness and inflammation in *Toxocara canis*-infected BALB/c mice. Clin. Exp. Allergy 35:826-832.

Summers R.W., Elliott D.E., Urban J.F., Thompson R.A. Jr., Weinstock J.V. 2005. *Trichuris suis* therapy for active ulcerative colitis: a randomized controlled trial. Gastroenterology 128:825-832.

Taylor M D., van der Werf N., Maizels R.M. 2012. T cells in helminth infection: the regulators and the regulated. Trends. Immunol. 33:181-9.

Yazdanbakhsh M., Kremsner P.G., van Ree R. 2002. Allergy, parasites, and the hygiene hypothesis. Science 296:490-494.

RICKETTSIA CONORII: AN OLD BUT NEWLY RECOGNIZED PATHOGEN IN DOGS

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Rickettsia conorii (*Rc*) is a spotted fever group *Rickettsia* that causes Mediterranean spotted fever (MSF) in people in Mediterranean countries and Sub-Saharan Africa. The onset of MSF is abrupt and typical human cases present with high fever, flu-like symptoms, a black eschar at the tick bite site and a maculo-papular rash. Severe forms of the disease may present with major neurological manifestations and multi-organ involvement may occur. The mortality rate is estimated at around 2.5% and classical risk factors for severe forms include elderly age, cirrhosis, chronic alcoholism and glucose-6-phosphate dehydrogenase deficiency (Parola et al., 2005; Brouqui et al., 2007). As an example, more than 1000 human cases of Mediterranean Spotted Fever are reported annually in Italy mainly in Sicily, Sardinia, Lazio and Liguria (Parola et al., 2005; Cascio and Iaria, 2006). Moreover, a more pathogenic subspecies of *Rc*, *R. conorii israelensis*, has been described in human cases in Israel, Sicily (Giammanco et al., 2005) and Portugal.
The organism is transmitted in the Mediterranean basin by the brown dog tick *Rhipicephalus sanguineus*. MSF is a human seasonal disease that is frequently diagnosed between April and October with a maximum peak in June-August (Parola et al., 2005) and it is well correlated with the presence of *R. sanguineus*. Ticks are possibly the main reservoir of *Rc* infection due to the existence of both transstadial and transovarial transmission in the tick (Parola et al., 2005; Brouqui et al., 2007). The role of dogs in maintaining zoonotic foci has been unclear for many years (Brouqui et al., 2007). Although dogs were the normal host of the vector, there was limited information on their susceptibility to Rickettsiae infection and on the development of long duration rickettsiemia able to allow transmission to a feeding vector tick. Recently, an experimental infection in dogs has demonstrated that dogs are capable of acquiring *R. conorii* from infected *Rh. sanguineus* ticks and transmitting infection to cohorts of uninfected ticks, thus confirming for the first time that dogs are indeed competent reservoirs for *R. conorii* (Levin et al., 2012).
Due to high levels of exposure to *R. sanguineus*, dogs have been used in epidemiological studies as sentinels for human MSF (Delgado and Carmenes, 1995; Harrus et al., 2007) and proximity to seroreactive dogs has been found as a risk factor for MSF in humans (Mannelli et al., 2003). Several studies have reported anti-*Rc* antibodies by the indirect fluorescent antibody test (IFA) in dogs in *Rc* endemic countries such as Italy with seroprevalences rates ranging from 15.5% to 74% (Torina and Caracappa, 2006). Studies reported the detection of *Rickettsia* DNA in the blood of Spanish and Italian dogs (Estrada-Pena and Venzal Bianchi, 2006; Solano-Gallego et al., 2006a; Torina et al., 2007; Solano-Gallego et al., 2008). However, evidence that *Rc* infection causes illness in dogs was lacking for many years.
Illness has been associated with *Rc* natural infection in a few dogs since human MSF was described in 1932 (Kelly et al., 1992). The possibility that *Rc* may cause a clinical disease in dogs was supported by the evidence of seroconversion in three sick dogs from Israel (Baneth et al., 1998) and the association between anemia and seroreactivity to *Rc* antigens (Solano-Gallego et al., 2006b). In addition, a study reported the association between male dogs and seroreactivity to *Rc* antigens as found in humans where males have higher rates of infection (Parola et al., 2005; Solano-Gallego et al., 2006b). Moreover, febrile illness was associated with *Rc* infection in three dogs from Sicily by means of seroconversion and PCR (Solano-Gallego et al., 2006a). Recently, febrile illness has been associated with detection of *R. conorii* DNA in seven dogs in Portugal (Alexandre et al., 2011).
A prospective matched case-control study was performed (Solano-Gallego et al., unpublished results): 1) to investigate the presence of *Rickettsia* spp. infection in febrile dogs from Sicily by means of serological and molecular tests when compared with matched case control dogs with normal body temperatures; 2) to evaluate whether *Rickettsia* spp. infection causes clinical disease and clinicopathological abnormalities by clinical history, physical examination and baseline laboratory tests and 3) to evaluate coinfections with other agents ofcanine vector borne diseases (CVBD) such as *Leishmania infantum*, *Ehrlichia* spp, and *Anaplasma* species. Febrile illness was statistically associated with acute and convalescent positive *R. conorii* antibody responses and seroconversion to *R. conorii*

antigens. The most common clinical signs and laboratory abnormalities of febrile dogs diagnosed with *Rickettsia* infection based on seroconversion and/or PCR were fever, lethargy, myalgia, lameness, thrombocytopenia, hypoalbuminemia, hyperproteinemia, hyperglobulinemia, decreased total iron serum levels, elevation of C-reactive protein and liver enzymes.
In conclusion, *R.conorii* is an old but newly recognized canine pathogen. *Rickettsia conorii* needs to be included in the differential diagnoses list of acute febrile illness in dogs. The most common clinical signs and clinicopathological abnormalities are similar to those described in human and canine spotted fever rickettsiosis.

References

Alexandre N., Santos A.S., Bacellar F., Boinas F.J., Nuncio M.S., De Sousa R. 2011. Detection of *Rickettsia conorii* strains in Portuguese dogs (*Canis familiaris*). Ticks Tick Borne Dis. 2(22):119-122.

Baneth G., Breitschwerdt E.B., Hegarty B.C., Pappalardo B., Ryan J. 1998. A survey of tick-borne bacteria and protozoa in naturally exposed dogs from Israel. Vet. Parasitol. 74:133-142.

Brouqui P., Parola P., Fournier P.E., Raoult D. 2007. Spotted fever rickettsioses in Southern and Eastern Europe. FEMS Immunol. Med. Microbiol. 49:2-12.

Cascio A., Iaria C. 2006. Epidemiology and clinical features of Mediterranean spotted fever in Italy. Parassitologia 48:131-133.

Delgado S., Carmenes P., 1995. Canine seroprevalence of *Rickettsia conorii* infection (Mediterranean spotted fever) in Castilla y Leon (northwest Spain). Eur. J. Epidemiol. 11:597-600.

Estrada-Pena A., Venzal Bianchi J.M. 2006. Efficacy of several anti-tick treatments to prevent the transmission of *Rickettsia conorii* under natural conditions. Ann. NY Acad. Sci. 1078:506-508.

Giammanco G.M., Vitale G., Mansueto S., Capra G., Caleca M.P., Ammatuna P. 2005. Presence of *Rickettsia conorii* subsp. *israelensis*, the causative agent of Israeli spotted fever, in Sicily, Italy, ascertained in a retrospective study. J. Clin. Microbiol. 43:6027-6031.

Harrus S., Lior Y., Ephros M., Grisaru-Soen G., Keysary A., Strenger C., Jongejan F., Waner T., Baneth G. 2007. *Rickettsia conorii* in humans and dogs: a seroepidemiologic survey of two rural villages in Israel. Am. J. Trop. Med. Hyg. 77:133-135.

Kelly P.J., Matthewman L.A., Mason P.R., Courtney S., Katsande C., Rukwava J. 1992. Experimental infection of dogs with a Zimbabwean strain of *Rickettsia conorii*. J. Trop. Med. Hyg. 95:322-326.

Levin, M.L., Killmaster, L.F., Zemtsova, G.E., 2012. Domestic dogs (*Canis familiaris*) as reservoir hosts for *Rickettsia conorii*. Vector Borne Zoonotic Dis.12:28-33.

Mannelli A., Mandola M.L., Pedri P., Tripoli M., Nebbia P. 2003. Associations between dogs that were serologically positive for *Rickettsia conorii* relative to the residences of two human cases of Mediterranean spotted fever in Piemonte (Italy). Prev. Vet. Med. 60:13-26.

Parola P., Paddock C.D., Raoult D. 2005. Tick-borne rickettsioses around the world: emerging diseases challenging old concepts. Clin. Microbiol. Rev. 18:719-756.

Solano-Gallego L., Kidd L., Trotta M., Di Marco M., Caldin M., Furlanello T., Breitschwerd E. 2006a. Febrile illness associated with *Rickettsia conorii* infection in dogs from Sicily. Emerg. Infect. Dis. 12:1985-1988.

Solano-Gallego L., Llull J., Osso M., Hegarty B., Breitschwerdt E. 2006b. A serological study of exposure to arthropod-borne pathogens in dogs from Northeastern Spain. Vet. Res. 37:231-244.

Solano-Gallego L., Trotta M., Caldin M., Furlanello T. 2008. Molecular survey of *Rickettsia* spp. in sick dogs in Italy. Zoonoses Public Health 55:521-525.

Torina A., Caracappa S., 2006. Dog tick-borne diseases in Sicily. Parassitologia 48:145-147.

Torina A., Vicente J., Alongi A., Scimeca S., Turla R., Nicosia S., Di Marco V., Caracappa S., de la Fuente J. 2007. Observed prevalence of tick-borne pathogens in domestic animals in Sicily, Italy during 2003-2005. Zoonoses Public Health 54:8-15.

CANINE EHRLICHIOSIS - AN OVERVIEW AND INSIGHTS FROM A RECENT VACCINATION STUDY

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Canine monocytic ehrlichiosis (CME) is an important disease of dogs worldwide. It is caused by the obligate intracellular rickettsia *Ehrlichia canis* and transmitted by the brown dog-tick, *Rhipicephalus sanguineus*. Most CME cases occur during the warm season when the vector ticks are abundant. Common clinical signs include depression, lethargy, anorexia, weight loss, lymphadenomegaly, splenomegaly and hemorrhagic tendencies (mainly petechiae, ecchymoses and epistaxis). The most common hematological sign in CME is thrombocytopenia occurring in most infected dogs (Harrus and Waner, 2011).

Diagnosis of the disease is challenging due to its different phases and multiple manifestations. The suspicion of CME should be considered when a compatible history (tick infestation, travel to or living in endemic region), typical clinical signs, typical hematological signs and biochemical abnormalities (hypoalbuminaemia, hyperglobulinaemia) are present. Classical diagnostic techniques (hematology, cytology, serology and isolation) are useful tools in the diagnosis of CME, however a definitive diagnosis of *E. canis* infection should be done by polymerase chain reaction (PCR) and sequencing. The latter are sensitive methods for detecting and characterizing *E. canis*-DNA, respectively. Detection of *E. canis* DNA in blood of infected dogs can be achieved as early as 4 to 10 days post-inoculation. Several assays are based on different target genes; however the 16S rRNA and the p30-based PCR assays are most commonly used. Real time PCR is a more sensitive assay than conventional PCR allowing quantitative analysis of specific DNA. It is rapidly becoming the preferred method for diagnosis of *E. canis* infection (Stich et al., 2002; Harrus and Waner, 2011).

Doxycycline is considered the drug of choice for the treatment of acute CME, a phase with a good prognosis if properly treated. The chronic phase however is characterized by severe peripheral pancytopenia due to bone marrow hypoplasia. It has a grave prognosis. To date, no commercial vaccine for CME exists. Daily treatment with low dose of doxycycline (100mg per working dog) has been shown effective in prevention of CME in endemic areas. Yet, tick control remains the best method of prevention (Mylonakis et al., 2004; Shipov et al., 2008).

A recent study has indicated that *E. canis* might be attenuated by multiple passages in tissue culture (DH82 cells). An attenuated Israeli strain of *E. canis* has been shown to have protective capability against challenge with a virulent strain. While challenge with the latter resulted in severe disease in all 4 control dogs, only a minority (3/8) of vaccinated dogs presented mild transient fever as a single clinical sign. Furthermore, the mean blood rickettsial load was significantly higher in the control group as compared to vaccinated dogs. The results of the latter study suggest that this strain may be used as a future vaccine for CME (Rudoler et al., in publication).

References

Harrus S., Waner T. 2011. Diagnosis of canine monocytotropic ehrlichiosis: An overview. Vet. J. 187:292-296.

Mylonakis M.E., Koutinas A.F., Breitschwerdt E.B., Hegarty B.C., Billinis C.D., Leontides L.S., Kontos V.S. Chronic canine ehrlichiosis (*Ehrlichia canis*): a retrospective study of 19 natural cases. 2004. J. Am. Anim. Hosp. 40:174-184.

Shipov A., Klement E., Reuveni-Tager L., Waner T., Harrus S. 2008. Prognostic indicators for canine monocytic ehrlichiosis. Vet. Parasitol. 153:131-138.

Stich R.W., Rikihisa Y., Ewing S.A., Needham G.R., Grover D..L, Jittapalapong S. 2002. Detection of *Ehrlichia canis* in canine carrier blood and in individual experimentally infected ticks with a p30-based PCR assay. J. Clin. Microbiol. 40:540-546.

VECTOR-BORNE ZOONOTIC DISEASES IN CENTRAL AND EASTERN EUROPE

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Since 1990s the number of publications on vector-borne pathogens (VBPs) of domestic and wild animals has increased considerably in Europe. The data of these studies provide important information for the potential of human infection in a particular geographic location. Most probably the increasing frequency of some vector-borne zoonotic diseases (VBZDs) is due to a joint action of several different factors. Anthropogenic climate change has been influencing the geographical distribution, density and seasonal activity of hard ticks, mosquitoes and other blood-sucking arthropods. Increasing temperatures shorten the life cycles of the vectors, potentially leading to larger populations which can transmit a number of pathogens between animals and humans. A northward range expansion of many arthropod species has also been observed in response to projected climate change, however, travel, trade, insecticide resistance and other drivers have also facilitated these processes (Rogers and Randolph, 2006; Jaenson and Lindgren, 2011). Changes in land-use, social and leisure activities of humans especially in Central and Eastern Europe have also affected the epidemiology of VBZDs. Moreover, during recent years Europe has experienced the introduction of new vector species (e.g., *Aedes albopictus*) and vector-borne agents (e.g., Chikungunya virus) from tropical regions. All of the mentioned events support the notion that this part of Europe is also a potential "hot spot" for emerging and re-emerging VBZDs.
For the last two decades the knowledge about VBZDs of domestic and wild animals as well as humans has been extended in Central and Eastern Europe. The introduction and the development of sophisticated diagnostic methods, mainly the polymerase chain reaction and nucleotide sequencing for VBPs allow rapid, sensitive and specific identification of pathogens in animals, human as well as in vectors collected from animals, humans and/or environment. In addition, the use of geographic information systems, remote-sensing technologies and risk analyses provide new tools for improving the epidemic knowledge of VBZDs.
The hard ticks are the most important vectors of zoonotic pathogens in Central and Eastern Europe. Our knowledge has also been extending about the vector competency of other vectors such as mosquitoes, sandflies and fleas which also play important roles in the transmission of zoonotic pathogens from domestic and wild animals to humans.

Tick-borne zoonotic diseases

Tick-Borne Encephalitis (TBE) is one of the most important and serious human infections occurring in Europe. *Ixodes ricinus* is the main vector in Europe. Apart from *I. ricinus,* several other tick species including *I. hexagonus, I. arboricola, Haemaphysalis concinna, Haemaphysalis inermis* and *Ha. punctata* are competent but secondary vectors of Tick-Borne Encephalitis Virus (TBEV) (Labuda and Nuttall, 2008). The TBEV is maintained in sylvatic cycles between *I. ricinus* and wild mammalian hosts, mainly rodents throughout the year. Larger animals (e.g., wild ruminants, horses) and dogs are not thought to have an important role in virus transmission between ticks. The most involved is the nymphal stage in human infections. The prevalence of virus infection in ticks varies in different regions and years. In TBE foci in Central Europe the prevalence of TBEV in nymphs ranges around 0.1-0.5% and in adults about 0.3-6.0% (Labuda and Nuttall, 2008). Although TBEV can be transmitted transovarially from the adults tick through the eggs to larvae, but it may be too low to make a significant contribution to the virus life cycle. Many cases of TBE in humans are unrecognized. In some cases the virus can cause life-threatening neurologic disease. In Central Europe nearly all deaths confined to patients above 60 years old. In some East European countries the incidence of TBE increased over the last two decades that is explained by climate change but socio-economic conditions (e.g., unemployment, political upheaval) have been shown to have an impact on the incidence of TBE. In some other countries the number of human TBE decreased in recent years because of a successful vaccination campaign (Donoso Mantke et al., 2008; Lindquist and Vapalahti, 2008).
Lyme borreliosis (LB) is a complex of several different zoonotic infections of which the etiological agents, spirochetes in the *Borrelia burgdorferi* sensu lato complex, are transmitted by mainly *I. ricinus* in Europe. *Ixodes hexagonus* and *I. uriae* are also considered vectors of LB spirochetes (Piesman and Gern, 2008). Although the level of infection in the adult populations of *I. ricinus* is higher (range: 3-58%) than in the nymphs (range: 2-43%), the nymphs are usually more important than the female adult ticks for transmission of the pathogens to humans. Larvae are rarely infected (range: 0-11%) (Hubálek and Halouzka, 1998). Lyme borreliosis is now the most commonly reported arthropod-borne disease in

Europe. The highest reported frequencies are in Central Europe. The number of ticks carrying the pathogens of LB, *B. burgdorferi* s.l. spirochetes also seems to have risen in many countries. Clinical symptoms associated with LB have been also reported in cattle, horses, dogs and cats. From the epidemiological point of view, dogs are very important since they are considered a suitable indicator of the spread of human LB pathogens. Specific antibodies of diseased and/or healthy, asymptomatic dogs to *B. burgdorferi* s.l. have been detected in almost all countries of Central and Eastern Europe. Cervids appear refractory to the infection and usually do not serve as *Borrelia* reservoirs (Jaenson and Tälleklint, 1992).

Human granulocytic anaplasmosis (HGE) is caused by *Anaplasma phagocytophilum*, an obligate intracellular bacterium with a tropism for leukocytes. The main vector of *A. phagocytophilum* is *Ixodes ricinus* (Strle, 2004). Only transstadial transmission occurs, the larvae and nymphs of *I. ricinus* acquire infection from a variety of wild animals. Prevalence of *A. phagocytophilum* in ticks varies widely from 3 to 25%, even in areas of endemicity and development stage of the tick (Stuen, 2007). This bacterium causes tick-borne fever in sheep, pasture fever in cattle, wild ruminants, granulocytic anaplasmosis in horses and dogs. The definitive reservoir(s) remains unknown, livestock such as cattle, sheep or horse may play roles in maintaining the organism in nature. HGE is a multisystemic disease that occurs more in adults than in children, especially in persons above the age of 60 years. In Europe, no fatal cases have been reported (Strle, 2004; Rymaszewska and Grenda, 2008).

Babesiosis is an emerging zoonotic problem caused by some *Babesia* species of protozoans (Genchi, 2007; Hunfeld et al., 2008). Most infected patients share splenectomy as a risk factor for acquiring the disease, but the rising number of HIV-positive individuals and the increasing population of immunocompromised patients may also serve to boost the number of human cases. The first confirmed case of human babesiosis was diagnosed in a splenectomized Yugoslavian cattle farmer who died of a fatal *B. divergens* infection in 1956 (Skrabalo and Deanovic, 1957). At least 70% of the human cases in Europe are associated with the cattle piroplasm *B. divergens* (Genchi, 2007). A new European *B. divergens*-like organism (EU1), named *Babesia venatorum*, has been described from deer and *I. ricinus* (Duh et al., 2005). This parasite species was involved in the first documented cases of human babesiosis in asplenic men in Italy, Austria and Germany (Herwaldt et al., 2003; Häselbarth et al., 2007). A human infection in a splenectomized patient, caused by a strain named *Babesia* EU3 that has high homology to *Babesia* EU1, was reported from Germany (Häselbarth et al., 2007). *Babesia microti* that is only transmitted transstadially by *I. ricinus* from small rodents has been found to be also an important species causing emerging infection of humans in Europe (Hildebrandt et al., 2007).

Tularemia is a rare, typically zoonotic disease of Central Europe. It is caused by *Francisella tularensis*, a Gram-negative obligate intracellular agent (Ellis et al., 2002; Tarnvik et al., 2004). The European brown hare *(Lepus europaeus)* is a common host of *F. tularensis* in Central Europe (Pikula et al., 2004). Ticks are believed to be the most important arthropods for *F. tularensis* as both mechanical and biological vectors (Hopla and Hopla, 1994). *Dermacentor reticulatus* plays an important role in the maintenance and transmission of *F. tularensis* among small and medium sized mammals in Central Europe (Hubalek et al., 1996). Other ticks, such as *I. ricinus, I. persulcatus, D. marginatus* and *Haemaphysalis inermis, Haemaphysalis concinna,* have also been found to be naturally infected with *F. tularensis* in Europe (Hopla and Hopla, 1994; Keim et al., 2007). The pathogen is tranmitted transtadially by the tick vectors.

The etiologic agent of **Q fever** is the rickettsial parasite, *Coxiella burnetii*. Ticks are one of a broad range of reservoirs for the organism. The boundary margin of Q fever corresponds to the northern end of the range for *D. marginatus* that is important for the maintenance of *C. burnetti* throughout Central Europe. After feeding on bacteremic hosts nymphs or adults can transmit the pathogen transstadially, and females can pass it transovarially (Lang, 1990). Other tick species such as *I. ricinus*, *Ha. punctata*, *Rhipicephalus sanguineus* and some *Hyalomma* species are also implicated as potential vectors. The most common reservoirs of the *C. burnetti* for human infections are cattle, sheep and goats. Dogs, cats, birds, and reptiles also are susceptible to infection and may play a role in maintaining the infection in natural habitats. Q fever may be epidemic especially on farms or in farming communities when infected domestic animals are being handled, such as during wool-shearing, lambing, calving, and slaughtering. Therefore farmers, abattoir workers, meat-packing workers, veterinarians and laboratory workers in contact with livestock are at high risk of infection (EFSA Journal, 2010).

Mosquito-borne zoonotic diseases

West Nile virus (WNV) is by far the most widely distributed arbovirus. The most competent host are birds. The virus is transmitted in an avian cycle by mosquitoes of the genus *Culex*. Vectors acquire infection by feeding on a viraemic birds (Hayes et al., 2005). Mammals can also be infected, but are considered dead end hosts because viraemia is generally too low to infect mosquitoes (Dauphin et al., 2004). Human infections have been reported in Romania (Tsai et al., 1998) and Hungary (Krisztalovics et al., 2008). Only a small portion of human infections are symptomatic (e.g., headache, swollen lymph glands). Neurological disease can occur very rarely in people of any age, but those over 50 years are at highest risk.

Human dirofilariosis is caused by *Dirofilaria immitis*, the causative agent of canine and feline heartworm disease and *D. repens* the main causative agent of subcutaneous filarial infections of dogs. Both species are transmitted by mosquitoes, mostly of the genera *Culex* and *Aedes*. Human infections are caused mainly by *D. repens* and the increasing numbers of autochthonous cases have been reported from Austria, Croatia, Hungary, Slovak Republic and Romania. There has been increasing concern over *Dirofilaria* spp. due to the spread of canine infections from well-known endemic areas of Southern Europe toward northern and eastern countries (Genchi et al., 2009; 2011).

Sandfly-borne zoonotic diseases

Zoonotic visceral leishmaniosis (ZVL) caused by *Leishmania infantum* is an important disease of humans in Mediterranean Europe. The increase of co-infections with human immunodeficiency virus (HIV) and *Leishmania* has been observed since the 1980s. The domestic dog is the main reservoir host, humans are considered as an accidental host that does not contribute to transmission. Therefore the introduction to northern Europe of *L. infantum* in dogs taken to the Mediterranean region on holiday or rescued from there as strays poses a significant risk. The principal transmission route for *L. infantum* is by the bite of blood feeding female phlebotomine sandflies. At least a dozen sandfly species have been incriminated as vectors of *L. infantum* in the Old World, of which *Phlebotomus perfiliewi*, *P. neglectus* and *P. tobbi* are the most important ones in Central and Eastern Europe. Leishmaniosis has become more apparent in northern latitudes where sandfly vectors are either absent or present in very low densities, such as in Germany. The occurrence of 'vectors without disease' poses a significant risk for the emergence of leishmaniosis in temperate regions of Europe (Ready, 2010).

Flea-borne zoonotic diseases

The main agent of **cat scratch disease** is a hemotropic Gram-negative bacterium, *Bartonella henselae*. Its principal reservoirs are domestic cats. Studies support an important role of cat fleas (*Ctenocephalides felis*) in the transmission of *B. henselae*. It has been shown that *B. henselae* can multiply in the digestive system of the flea and remains reproductively viable in flea faeces within the environment for several days. The primary mode of transmission of *B. henselae* to humans appears to be the flea faeces that are inoculated by contaminated cat claws, through a cutaneous trauma caused mainly by the scratch of a cat. There is no evidence that fleas can transmit the pathogen to humans (Breitschwerdt, 2008).

The risk of pathogen transmission between domestic/wild animals and human populations with vectors should be reduced. For this reason research and effective surveillance of VBZDs are needed to continue in Central and Eastern European countries. Veterinarians and physicians should improve their knowledge about the different vector species of veterinary and medical importance and the pathogens transmitted by these arthropods to improve the health and well-being of humans as well as animals. Last but not least public education can be also very helpful and important for the prevention and control of human infections with many of vector-borne zoonotic pathogens.

References

Breitschwerdt E.B. 2008. Feline bartonellosis and cat scratch disease. Short survey. Vet. Immunol. Immunop. 123:167-171.

Dauphin G., Zientara S., Zeller H., Murgue B. 2004. West Nile: worldwide current situation in animals and humans. Comp. Immunol. Microb. 27(5):343-355.

Donoso Mantke O., Schädler R., Niedrig M. 2008. A survey on cases of tick-borne encephalitis in European countries. Euro Surveill. 13:1-9.

Duh D., Petrovec M., Avsic-Zupanc T. 2005. Molecular characterization of human pathogen *Babesia* EU1 in *Ixodes ricinus* ticks from Slovenia. J. Parasitol. 91: 463-465.

EFSA Journal. 2010. Scientific Opinion on Q fever. 8(5):1595.

Ellis J., Oyston P.C, Green M., Titball R.W. 2002. Tularemia. Clin. Microbiol. Rev. 15: 631-646.

Genchi C. 2007. Human babesiosis, an emerging zoonosis. Parassitologia 49(suppl. 1):22-31.

Genchi C., Rinaldi L., Mortarino M., Genchi M., Cringoli G. 2009. Climate and Dirofilaria infection in Europe. Vet. Parasitol. 163:286-292.

Genchi C., Rinaldi L., Rivasi F. 2011. Dirofilarial Infections in Europe. Vector Borne Dis. 11(10):1307-1317.

Hayes E., Komar N., Nasci R., Montgomery S., O'Leary D., Campbell G. 2005. Epidemiology and transmission dynamics of West Nile virus disease. Emerg. Infect. Dis. 11(8):1167-1173.

Häselbarth K., Tenter A.M., Brade V., Krieger G., Hunfeld K.P. 2007. First case of human babesiosis in Germany – Clinical presentation and molecular characterization of the pathogen. Int. J. Med. Microbiol. 297:197-204.

Herwaldt B.L., Caccio S., Gherlinzoni F., Aspöck H., Slemenda S.B., Piccaluga, P., Martinelli G., Edelhofer R., Hollenstein U., Poletti G., Pampiglione S., Loschenberger K., Tura S., Pieniazek N.J. 2003. Molecular characterization of a non-*Babesia divergens* organism causing zoonotic babesiosis in Europe. Emerg. Infect. Dis. 9:942-948.

Hildebrandt A., Hunfeld K.P., Baier M., Krumbholz A., Sachse S., Lorenzen T., Kiehntopf M., Fricke H.J., Straube E. 2007. First confirmed autochthonous case of human *Babesia microti* infection in Europe. Eur. J. Clin. Microbiol. 26:595-601.

Hopla C.E., Hopla A.K. 1994. Tularemia. In: Beran, G.W. (Ed.) *CRC handbook of zoonoses, Section A, Bacterial, rickettsial and mycotic diseases*, 2nd ed., CRC Press Inc., Boca Raton, Florida, pp. 113-125.

Hubálek Z., Halouzka J. 1998. Prevalence rates of *Borrelia burgdorferi* sensu lato in host-seeking *Ixodes ricinus* ticks in Europe. Parasitol. Res. 84:167-172.

Hubalek Z., Treml F., Halouzka J., Juricova Z., Huňady M., Janík V. 1996. Frequent isolation of *Francisella tularensis* from *Dermacentor reticulatus* ticks in an enzootic focus of tularaemia. Med. Vet. Entomol. 10:241-246.

Hunfeld K.P., Hildebrandt A., Gray J.S. 2008. Babesiosis: recent insights into an ancient disease. Int. J. Parasitol. 38:1219-1237.

Jaenson T.G., Lindgren E. 2011. The range of *Ixodes ricinus* and the risk of contracting Lyme borreliosis will increase northwards when the vegetation period becomes longer. Ticks Tick Borne Dis. 2:44-49

Jaenson T.G.T., Tälleklint L. 1992. Incompetence of roe deer as reservoirs of the Lyme borreliosis spirochete. J. Med. Entomol. 29:813-817.

Keim P.S., Johansson A., Wagner D.M. 2007. Molecular epidemiology, evolution and ecology of *Francisella.* Ann. NY Acad. Sci. 1105:30-66.

Krisztalovics K., Ferenczi E., Molnár Z., Csohán Á., Bán E., Zöldi V., Kaszás K. 2008. West Nile virus infections in Hungary, Euro Surveill. 13(45):1-3.

Labuda M., Nuttall P.A. 2008. Viruses transmitted by ticks. In: Ticks. Biology, Disease and Control. Bowman AS Nuttall PA (Eds.). Cambridge University Press, pp. 253-280.

Lang G.H. 1990. Coxiellosis (Q fever) in animals**.** In Marrie, T.J. (Ed.) Q Fever. Volume 1. The Disease. CRC Press, Boca Raton, USA, pp. 23-48.

Lindquist L., Vapalahti O. 2008. Tick-borne encephalitis. Lancet 371:1861-1871.

Piesman J., Gern L. 2008. Lyme borreliosis in Europe and North America. In: Ticks, Biology, Diseae and Control. Bowman AS and Nuttall P, Cambridge University Press, U.K, pp. 220-252.

Pikula J., Beklova M., Holesovoska Z., Treml F. 2004. Ecology of European brown hare and distribution of natural foci of tularemia in the Czech Republic. Acta Veterinaria Brno 73: 267-273.

Ready P. 2010. Leishmaniasis emergence in Europe. Review Article. Euro Surveill. 15(10):1-11.

Rogers, D.J., Randolph, S.E. 2006. Climate change and vector-borne diseases. Adv. Parasit. 62:345-381.

Rymaszewska A., Grenda S. 2008. Bacteria of the genus *Anaplasma*-characteristics of Anaplasma and their vectors: a review. Vet. Med-Czech. 53:573-584.

Strle F. 2004. Human granulocytic ehrlichiosis in Europe. Int. J. Med. Microbiol. 293:27-35.

Skrabalo Z., Deanovic Z. 1957. Piroplasmosis in man; report of a case. Documenta de Medicina Geographica et Tropica 9:11-16.

Strle F. 2004. Human granulocytic ehrlichiosis in Europe. Int. J. Med. Microbiol. 293:27-35.

Stuen S. 2007. *Anaplasma phagocytophilum* – the most widespread tick-borne infection in animals in Europe. Vet. Res. Commun. 31:79-84.

Tarnvik A., Priebe, H.S., Grunow, R. 2004. Tularemia in Europe: An epidemiological overview. Scand. J. Infect. Dis. 36:350-355.

Tsai T.F., Popovici F., Cernescu C., Campbell G.L., Nedelcu N.I. 1998. West Nile encephalitis epidemic in Southeastern Romania. Lancet 352(9130):767-771.

TICKS AND TICK-BORNE PATHOGENS IN PUBLIC CITY PARKS

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In recent years, public parks have attracted – in parallel with the increasing recreational activities of the modern working society – an increasing interest for ticks and therewith transmitted infections (i.e., protozoa, bacteria and virus). With the objective to get more information about the occurrence of ticks and therewith transmitted pathogens, we started a project in Bavarian cities or greater city areas and collected ticks in monthly intervals by using the flagging method. Altogether, nine different public parks in five Bavarian cities were integrated in this study. Thereby, the tick abundance (adults and nymphs/100m^2) was determined and nine sampling sites (5 sampling sites in 2010) were screened for DNA of *Babesia* spp., *A. phagocytophilum*, *Rickettsia* spp. and *Babesia* spp. by specific conventional and real-time PCR. In 2010 the ticks were only screened for *Babesia* spp. and *A. phagocytophilum*. A total of 13403 *I. ricinus*, one *I. frontalis* as well as one *I. hexagonus* were collected. The tick abundance varied between 15-53 ticks/100 m^2 depending on the sampling site. 6593 ticks (5569 for *A. phagocytophilum)* were investigated with the following results: *Babesia* spp. (2009: 0.4% with one pool of larvae à 2 larvae; 2010: 0.5-0.7% with one pool of larvae à 5 larvae); *A. phagocytophilum* (2009: 9.5%; 2010: 6.6%); *Rickettsia* spp. (2009: 6.4-7.7% with 16 pools of larvae including 76 larvae). Sequence analysis revealed *Babesia* sp. EU1 (n=25), *B. divergens* (n=1), *B. divergens/capreoli* (n=1), *Babesia gibsoni*-like (n=1), *R. helvetica* (n=272), *R. monacensis* strain IrR/Munich (n=12) and *R. monacensis* (n=1). The occurrence of DNA of *Bartonella* spp. in *I. ricinus* could not be demonstrated. Co-infections were detected in 0.7% of all investigated ticks in 2009. Prevalence variation between the years and sampling sites as well as a noticeable species arrangement of *Babesia* spp. in public parks showed that the occurrence of tick-borne pathogens depends on different biotic and abiotic factors. Thereby the city park as habitat for ticks seems to take an eminent position on tick-borne pathogen topics.

EMERGING VECTOR BORNE DISEASES OF LIVESTOCK

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Introduction

In Europe, a low level of priority was generally given to vector borne diseases by national and European animal health organizations. The introduction and rapid spreading in Northern Europe of bluetongue virus in 2006 and more recently (2011) of Schmallenberg virus has changed dramatically our present perception of vector borne diseases and the thread they represent for the European livestock.

Several factors probably play a critical role in the introduction, persistence and spreading of many vector borne diseases in Europe and elsewhere: 1) an increase of commercial exchanges between EU countries but also between EU and other continents, 2) in some areas a sharp increase of wildlife populations (such as red deer, roe deer or wild boar) and consequently a higher risk of transfer of pathogens from cattle to wildlife and vice versa, 3) global warming and environmental degradation (pollution, loss of biodiversity) which could have an important impact on the distribution and abundance of some vectors, 4) in some areas a tendency towards extensive cattle breeding which could lead to a higher exposure to some vectors and associated pathogens and 5) the intensive selection for some production traits such as meat and milk could also result in an increased susceptibility to different arthropod pests in some very susceptible genotypes, this latter point being well documented in tropical and sub-tropical countries.

A few relevant and recent examples are developed in the text below. They deal with truly emergent or sometimes re-emergent vector borne diseases of livestock at national or European levels. Besides the clinical signs and epidemiology some key information is provided on the specific vector(s).

Diseases transmitted by Diptera

Infection by the Schmallenberg virus

The Schmallenberg virus (SBV) was identified for the first time in Germany in 2011. The first detection of the virus was achieved on a pooled blood samples from a cattle farm in Schmallengerg (Rheinland Nord Westphalen). This virus belongs to the *Bunyaviridae*, genus *Orthobunyaviridae*, sero group *Simbu*. It is related to *Akabane*, *Aino* and *Shamonda* viruses. Those viruses are not contagious being transmitted by biting arthropods namely mosquitoes and biting midges (*Culicoïdes* spp.). Infection by SBV is characterized in cattle by a marked drop of milk yield, fever and diarrhoea and sometimes abortions. Between November 2011 and April 2012, the virus was found in sheep, goats and cattle in Germany, the Netherlands, Belgium, Luxembourg, Great Britain and France. Cases in a goat and in a lamb were recorded in Italy and Spain, respectively (Dominguez, 2012).

Infection during pregnancy can lead to abortion or the birth of congenitally infected lambs, kids and calves suffering from an arthrogryposis/hydranencephaly syndrome. Initially the differential diagnosis included Bluetongue, foot-and-mouth disease, Bovine Viral Diarrhoea, Infectious Bovine Rhinotracheitis, Rift Valley Fever and Bovine Ephemeral Fever.

Preliminary data indicate that SBV is transmitted by biting midges (family Ceratopogonidae). Those tiny diptera (1-2 mm) are important vectors of arbovirus, nematodes and protozoa. There are over 1300 known species with a worldwide distribution. The female are haematophagous whereas the males feed on plant material. Breeding sites are extremely variable depending on species: dung cattle, manure, decaying plant material such as silage, hay, straw, wood…, still water, algae… The monitoring of biting midges population which started in 2006 has allowed the identification of 51 species. The most abundant and important species belong to the sub groups Avaritia (*C. obsoletus/scoticus; C*. *dewulfi*, *C. chiopterus*) and Culicoides (*C. pulicaris*) the former representing at least 80% of the captures most of the time. RTPCR tests carried at CODA-CERVA (Brussels) on different pools of distinct species of biting midges captured in summer and autumn 2011 in Belgium indicate that *C. obsoletus/scoticus*, *C. dewulfi* and *C. chiopterus* are most probably vectors of SBV.

At the time being there is no serological test for the diagnosis of SBV infection in ruminants; RTqPCR is available and allow a fast and reliable detection of viral material. No vaccines are available and the zoonotic risk is considered to be very low.

Parafilariosis

This disease ("Bleeding spots") is due to the development and migration of the nematode *Parafilaria bovicola* in the connective subcutaneous and intermuscular tissues of cattle (Losson and Saegerman, 2009). A few days before the apparition of the nodules and bleeding spots, an abnormal gait is often recorded which is probably due to the pain due to a marked local inflammation. Thereafter painless nodules measuring approximately 15 mm in diameter appear mainly on the withers, shoulders, rumps and sometimes on the lateral aspects of the neck. Within a few hours a tiny hole is visible which produces an abundant serohemorrhagic exudate which contains the embryonated eggs produced by the females and dries up quickly giving a far less typical image. New nodules can appear during the next 3 to 4 weeks. The adults feed on the inflammatory exudate. This disease is mainly observed in young animals and bulls, the cows being less often clinically affected. In an affected herd, some individual (particularly the bulls) can exhibit lesions for several consecutive years. Inflammatory subcutaneous lesions are often extensive. They are characterized by a yellowish coloration of the subcutaneous tissues and the presence of hemorrhages which must be differentiated from traumatic lesions. Due to the chronobiology of this infection lesions are observed essentially between December and June.

Musca autumnalis (The Face Fly) is considered as the main vector in Europe. This was demonstrated in Sweden. This fly is very abundant during the grazing season. It has also the ability to overwinter indoors as adults. This non-biting fly acquires the infection when feeding on the exudate produced at the skin level. After tow molts, the infective third stage larvae is found in the mouthparts of the vector. In summer this step takes approximately 2 to 3 weeks. The L3 is then deposited onto the conjunctiva as the fly is highly attracted by lacrymal secretions. After a long migration through the subcutaneous tissues, the adults mate, induce the formation of a nodule and embryonated eggs are evacuated with the abundant exudate. The entire life cycle takes one year which explains the marked seasonality of this condition. In Belgium in 2012 the first bleeding spots were detected on February 28th. The clinical impact of this infection is very low. However, the migration of larvae is responsible for severe inflammatory response. The carcass is downgraded and the removal of the lesions induces fairly high losses (during the eighties and nineties in Sweden, this was estimated around 7 kilos of meat par animal). In the non endemic areas, the disease is not easily identified by the breeders and veterinarians and it can spread rapidly, the vector being extremely common abundant and able to fly over fairly long distances. The disease was first described in South Africa in 1934. In France, it is endemic in the South West and seems to be spreading. In Sweden, it was probably introduced through the importation of French cattle. It spread very rapidly in this country and was responsible for marked economical losses. More recently, this parasitic infection was reported in the Netherlands in one Charolais bull imported from France; the animal was slaughtered to avoid a rapid extension of the disease via the vector. In Belgium, the disease was reported in 2008 and is spreading, being present in several provinces. Since then it has been identified in several herds in Central Northern Italy and in South Western and Southern Germany.

The microscopic examination of the exudate allows the observation of the typical embryonated eggs and will confirm the clinical diagnosis based on the localization, aspect and time of appearance of skin lesions.

Control relies on the use of macrocyclic lactones (ivermectin, doramectin, moxidectin at 200 µg/kg body weight) or nitroxinil (20 mg/kg b.w.). However macrocyclic lactones seem to be active only on the adult worms and this is in agreement with recent observations in the field in Belgium. Prevention is based on the use of insecticides to control fly proliferation in the spring. There is no zoonotic impact.

Besnoitiosis

Until the nineties, bovine besnoitiosis was limited to a few foci in Spain and Southern France. It is now considered as a potentially emerging disease of cattle in Europe. Indeed in France, Spain and Portugal the disease is spreading rapidly and several outbreaks have been recently reported in Italy and Germany. Due to its marked clinical and economic impact, it must be considered as a serious threat to the cattle industry (Jacquiet et al., 2010).

Besnoitia besnoiti is a protozoan belonging to the family Sarcocystidae. The life cycle is considered as heteroxenous. Since its first description by Besnoit in France at the beginning of the 20th century, the cat was considered as the final host shedding fecal oocysts in the environment. However, there is still a lot of controversy and clearly additional data are needed. After a putative oral infection in cattle, the disease is characterized first by an acute phase (lasting 2-3 weeks) due to the multiplication of the

parasite in many cell types and thereafter by the development of tissue cysts measuring 2 to 4mm in diameter and containing thousands of bradyzoites. These cysts may remain viable probably during several years. Those cysts are usually very numerous and are observed in different mucosa (nasal, tracheal, vaginal), in the intermuscular and subcutaneous fibroblasts, in the skin and in the testis. Venereal and vertical transmissions are not recorded. The bradyzoites are most probably transmitted mechanically through the bites of different haematophagous flies (stomoxes, tabanids) or the use of non disposable needles. In a given herd, there is a rapid extension of the disease as judged by serology. However, only a few animals are clinically infected (5 to 20%); in those, mortalities can occur. After a short incubation period (6 to 10 days), three successive phases are described: 1) an acute phase with high fever (40-42 °C), epiphora, nasal discharge and generalized congestion. The skin is painful. This phase lasts about one week and the differential diagnosis is difficult due to the unspecific nature of the clinical signs; 2) a sub acute phase with oedema of the head, dewlap and members. Fever is not present anymore; the udder and the testis are painful and congested. Locomotion is impaired; 3) a final phase which is characterized by scleroderma and hair loss. The skin is thickened; the locomotion is more and more difficult. The animals are losing weight and most of them must be euthanized. In about 25% of the infected animals, the typical cysts develop on the sclerotic and cornea. These are pathognomonic of the disease.

The disease has been observed in young animals under one year of age. However, the heifers and the bulls seem to be the most susceptible groups. There is a fairly marked seasonality in relation with the proliferation of the vectors. Indoors*, Stomoxis calcitrans* can play a role in early spring. Thereafter, the tabanids are mainly responsible for the rapid spreading of the infection. In endemic areas, clinical cases are sporadic but the seroprevalence is very high; in contrast, in newly infected herds, clinical cases are frequently observed and the disease can take a pseudo-epizootic pattern. The differential diagnosis is difficult during the acute and subacute phases. It includes bluetongue virus infection, photosensitization, malignant catarrhal fever, anaplasmosis (*Anaplasma phagocytophilum*) and different infectious diseases of the respiratory tract (Tables 1 and 2). The chronic phase is very typical; differential diagnosis may include zinc deficiency and chronic psoroptic mange (Losson et al., 2010) (Table 3).

Table 1. Clinical differential diagnosis of besnoitiosis (acute phase) in cattle

Disease	Fever	Tachypnoea	Skin congestion	Nasal discharge
Besnoitiosis	++++	+++	+++	+++
Malignant catarrhal fever	++++	+++	+++	+++
Bluetongue	+++	++	++	++
IBR, RSV,PI3 infections	++++	+++	(+)	+++
Photosensitization	+(+)	+(+)	++++	+

Table 2. Clinical differential diagnosis of besnoitiosis (sub acute phase) in cattle

Disease	Skin thickening and congestion	Oedema
Besnoitiosis	+++	++(+)
Blue tongue	(+)	+(+)
Anaplasmosis	0	+(+)
Photosensitization (sub acute phase)	++	

Table 3. Clinical differential diagnosis of besnoitiosis (chronic phase) in cattle

Disease	Hyperkeratosis and skin foldings	Hair loss	Itch
Besnoitiosis	++++	+++	0
Zinc deficiency	+	+++	0
Psoroptic mange	++(+)	++	+++

At the laboratory, the clinical diagnosis can be confirmed through histopathology carried out on a skin or scleral biopsy. Different serological (ELISA, Western blot) and molecular (PCR) tests are also available. They are more efficient during the chronic phase. At the time being, the diagnosis during the acute phase remains very difficult.

High dosages of different sulfamids are useful during the acute phase; however, the infected animals remain carriers probably during the rest of their life. There is no available vaccine. The culling of seropositive animals and the serological screening of any recently purchased animal are recommended in infected areas or countries. There is no zoonotic impact of the disease.

Diseases transmitted by hard ticks (Ixodidae)

Anaplasmosis

Tick-borne fever (TBF) is caused by *Anaplasma phagocytophilum* (formerly *Rickettsia phagocytophila*, *Cytoecetes phagocytophila* and *Ehrlichia phagocytophilum*). The disease was first described in the UK in 1940 in lambs exposed to the bites of *Ixodes ricinus*.

Since then, the disease was described in different species of ruminants in many European countries: Ireland, Scandinavia, the Netherlands, Belgium, Austria, Switzerland, Spain and France. Following a recent reclassification, TBF agent is considered now as a variant of one species responsible for granulocytic anaplasmosis of ruminants, horses and humans. *A. phagocytophilum* multiplies mainly in the neutrophils and eosinophils; monocytes can be infected also. Within the cytoplasm of its host cells, *A. phagocytophilum* multiplies to form typical morulae.

In cattle, TBF is characterized by high fever, neutropenia, lymphocytopenia, thrombocytopenia and general immunosuppression. Septic complications are frequent (pasteurellosis, listeriosis, enterotoxemia). Animals in late pregnancy may abort. The presence of hyperthermia, respiratory symptoms (coughing, nasal discharge), oedema (especially at the level of the hind legs) and a marked drop of milk production in animals recently moved to tick infested pastures are suggestive of TBF.

Anaplasma phagocytophilum is transmitted transtadially by the three-host tick *Ixodes ricinus* and possibly other species of ticks. This tick is widely distributed in Western and Eastern Europe. It is particularly abundant in biotopes characterized by deciduous forest and pastures grazed by domestic ruminants. Under such circumstances contacts between cattle and wild ungulates are frequent and the transfer of pathogens is enhanced. The life cycle is long and can take up to 3 years. Depending to the local climatic conditions, the phenology of this tick can vary greatly. In Belgium, questing *I. ricinus* ticks are most abundant in May-June with a lower peak in September and early October. There issome evidence that the geographical distribution of this tick in Europe is on the rise both in altitude and longitude. These changes are probably linked to global warming (Jaenson et al., 2010). Transovarian transfer in the tick vector is not recorded. After infection, domestic ruminants remain clinically silent carriers for a long period of time (possibly years). Wild ruminants (red deer, roe deer and fallow deer) and small rodents are good reservoirs and are a potential source of infection for *I.ricinus*. Several studies suggested a high degree of antigenic diversity among the different *A. phagocytophilum* field isolates. Also, recent evidence suggests sequential changes in some of the major surface proteins of *Anaplasma* spp. during recurrent bacteriemia. These observations could explain, at least in part, the relapses which are observed in previously infected cattle. All these data demonstrate that the epidemiology of TBF is very complex (Woldehiwet, 2006).

The diagnosis of TBF relies on the observation of the typical morulae in peripheral blood granulocytes or monocytes or the detection of specific DNA by PCR. Serology (Indirect Immunofluorescence) is useful to assess the degree of exposure to *A. phagocytophilum* in a herd or a region. The agent can be cultivated in tick cell lines. The zoonotic impact of TBF agent is still a matter of discussion. It remains to be established wether the variants causing Human Granulocytic Anaplasmosis are genetically and biologically different from those causing TBF in ruminants.

Current control strategies relies on the use of acaricides to limit *I. ricinus* (and possibly other species) populations on the host and of long acting tetracyclines given before the animals are moved to tick infested areas. Currently there is no commercially available vaccine for TBF (Woldehiwet, 2006).

References

Dominguez M. Virus Schmallenberg: point de la situation. http://www.survepi.org/cerepi/consulted April 15th 2012.

Jacquiet P., Liénard E., Franc M. 2010. Bovine besnoitiosis: Epidemiological and clinical aspects. Vet. Parasitol. 174:30-36.

Jaenson T.G., Jaenson D.G., Eisen L., Petersson E. 2012. Changes in the geographical distribution and abundance of the tick *Ixodes ricinus* during the last 30 years in Sweden. Parasit. Vectors 10:4-8.

Losson B., Saegerman C. 2009. First emergence of bovine parafilariosis in Belgium. Vet. Rec. 164:623-629.

Losson B., O'Brien D., Jemli M.H. 2010. Mange and demodicosis in livestock. In: P.-C. Lefèvre, J. Blancou, R. Chermette and G. Uilenberg (Ed.). Infectious and parasitic diseases of livestock, volume 2, Bacterial diseases, fungal diseases, parasitic diseases. Editions Tec et Doc, Paris, 1427-1444.

Woldehiwet Z. 2006. *Anaplasma phagocytophilum* in ruminants in Europe. Ann. NY Acad. Sci. 1078:446-460.

ECHINOCOCCUS SPP.: EPIDEMIOLOGICAL IMPLICATIONS OF INTER- AND INTRASPECIFIC DIVERSITY

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An impressive number of recent studies on the genetic identity of *Echinococcus* spp. – from the species to the haplotype level – has provided new insights into relationships within the genus and has reshaped taxonomy. As a result of this process, the conventional strain-based nomenclature of *E. granulosus* (sensu lato) is increasingly replaced by the use of resurrected species names, which provide a more realistic system by avoiding overdiscrimination of minor variants (e.g., G1-3) and emphasizing the distinctness of distantly related taxa (e.g., *E. equinus*) (Nakao et al., 2007). Although still controversial in details, this has provided firm ground from which (1) to explore the genetic variability within species / strains and thereby fine-tune the limits of those taxa, and (2) to evaluate the epidemiological, medical and economic consequences of this diversity on a world wide scale. A range of diagnostic tools, including mitochondrial markers, microsatellite profiles, and even complete mt genomes, are now available to investigate appropriate numbers of field isolates, linking the genetic information with biological, morphological and medical/veterinary characters (Nakao et al., 2010). Although molecular surveys are still spoardic and limited to few countries, they already demonstrated a previously underrecognized epidemiological complexity. As an example, the public health impact and clinical presentation of the pig/camel strain (G6/7) of *E. canadensis* may have to be re-appreciated in view of a considerable number of patients infected with this taxon in Central-Eastern Europe (Schneider et al., 2010) as well as other parts of the world. A concerted survey is currently underway to study the epidemiology of cystic echinococcosis (CE) in Eastern and Southern Africa, where the diversity of *Echinococcus* is arguably larger than in any other region of the world. Preliminary results show that both domestic and silvatic parasite cycles occur and are interlinked in Africa (Hüttner et al., 2009). The situation is complex and can differ fundamentally even between adjacent countries (Romig et al., 2011). Cattle appear to be dead-end hosts for the parasite in many regions. Sheep-dog-cycles are the main source for human CE in some regions, but not in others. The presence or absence of *E. granulosus* G1 seems e.g., to provide at least a partial explanation for the curiously focal distribution of high-prevalence human CE on the African continent. The information gained by our understanding of the biological traits of these species has also practical consequences for control, as different host species will have to be targeted in different regions, and existing livestock vaccines against *E. granulosus* G1 may not work against CE caused by other taxa (Chow et al., 2008). In addition to such interspecific differences between CE agents, the study of microdiversity (e.g., within *E. granulosus* sensu stricto, G1-3) is expected to lead to a better understanding of natural and anthropogenic parasite dispersal and introductions (Casulli et al., 2012). *E. multilocularis*, the causative agent of alveolar echinococcosis (AE), is known to be genetically rather homogeneous on a world-wide cale. Still, a certain level of diversity was demonstrated using microsatellite profiles and mitochondrial gene sequences not only between continents, but also within regions of Europe and even on local sites (Knapp et al., 2009). The unequal distribution of mt haplotypes and ms profiles has led to hypotheses on the dispersal history of *E. multilocularis* in Europe. Regions with higher diversity (e.g., southern Germany and northern Switzerland) may constitute ancient refugia, whereas low genetic diversity (including founder effects) e.g., in the Northeast of Central Europe indicates a more recent establishment. As humans seems to exhibit a certain resistance against infection wih *E. multilocularis* (or the development of AE), the question arises whether certain variants are more frequent in humans than in animals within a certain region. The almost complete absence of human AE in central North America, despite frequent animal infections, have been ascribed to hypothetically non-infective (or non-pathogenic) genotypes in that region, which may or may not be verifiable in future (Davidson et al., 2012). The recent appearance of *E. multilocularis* in non-endemic regions (e.g., Scandinavia) has led to a debate over introduction routes with domestic or wild animals, and the genetic identity of the introduced isolates will certainly help to recognize routes and risk factors (Davidson et al., 2012). In the case of the recently recorded presence of *E. multilocularis* in northern Italy, unique ms profiles of the isolates suggest an ancient unrecognízed presence of this parasite rather that introductions from neighbouring endemic regions (Casulli et al., 2009). In case of the

parasite's emergence on the arctic islands of Svalbard, the origin of the parasite could be shortlisted to some arctic region rather than Central or Eastern Europe due to its genetic similarity to isolates from St. Lawrence Island in the Bering Strait (Knapp et al., 2012). Despite these examples, the data gathered on any *Echinococcus* species so far still amount to rather few pieces of a large puzzle, and further concerted, interdisciplinary efforts are necessary to provide a comprehensive understanding of the epidemiological characteristics of the various species and genotypes of *Echinococcus* spp. Such an understanding, however, will be necessary for the successful implementation of prevention and control.

References

Casulli A., Bart J.M., Knapp J., La Rosa G., Duscher G., Gottstein B., Di Cerbo A., Manfredi M.T., Genchi C., Piarroux R., Pozio E. 2009. Multi-locus microsatellite analysis supports the hypothesis of an autochthonous focus of *Echinococcus multilocularis* in northern Italy. Int. J. Parasitol. 39:837-842.

Casulli A., Interisano M., Sreter T., Chitimia L., Kirkova Z., La Rosa G., Pozio E. 2012. Genetic variability of *Echinococcus granulosus* sensu stricto in Europe inferred by mitochondrial DNA sequences. Infect. Genet. Evol. 12:377-383.

Chow C., Gauci C.G., Vural G., Jenkins D.J., Heath D.D., Rosenzvit M.C., Harandi M.F., Lightowlers M.W. 2008. *Echinococcus granulosus*: variability of the host-protective EG95 vaccine antigen in G6 and G7 genotypic variants. Exp. Parasitol. 119:499-505.

Davidson R.K., Romig T., Jenkins E., Tryland M., Robertson L.J. 2012. The impact of globalisation on the distribution of *Echinococcus multilocularis*. Trends Parasitol. 28:239-247.

Hüttner M., Siefert L., Mackenstedt U., Romig T. 2009. A survey of *Echinococcus* species in wild carnivores and livestock in East Africa. Int. J. Parasitol. 39:1269-1276.

Knapp J., Bart J.M., Giraudoux P., Glowatzki M.L., Breyer I., Raoul F., Deplazes P., Duscher G., Martinek K., Dubinsky P., Guislain M.H., Cliquet F., Romig T., Malczewski A., Gottstein B., Piarroux R. 2009. Genetic diversity of the cestode *Echinococcus multilocularis* in red foxes at a continental scale in Europe. PloS Negl. Trop. Dis. 3:e452.

Knapp J., Staebler S., Bart J.M., Stien A., Yoccoz N.G., Drögemüller C., Gottstein B., Deplazes P. 2012. *Echinococcus multilocularis* in Svalbard, Norway: microsatellite genotyping to investigate the origin of a highly focal contamination. Infect. Genet. Evol. 12:1270-1274.

Nakao M., McManus D.P., Schantz P.M., Craig P.S., Ito A. 2007. A molecular phylogeny of the genus *Echinococcus* inferred from complete mitochondrial genomes. Parasitology 134:713-722.

Nakao M., Yanagida T., Okamoto M., Knapp J., Nkouawa A., Sako Y., Ito A. 2010. State-of-the-art *Echinococcus* and *Taenia*: phylogenetic taxonomy of human-pathogenic tapeworms and its application to molecular diagnosis. Infect. Genet. Evol. 10:444-452.

Romig T., Omer R.A., Zeyhle E., Hüttner M., Dinkel A., Siefert L., Elmahdi I.E. Magambo J., Ocaido M., Menezes C.N., Ahmed M.E., Mbae C., Grobusch M.P., Kern P. 2011. Echinococcosis in sub-Saharan Africa: emerging complexity. Vet. Parasitol. 181:43-47.

Schneider R., Gollackner B., Schindl M., Tucek G., Auer H. 2010. *Echinococcus canadensis* G7 (pig strain): an underestimated cause of cystic echinococcosis in Austria. Am. J. Trop. Med. Hyg. 82:871-874.

HYDATIDOSIS IN EUROPE: PRESENT SITUATION AND FUTURE PERSPECTIVES

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Cystic echinococcosis (CE) remains an important health problem in many regions of the world, both where no control measures have been implemented, and where control programs have been incompletely successful with ensuing re-emergence of the disease.
Official data on CE in Europe collected by the European Food Safety Authority show a general increase in the proportion of intermediate hosts with CE during the last few years, and autochthonous pediatric patients have been reported in some areas, a sign of active local transmission of disease. A similar picture emerges from data collected by different CE specialists in several European countries. In this respect, data are conflicting because prevalences found in surveys and reported to specialized journals are higher than those reported to the European Food Safety Authority by the same country. For some areas, official data are lacking, due to the fact that CE is not a notifiable disease.
Published data suggest that prevalence is rather high in several European regions, and the overall economic losses due to human and animal CE calculated for some European areas is very high.
Due to the data suggesting that CE is an increasing public health and socio-economic concern in Europe, improved disease surveillance and greater cooperation between agencies should be fostered. The introduction of national registries for CE with online data entry, following the example set by the European Registry for Alveolar Echinococcosis, would help streamline data collection on CE, and could be the base to provide much needed clinical and epidemiological data.

ECHINOCOCCOSIS- DIFFERENT CLINICAL PATHWAYS FOR TWO DISEASES

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The term “Echinococcosis” is used for two diseases which are caused by different species of *Echinococcus*; most importantly, these two diseases differ widely in the human host by presentation, and clinical management. In alveolar echinococcosis (AE) – caused by *Echinococcus multilocularis* – a multi-vesiculated tumor is formed mainly in the liver; whereas in cystic echinococcosis (CE) – caused by *E. granulosus* sensu strictu – a circumscribed, fluid-filled cyst(s) (also named as hydatid) develop(s) predominantly in the liver and/or in the lung. The growth of the two metacestodes clearly separates the “malignant” AE from the “benign” CE. Recently, experts have updated the present knowledge for diagnosis and treatment of AE and CE, and these recommendations are aimed to offer guidance to clinicians in regions where these diseases occur less frequently (Brunetti et al., 2010). A multi-disciplinary approach is the fundamental basis to deal with the diseases, and is displayed for diagnosis, treatment and follow-up.

CE, also known as hydatid disease, is an important public health issue in many countries around the world, and is targeted by World Health Organization (WHO) as one of the important Neglected Zoonotic Disease (WHO, 2012). Unfortunately, few clinicians are familiar with the disease. The incubation period is ill-defined, since exposure is not well memorized. Signs and symptoms are often reported within months to years, and depend on the localization of the cyst(s). Beside liver and lung other organs are potentially affected. The larva forms a single or several, fluid-filled cyst(s) – since ancient times named as hydatid – which are always surrounded by a well-organized, compact capsule of host origin. Larval growth occurs inside the cyst (endogenous budding). This strong enclosure of the parasitic endocyst by the host might explain the still unsatisfactory confirmation of CE by serological tools. The WHO-IWGE classification of hepatic cysts (WHO-Informal Working Group on Echinococcosis) by ultrasound imaging has become a benchmark, and its application helps to simplify the clinical pathway and the management of a case (WHO, 2003). Experiences with the interventional procedures, such as percutaneous puncture methods, are encouraging, and have shown to be safe for hepatic cysts (Neumayr et al., 2011). Surgery is not anymore the treatment of choice. Instead, many clinicians treat with benzimidazoles, which kill the larva, leads to a degeneration of the endocyst, and subsequently to a regressive course of CE. Others favor the “Watch and Wait” concept, and carefully observe the natural degeneration of the cyst(s) (Junghanss et al., 2008). However, diagnosis of regressive, hepatic CE is still a challenge; serology is not well suited to identify neither the remnants nor the viability of the parasite, and most importantly, imaging findings result in unnecessary surgery, and expose patients to high risk procedures. This occurs particularly in regions where both CE and AE are being diagnosed concomitantly. Individual treatment approaches need to be applied for rare manifestations of extra-hepatic or extra-pulmonary CE (Brunetti et al., 2010).

In contrast to CE, alveolar echinococcosis (AE) is a rare disease, and restricted to some regions in the northern hemisphere from Central Europe extending to the East and South, Turkey to Western China/Japan/Alaska. The disorder is insidious, and becomes apparent after a variable asymptomatic incubation period of > 10 years. The precise diagnosis remains a true challenge for clinicians and radiologists. Insufficient knowledge leads to misinterpretation of findings, and to unnecessary diagnostic measures, and inappropriate treatment attempts. Serology is helpful, but again, clinicians and/or microbiologists stumble on the cross-reactivity of antibodies. By exogenous budding the larva infiltrates the liver, and metastases may be formed in organs adjacent to or in distant localizations. The PNM-classification (Primary-tumor-Nodes-Metastases-system (WHO-IWGE) has become an international benchmark (Kern et al., 2006). Due to the potential “malignant” features of AE continuous larval suppression with benzimidazoles remains the backbone of a life-long treatment of AE. If the lesion is confined to the liver, radical surgery (R0 = resection and demonstration of 2 cm margin without larval infiltration as judged by histopathology) offers cure. Unfortunately, few patients are diagnosed when curative surgery is feasible, most patients present with an advanced stage of disease. Continuous suppressive treatment with benzimidazoles leads to clinical stabilization and a life-expectancy comparable to the regular population of the particular area (Torgerson et al., 2008;

Piarroux et al., 2011). Unfortunately, many patients undergo palliative surgery (R1 or even R2) without or with incomplete coverage of benzimidazoles, and thus, suffer from relapses. Some patients benefit from liver transplantation. Immune deficiency seems to have a strong negative impact according to anecdotal observations; molecular mechanisms are still undiscovered leading to progressive disease.

References

Brunetti E., Kern P., Vuitton D.A. 2010. Expert consensus for diagnosis and treatment of cystic and alveolar echinococcosis in humans. Acta Tropica 114:1-16.

Junghanss T., da Silva A.M., HORTON J. 2008. Clinical management of cystic echinococcosis: State of the art, problems, and perspective. Am. J. Trop. Med. Hyg. 79:301-11.

Kern P, Wen H., Sato N., Vuitton D.A. 2006. WHO Classification of alveolar echinococcosis: Priniciples and application. Parasitol. Int. 55:283-S287.

Neumayr A., Troia G., de Bernardis C. 2011. Justified concern or exaggerated fear: The risk of anaphylaxis in percutaneous treatment of cystic echinococcosis – a systematic literature review. PloS NTD. 5:e1154.

Piarroux M., Renaud P., Roch G., Jenny K. 2011. Clinical features and evolution of alveolar echinococcosis in France from 1982- 2007: Results of a survey of 387 patients. J. Hepatol. 55:1025-1033.

Torgerson P., Schweiger A., Deplazes A. 2008. Alveolar echinococcosis. From a deadly disease to a well-controlled disease. Relative survival and economic analyses in Switzerland over the past 35 years. J. Hepatol. 49:72-79.

WHO Informal Working Group. 2003. International classification of ultrasound images in cystic echinococcosis for application in clinical and field epidemiological settings. Acta Tropica 85: 273-285.

WHO. 2012: Diseases covered by NTD Department. As accessed in May 2012 from http://www.who.int/neglected_diseases/diseases/en/.

CYSTIC ECHINOCOCCOSIS: CHRONIC, COMPLEX AND STILL NEGLECTED

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Cystic echinococcosis is a chronic, complex, and neglected disease.
It is chronic because none of the three available treatment options – surgery, percutaneous treatment, and benzimidazoles – can completely extirpate the cyst, with the exception of radical surgery in selected cases.
It is complex as its clinical presentation may range from none in the case of asymptomatic carriers to severe, even life-threatening complications such as embolism, anaphylactic shock and paralysis.
Diagnosis may be difficult, as it based mainly on imaging techniques – with ultrasound being the most widely used – along with serology. CE is one the few parasitic diseases where the laboratory diagnosis is based on serology instead of direct demonstration of parasites. The latter is only possible by sampling the cyst fluid, which is possible during surgery or percutaneous treatment, but not as a routine test.
Unfortunately, serological tests are not standardized and their clinical performance depends on cyst location, stage, size, and complications. Unfortunately, the available studies do not take the effects of these variables into account.
Medical decision making depends on the same variables and often on the availability of treatment and medical expertise. Furthermore, recommendations for diagnosis and treatment have not progressed beyond expert opinion and are not necessarily adopted by clinicians due to a lack of grade I evidence.
Against this backdrop, clinical research on cystic echinococcosis receives very little funding compared to other diseases that have a similar burden measured in DALYs and this contributes to maintaining the vicious circle of neglect.
The presentation will detail and discuss these problems and will suggest a possible way forward.

INSIDE OF CLINIUCAL ECHINOCOCCOSIS: ROMANIAN EXPERIENCE

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Echinococcosis/hydatidosis is one of the major local parasitic disease, being spread all over the country. The real statistical parameters are difficult to estimate, as a big number of cases remain unreported. The classical locations of CE are liver, in adults and lungs in children, but the uncommon locations (thyroid, spleen, CNS, muscles, bones, etc.) are still present, actually representing the tricky diagnosis. The clinical manifestations are closely related to the number, size, location, or, the viability of the cyst, correlated with the host immune response. The course of the diseases can be salient, progressive, or, by contrary, very severe, associated with complications which can put in danger the patient's life. The diagnosis combine the imaging and serological methods. Surgery will confirm in some situations the clinical suspicion. The present therapeutic attitude are represented by antiparasitic medication alone, or associated to surgery, evacuation of the cyst under US guidance, or, in the case of inactive cysts, the "watch and wait" attitude. Early diagnosis and appropriate treatment, according to the patients' desire and WHO guidelines, are crucial for the good prognosis and the cure of the disease. The appropriate therapeutic option should be decided in accordance with the characteristics of the cysts and patients as well. The relapses are common when the medical treatment associated is absent or delayed.
The necessities of improving the notifications of the cases, the surveillance end control system, the correct detection and monitoring of the cases, represent the most important steps in respect of estimation the burden of the disease and its complications.

HUMAN ECHINOCOCCOSIS IN NORTH-WEST ROMANIA: CLINIC, EPIDEMIOLOGY AND DIAGNOSIS

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Hydatidosis or cystic echinococcosis in humans represents a major problem of public health, responsible of hospitalizations and surgical interventions, each year. Latest years studies reveal that the prevalence of echinococcosis has increased in many countries. Human echinococcosis is still a serious problem for the public health in Romania, despite the measures taken for its prophylaxis. The surgery is no more a first choice treatment for it. The modern management of cystic echinococcosis needs the contribution of all the diagnosis procedures: the medical procedures, the parasitological and the surgical ones. Studies objectives are to present the clinic, the epidemiologic and the diagnosis aspects of human echinococcosis in Romania: there were assessed 60 patients hospitalized during 2004-2006. Ultrasonography, radiologic and serologic examinations had been made: echography (78.3%), echography and clinic exams (21.7%), computerised tomography – CT (1.7%), CT and echography (3.3%), established the diagnosis, confirmed during surgery. Detection of the serum antibodies anti *Echinococcus granulosus* IgG class by the ELISA method was used for the etiologic confirmation of the pseudotumoral forms that were ultrasound suggestive for the hydatid cyst in 42 patients. We underline that most of the hydatid cysts were found by ultrasound (95%) and in a small percentage of the patients (5%) it was necessary to make also a CT in order to be able to confirm the ultrasound suspicion.

Concerning the epidemiologic and the clinic aspects of the human echinococcosis, the results obtained demonstrated that the hydatid cyst had been emphasized in adults (46.5%) and in children (53.5%), with an alarming high incidence in children between 7-14 years of age (70.1% being diagnosed in children). In adults, it prevailed in young adults (47%), having a medium incidence (34%) in those between 40-60 years and a low incidence in old people (6.5%). Hydatid cyst was more common in females (53%) than in males (47%). Although the total incidence of the hydatid cyst in rural communities was only slightly increased (57.9%) comparative to the urban ones (42.1%), in some counties the incidence in the villages was significantly higher.

In adults, the most common locations of the hydatid cyst were the liver (83.9%), the lungs (9.7%), the spleen (1.1%) and the retroperitoneal space. In children, the most common locations were the liver (60.7%), the lungs (20.6%), the liver and the lungs (14%), the spleen (1.9%), the kidneys (0.9%). Most of the cases (71.5%) were uncomplicated (75.4% in children and 64.1% in adults), and only 28.5% were diagnosed due to the complications produced.

The evolution of uncomplicated hepatic hydatidosis was like a biliary dyspeptic syndrome (64.3%), and with portal hypertension (2.1%). Toxic and allergic reactions occurred, especially in children (6.2%). The complications of the hepatic hydatidosis were cholangitis (8.4%), jaundice (4.9%), the fissure (4.9%) or the rupture of the hydatid cyst (18.8%). The rupture occurred in the biliary ducts (9%) with concomitant hydatid lithiasis (2.8%); in pleura, with the formation of a pneumopericyst (6.4%), of one abscess in the abdominal wall (1.5%); in the peritoneal cavity (1.4%) with the occurrence of the anaphylactic shock (2.5% in adults). In adults the cirrhotic signs (3.8%) have been noted.

Pulmonary hydatidosis ordinarily are uncomplicated (77%) and give rise to cough (63.6%), shortness of breath (54.5%) and chest pain (36.4%). The complications of the pulmonary hydatidosis are the fissure of the hydatid cyst and the occurrence of an infectious syndrome with fever (36.4%), anorexia (18.2%), and asthenia (9.1%). No severe complications like anaphylactic reactions, due to cyst rupture, had been mentioned.

Either surgery or chemotherapy determined a favourable evolution (90.5%). The unfavourable postsurgical evolution (9.5%) was due to relapses (4.5%), secondary hydatidosis (1%) and postsurgical infections (3.5%).

These data revealed that hydatidosis is responsible for 200 hospitalizations, representing 0.6% of the surgical interventions per year. Hepatic (71.5%) and pulmonary (15.5%) locations are frequent,

leading by their chronic evolution to severe complications (1.5%), reserved prognosis (9.5%), especially in adults (14%) and even to death (0.5%).
Concerning the diagnostic methods, the results prove that ultrasonography represents an important method in detecting the hydatid cyst. The serologic diagnosis allows the detection of the hydatid cyst in early stages, non-detectable by imaging techniques, fact that represents a premise of early hydatid cyst diagnosis. The diagnosis should be interpreted in correlation with the clinical evolution and the imaging methods. This early diagnosis makes possible the introduction of chemotherapy and it can avoid intra/post surgery complications.
Our studies reveal the necessity of the active surveillance of hydatidosis, disease with important social and economic aspects, also called the “white cancer”. In consequence we must emphasize the need of a national screening program for the control of this infection in humans and in animals.

ECHINOCOCCUS MULTILOCULARIS IN EASTERN EUROPE GENERALLY WITH SPECIAL REFERENCE TO ROMANIA

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The presence of the parasite is reported more frequently in Eastern Europe. In most countries alveolar echinococcosis has already an endemic character and is still expanding with alarming rapidity. In Europe in 1980 it was known in only four countries (Austria, France, Germany and Switzerland) where it was described the *E. multilocularis*. In 1996 the number of these countries has reached 9. The presence of the parasite is reported more frequently in Eastern Europe (Slovakia, Hungary, Ukraine, Bulgaria), Turkey, Asia (Russia, Kazakhstan and China), Amedia North (Dakota) and Japan. In most countries alveolar echinococcosis has already an endemic character and is still expanding with alarming rapidity. If in 1982 the extension was limited to a small part of France and Central Europe, in 2003 it is expanding throughout Central and Eastern Europe. So for example if in Poland in 1999 the prevalence of *E. multilocularis* in examined foxes was 2.6%, in 2003 it reached 29.4%. Also, in 1999 in Slovakia the first case of *E. multilocularis* in fox was described, and then in 2000 the prevalence in the examined foxes reached 24.8% and 33.9% in 2001. While in Hungary the first case of alveolar echinococcosis in humans was described in 1988 still the parasite responsible was not identified in the country until 2003, when parasite prevalence was already set to be 29% in examined foxes. Foxes came from border territories of the Slovak Republic. Moreover, the Eastern European reports on *E. multilocularis* are abundant in these countries (Table 1) (Sikó Barabási et al., 2010a; 2010b; Sikó Barabási, 2011).

Table 1. The documented *E. multilocularis* or the alveolar echinococcosis presence in east European countries (Sikó Barabási, 2011)

Country	Year	Species
Poland	2001-2004	Fox
Slovenia	2006-2007	Man
Hungary	1988	Man
	2003	Fox
Bulgaria	1951	Man
	1951	Rodents
Greece	1998	Man
Turkey	1934	Man
	1939	Man
	1965	Fox
Moldavia	1961	House mouse
Ukraine	1957, 2006, 2008	Fox
Armenia	1958	Man
Byelorussia	1957, 1958	Rodents
	2001, 2003	Fox
Lithuania	2003	Muskrat
	2003	Dog, pig
	2007	Fox and man
Latvia	2008	Fox
Estonia	2005	Fox
West part of Russia	1957	Fox
	1966	Wild cat
	1970, 1972, 1998	Fox and man

In Romania, the first cases of alveolar echinococcosis were described in 1991 in cattle (0.01%) and in wild rodents – *Microtus Chionomis nivalis ulpius Brehm* (0.4%). Then, in 1998 was a case of hepatic alveolar echinococcosis in a sheep, and in 1999 a case of hepatic localized alveolar echinococcosis in humans was described in 1999. An aberrant intermediate host has been reported in the liver of a horse (2004). However the species diagnosis of these these findings were not confirmed on a species level (Sikó Barabási, 2011).

The investigations about adult stage of *E.multilocularis* in Romania in dogs and foxes between 2002-2007, don't confirm the presence of parasite.

Starting from these reasons between August 2007-March 2010 were examined a number of 561 samples of European red fox (*Vulpes vulpes*) small intestine. The samples came from North-West and Central region of Romania.

Echinococcus multilocularis was isolated from 27 samples, showing a 4.8% prevalence, and confidence interval (CI 95%) [3.0; 6.5]. The prevalence of infection was higher in female foxes (59%) than in male foxes (41%). Four year old foxes were the most infected (52%). The intensivity of infestation was 100 cestodes per sample in 70%, and in 8 cases (30%) there number was between 114 and 312. Teysseyre (2005) states that average infestation in the European red foxes with *E. multilocularis* varies between 50 and 2000 specimen/sample. During the examination of the 561 samples 2012 specimen of *E. multilocularis* were isolated. This represents the *E. multilocularis* biomass of the 27 positive samples. 1482 were preserved in formalin and 530 specimens in ethanol 70% for further examenes and analyses, including PCR.

Same results are described by Knapp et al. (2008), who finds a prevalence of 53% after examining 571 red fox bodies from Central Europe. Concerning the intensity of infestation, 91% from the samples had till 10000 *E. multilocularis*/sample, and in 9% of the bodies the number of the cestode exceded 10000/examined sample. Also high values of infestation 45.7% reported Reperant (2005) after the examination of 267 red foxes in Switzerland. The value of infestation varies from 1 to 120020 specimen per examined. In 88% of the samples they have found less than 100 *E. multilocularis*, in 9.7% of the samples between 1000 and 55000 specimens, and in 1.9% had over 55000 specimens per examined sample. This last category gave 76% of total biomass of *E. multilocularis*.

Regarding the parasitic dispersal on the studied area, has to be noted that from the 15 counties, the parasite was found 8 (53.3%), respectively in 26 locations. The most infected samples were from Satu Mare – (31%), Maramureş – (23%) and Bihor counties (23%). Exact location of the origin of positive samples were made using GPS. Analyzing environmental conditions from infected areas, it was found that elevation of these areas ranged between 116 m (Căuaş, Satu Mare County) and 586 m (Aleşd, Bihor County). In infected areas average annual temperature was between 9-11 °C, and average annual rainfall was 700-900 mm.

Morphology analysis of *E. multilocularis* samples showed that individuals had 4-5 small segments, with the opening of genital pore in the first third of the mature proglotid. The gravid uterus was sac-like. Most of the *E. multilocularis* specimens were gravid. The eggs from the gravid proglotids ranged in size 30-50 x 44 µm. The average number of eggs in a gravid proglotid was 160-210, which is consistent with the data of Teysseyre (2005) who is setting this number around 200. In a few mature specimens the hooks of *E. multilocularis* were examined, which were situated on the rostrum or detached from this. The size of these hooks was: length of large hooks 30.3-30.4 µm; length of anteriorthird of large hooks 16.3 µm; length of posterior third of large hooks 17.4 µm; length of small hooks 24.5-25.8 µm; length of anterior third of small hooks 8.6-10.5 µm; length of posterior third of small hooks 18.2-19.6 µm. All dimensions are within the ranges given in OIE (2008).

To confirm the taxonomic status of the morphologically identified cestode like *E. multilocularis*, the Multiplex PCR method was used. For the confirmation of the *E. multilocularis* cestode, primers were used which were corresponding the 12S-ARN mitochondrial gene, with the amplicon size 395 pb, for *E. granulosus* primers with amplicon size 117 pb, and for *Taenia* spp. primers with amplicon size 267 pb.

After working protocol compliance and sequencing: PCR mix preparation, amplification and electrophoresis performed in an integrated PCR equipment, by migration in agarose gel, an amplicon size 395 pb was obtained in all examinated samples, confirming the taxonomic affiliation of isolate *E. multilocularis* cestoda species.

Investigations carried out in Hungary revealed the existence of four genotypes, suggesting that this country may be the boundary to the east of a European outbreak. However, given that the counties

bordering with Hungary presents a significant infection with *E. multilocularis* of the fox populations the question is whether this boundary is not larger, including a part of Romania. To elucidate this aspect is required to accurately determine the genotypes of *E. multilocularis* in Romania.
Alveolar hydatidosis produced by the larval form of the *E. multilocularis* in wild rodents as main intermediate hosts, was described in France, Switzerland and Austria with a high incidence. Ecoregions of central and north-west region of Romania inhabited by these rodents are identical in terms of weather and pedoclimatic conditions to those in Western Europe.
Following morphological examinations performed immediately after the capture of a total number of 552 rodents macroscopic changes were detected in the liver of 17 (3.1%) animals. In 13 (prevalence: 3.6%) sample alveolar echinococcosis was confirmed. The macroscopically glomerular formations were found just below the liver surface under the Glisson's capsule, like a conglomerate of cysts, similar to a cauliflower, with well highlighted area, dirty yellowishwhite color; with vesicle diameter ranging from 1-6 mm. Extrahepatic disease was very rare (1%). The most frequent morphological profile of alveolar echinococcosis was represented by an intrahepatic heterogeneous, infiltrative and destructive mass, with irregular outlines and a non-vascular and necrotic center. The cyst appears as a spongy structure, resembling a crumb or cheese, with empty cavities or with a grey and jelly content. Most of the cavities do not have a well-defined content. In other samples, the formation had mainly a cystic structure, without a divided multivesicular character. The gross lesions consisted of unilocular cysts (in the case of young cysts), circular or irregular oval forms. The small cysts had dimensions between 100-200 x 250-350 µm. The biggest cysts (1200-1500 µm) usually contained a calcareous deposit, or a jelly substance, moreover an unidentifiable content. The greatest number of them had a complex structure divided by the cuticula in a multivesicular, cavernous or spongy formation.
Histologically the larval cestodes of *E. multilocularis* from the liver of voles produce a typical infiltration and induce destruction of the organ. The intermediate hosts respond with a granulomatous inflammatory reaction and peripheral formation of granulation tissue. In centripetal direction, the adventitial layer's components were formed by the fibroconjunctive reaction; the inflammatory cells were present. In the peripheral areas the lymphocytes and monocytes were dominating. They were followed by a rich infiltration of the giant cells and a decrease of the inflammatory local reaction. These structures were gradually changed by the fibroconjunctive reaction. The cuticular layer was mono- or multistratified, formed by concentric conjunctival tissue layers, which penetrated in the cystic cavity and developed many anastomoses conferring it a multi-vesicular aspect. In the spaces between these blade cells similar to giant cells we could also notice rare histiocytes and eosinophils. Due to the multitude of anastomoses, the cyst is a structure with an invasive character. In most cases there were collapsed cysts characterized by a fragmented or folded laminar layer and massive necrosis. The germinative layer which lines the new formed cavities has a plasmatic structure with rarely multinucleate cells. This layer had many young cells and gave a rhizoid aspect characteristic for the malignant tissues. Some cysts contained remnants of a single germinative layer.
The found cystic structure, their size and location look alike the wild rodents cysticercosis produced by *Taenia taeniaeformis*. The differential diagnosis is based on the gross and histological characteristics. The final results of natural infected wild rodents (n=552) respectively the confirmation of the *E. multilocularis* metacestode presence show the 2.4% (n=13) prevalence and the 53.8% fertility of the cysts.
The confirmed *E. multilocularis* metacestode samples were originating from the same geographic area where the adult stage of the parasite was found in foxes.

References

Knapp J., Guislain M.H., Bart J.M., Raoul F., Gottstein B., Giraudoux P., Piarroux R. 2008. Genetic diversity of *Echinococcus multilocularis* on local scale. Infect. Genet. Evol. 8:367-373.

OIE. 2008. Manual of diagnostic tests and vaccines for terrestrial animals (mammals, birds and bees), Sixth Edition. Volume 1:598.

Reperant L.A. 2005. Ecologie parasitaire *d'Echinococcus multilocularis* et autres helminthes du systeme renard roux-rongeurs en milieu urban et peri-urbain: Implications zoonotiques dans le canton de Geneve, Suisse. Ecole Nationale Veterinaire de Lyon, Thèse nr. 52 pour obtenir le grade de Docteur Vétérinaire.

Sikó Barabási S., Deplazes P., Cozma V., Pop S., Tivadar Cs., Bogolin I., Popescu R. 2010a. *Echinococcus multilocularis* confirmed in Romania. Sci. Parasitol. 11(2):89-96.

Sikó Barabási S., Deplazes P., Ceica C., Tivadar Cs., Bogolin I., Popescu S., Cozma V. 2010b. *Echinococcus multilocularis* in South-Eastern Europe (Romania). Parasitol. Res. 108(5):1093-1097.

Sikó Barabási S. 2011. Echinococcosis with *Echinococcus multilocularis* in Romania. PhD Thesis. University of Agricultural Science and Veterinary Medicine Cluj-Napoca, Romania.

Teysseyre A.J.F. 2005. Contribution à l'étude du parasitisme intestinal du renard roux (*Vulpes vulpes*) en Midi-Pirénées: Recherche *d'Echinococcus multilocularis*. These pour obtenir le grade de Docteur Vétérinaire. École Nationale Veterinaire Toulouse.

ECHINOCOCCUS MULTILOCULARIS AND ALVEOLAR ECHINOCOCCOSIS: OUTLOOKS AND RESEARCH NEEDS

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It is well known that in the last two decades the information available on the fox tapeworm, *Echinococcus multilocularis,* as well as on the disease, alveolar echinococcosis, caused by its metacestode in humans and animals underwent very substantial extension, especially in the European region.
Selected aspects of these parasitic conditions will be critically considered. 1/ It is of interest whether recent data on the rapid expansion of prevalence reflect the detection of neozotic or rather formerly unrecognized infections. 2/ The important pathological and epidemiological implications of the existence of host-adapted strains of *E. granulosus* are well known; however, the identification of possible strain differences (intraspecific diversity) in the case of *E. multilocularis* developing in different host and/or intermediate host species has not been appropriately attempted. 3/ The value of methods of mucosal scrapings, washing and sedimentation of the small intestinal content, and coproantigen detection, used for survey studies of fox tapeworm infections, will be comparatively assessed. 4/ Pros and cons will be considered concerning the development of protective immunity against secondary *E. multilocularis* infections of foxes and against the metacestode developing in humans. 5/ As a rule, the life cycle of *E. multilocularis* takes its course unobserved between foxes and small rodents in the sylvatic cycle; possible measures for controlling transmission to humans (such as deworming of foxes by medicated baits) offer little hope for wide-scale application. Suggestions will be put forward for related topic areas where future research is primarily needed.

VARIABILITY IN THE ACANTHOCEPHALA

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Unique and unusual features in the many species of acanthocephalans described and/or studied by Amin from fish, amphibians, reptiles, birds, and mammals, in various parts of the world including South America, Vietnam, Japan, the United States, Germany, the Middle East, Turkey, Iran, Thailand, Peru, Chili, Indonesia, Taiwan, and North, South and East Africa, are presented. The presentation includes highlights of about 35 publications by Amin many of which use SEM images, and is in five parts. (1) An introductory section dealing with the classification, general morphology, ecology, and life cycles of the Acanthocephala. The phylum now includes four classes and not three. Aquatic crustacean and terrestrial arthropod intermediate hosts are included and the time span spent by juvenile forms and adults in their respective hosts totaling no more than one year is shown. (2) Unusual anatomical features of taxonomic or of questionable taxonomic importance addressing variations in the proboscis, proboscis hooks, proboscis receptacle, epidermis, male and female reproductive organs, and lemnisci. Newly described structures include (a) Para-receptacle structure (PRS) in *Eoacanthocephalans* especially in the genus *Neoechinorhynchus,* and hoods in certain species from Vietnam. The use of ampulla and para-receptacle structures to enhance the aversion and retraction of the proboscis into the proboscis receptacle in worms with weak receptacle wall made up of only one layer of muscles. This function is accomplished by the additional pumping of body cavity fluids directly into the proboscis receptacle through the proboscis receptacle wall, (b) a new order of *Acanthocephala* from Vietnamese birds which combines features from two different families, Polymorphidae and Heteracanthocephalidae, and (c) the unusual presence of microtrichs in one species of acanthocephalans from skipjack tuna in the Pacific Ocean. The latter report argues for the closer relationship between acanthocephalans and tapeworms. (3) Structural and functional relationships explaining the relationship between the metamorphosis of the giant nuclei in Eoacanthocephala (from rounded forms in juveniles to elongated-lobulated forms in adults and worm reproductive cycle). This relationship has been demonstrated in at least 3 eoacanthocephalan genera and is also demonstrated using TEM. (4) Host-parasite relationships elucidating the relationships between worm anatomy and biology during worm growth which include changes in the position of the female gonopore. The relative size of proboscis, proboscis hooks, and bulb in the genus *Pomphorhynchus*, among other anatomical features, were shown to change with increase in worm length. Those changes have biological and taxonomic implications. (5) Curiosities in reviews and revisions highlighting taxonomically based zoo-geographical patterns and trends in the genera *Neoechinorhynchus*, *Polymorphus*, and *Pallisentis*.
These relationships are usually a function of host species and/or geographical location involving differential habitat utilization. A comprehensive treatment of the acanthocephalans of South America and those marine forms off the Eastern United States is also included here. The overall conclusion is that acanthocephalans are not just one homogeneous group of worm but rather a very variable heterogeneous assemblage. Many adaptations are shown using different strategies to resolve the same problems.

VECTOR SPECIES OF THE GENUS *TRIATOMA*: A MOLECULAR APPROACH

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American trypanosomiasis or Chagas disease is widespread in Latin America from Mexico to Chile and Southern Argentina. Although present estimates of 10 to 12 million people infected with the haemoflagellate protozoan species *Trypanosoma cruzi* represent 6-8 million fewer cases than those reported in the 1980s (Schmunis, 2004), it remains one of the most serious parasitic diseases of the Americas for its social and economic impact (World Bank, 1993; WHO, 2010). Over the years, sustained vector control has largely contributed to reducing Chagas disease transmission in Latin America – and saving millions of people from chronic impairments. The objective now is to interrupt transmission via intra-domiciliary vectors in Latin America and transmission via blood transfusion in Latin America, Europe and the Western Pacific by 2015 (WHO, 2012). Although it can also be transmitted by blood transfusion or across the placenta from infected mothers or through organ transplantation, most human contamination is attributed to insect vectors in poor rural or periurban areas of Central and South America (Schmunis, 2004).

Chagas disease vectors are haematophagous reduviid (Hemiptera: Heteroptera) insects belonging to the subfamily Triatominae. Species of Triatominae are usually grouped into 17 genera forming five tribes, although other arrangements have been proposed. Of these, *Alberproseniini*, *Bolboderini*, *Cavernicolini* and *Rhodniini* are considered monophyletic, whereas *Triatomini* is considered polyphyletic (Dujardin et al., 2000). Among the latter, most of the species (over 70) are included in the genus *Triatoma*, among which two main clades appear in ribosomal DNA (rDNA) sequence phylogenies, corresponding to species of North and Central America and species of South America separated prior to the closing of the isthmus of Panama about 3 million years ago (Bargues et al., 2000, Marcilla et al., 2001; Bargues et al., 2002). Moreover, *Triatoma* species are distributed in three main groupings: the Rubrofasciata group (mainly North Americanand Old World species), the Phyllosoma group (mainly Mesoamerican and Caribbean), and the Infestans group (mainly South American), each including different complexes and subcomplexes in a classification which is progressively updated according to new genetic and morphometric data (Dujardin and Schofield, 2004; Bargues et al., 2010).

Over half of the 141 currently-recognised species of Triatominae have been shown to be naturally or experimentally infected with *Trypanosoma cruzi* and all are suspected to have this capacity. However, although of great epidemiological relevance (and a primary stimulus to research on the group), this characteristic is not used in the definition of the subfamily because of the capacity of *T. cruzi* to infect a wide range of other arthropods (Schofield et al., 2009) even though other arthropods appear to have no epidemiological significance as vectors.

Among the species of greatest epidemiological significance as domestic vectors, three belong to the genus *Triatoma*: *T. infestans* and *T. brasiliensis* from South America, and *T. dimidiata*, distributed in Meso- and Central America from Mexico down to Colombia, Venezuela, Ecuador and northern Peru (Dujardin et al., 2000).

The broad usefulness of nuclear rDNA and mtDNA sequences explains why the number of triatomine studies using these neutral markers published in recent years has increased so pronouncedly (Mas-Coma and Bargues, 2009). A comparative analysis is presented on the efficiency, weight of their different characteristics, limitations and problems of each of the different DNA markers in the light of the results obtained in studies on populations, hybrids, subspecies and species of the subfamily Triatominae. A large number of sequences of ribosomal DNA (rDNA) and mitochondrial DNA (mtDNA) from numerous populations of *Triatoma* of the Phyllosoma, Rubrofasciata and Infestans groups of species have analysed with emphasis on the intraspecific variability, haplotype profiling, phylogeography and genetic polymorphism.

Phylogenetic and population genetics analyses based on sequences of the ITS-2 rDNA of *T. dimidiata* confirmed the existence of four groups, three of them proposed to be subspecies of *T. dimidiata*, named after *T. dimidiata dimidiata* for Central American populations, *T. d. capitata* for Colombian populations, and *T. d. maculipennis* for Mexican populations; and a fourth group belong to a cryptic species (*Triatoma sp. aff. dimidiata*) confined to the Yucatan peninsula and specific locations in

Chiapas, Guatemala and Honduras. This distinction agrees with results from multidisciplinary studies using RAPD-PCR, genital structures, morphometrics, antennal phenotypes, cuticular hydrocarbons and chromosomes (Bargues et al., 2008) and has been confirmed by ND1 analyses and the absence of introgressed sequences in overlapping zones (Mas-Coma and Bargues, 2009). Central American populations constitute a group that its main characteristic is the capacity to occupy sylvatic, peridomestic, and domestic habitats and, therefore, it represents a serious challenge to the control programs as the extra-domiciliary populations act as reservoirs to repopulate the treated houses (Bargues et al., 2008; Dorn et al., 2009). Combined rDNA and mtDNA haplotype profiling was described for *T. dimidiata* populations from Costa Rica and other Mesoamerican countries (Blandon-Naranjo et al., 2010). Regarding the vectorial control in this country it must be highlighted that Costa Rican populations are identified as the subspecies *T. dimidiata dimidiata*, which is the most common variant in Mesoamerica and the origin of the group of species and subspecies known as *T. dimidiata sensu lato* (Bargues et al., 2008). This subspecies is characterized by its capacity of occupying all kind of habitats, which permits the re-infestation of the houses by peridomestic and wild insect populations shortly after the fumigation, compromising the effectiveness of control campaigns. In Costa Rica, a high degree of recolonization by wild adults has been reported (Zeledón and Rojas 2006), and chromosomal variation on *T. dimidiata* has shown the existence of gene flow among domestic, peridomestic, and sylvatic populations in Central America (Panzera et al., 2006). Although the relationship between the habitat and the haplotype profiling is less clear, there are different patterns of haplotype distribution in each geographic area between the two habitat-related ecotopes studied (domestic/peridomestic and sylvatic), some of them reflected in the phylogenetic relationships analyzed. Consistently, there is evidence of genetic flow among domestic, peridomestic, and sylvatic populations in the country (Blandón-Naranjo et al., 2010). The intraspecific variability detected may underlie the known plasticity of *T. dimidiata*, an important vector for Chagas disease transmission, suggesting that this species must be continuously monitored and alternatives to the fumigation with insecticides, as environmental management, must be taken into account to improve the control (Zeledón et al., 2008).

The *infestans-platensis-delpontei* question has been addressed again by considering genetic distances. They do not show significant mtDNA divergence, but the genetic differences at the nuclear level (Bargues et al., 2006) were among the arguments to decide about their species status, although in this case the ecological specialization also provided a very strong argument.

The use of a standardized composite haplotype code nomenclature for both nuclear rDNA and mtDNA markers is strongly encouraged to avoid difficulties in comparative studies. Triatomine aspects related to concerted evolution, microsatellites, minisatellites and insertions/deletions in nuclear rDNA and silent/non-silent mutations, pseudogenes and weaknesses of partial sequences in mtDNA are analysed. Concerning the contributions of nuclear rDNA and mtDNA to the systematic concept of species in *Triatominae*, their respective, above-analysed, different characteristics indicate that nuclear DNA shall have more weight and be systematically prioritary and with mtDNA as complementary, independently of the better usefulness of mtDNA for other purposes. This work offer a baseline for future fundamental research on triatomines and applied research on transmission, epidemiology and control measures related to Chagas disease vectors.

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References

Bargues M.D., Marcilla A., Ramsey J., Dujardin J.P., Schofield C.J., Mas-Coma S. 2000. Nuclear rDNA-based molecular clock of the evolution of Triatominae (Hemiptera: Reduviidae), vectors of Chagas disease. Mem. Inst. Oswaldo Cruz 95:567-573.

Bargues M.D., Marcilla A., Dujardin J.P., Mas-Coma S. 2002. Triatominae vectors of Chagas disease: a molecular perspective based on nuclear ribosomal DNA markers. Trans. Roy. Soc. Trop. Med. Hyg. 96:159-164.

Bargues M.D., Klisiowicz D.R., Panzera F., Noireau F., Marcilla A., Perez R., Rojas M.G., O'Connor J..E, Gonzalez-Candelas F., Galvao C., Jurberg J., Carcavallo R.U., Dujardin J.P., Mas-Coma S.

2006. Origin and phylogeography of the Chagas disease main vector *Triatoma* infestans based on nuclear rDNA sequences and genome size. Infect. Genet. Evol. 6(1):46-62.

Bargues M.D., Klisiowicz D.R., Gonzalez-Candelas F., Ramsey J.M., Monroy C, Ponce C., Salazar-Schettino P.M., Panzera F., Abad-Franch F., Sousa O.E., Schofield C.J., Dujardin J.P., Guhl F., Mas-Coma S. 2008. Phylogeography and genetic variation of *Triatoma dimidiata*, the main Chagas disease vector in Central America, and its position within the genus *Triatoma*. PloS Negl. Trop. Dis. 2(5):e233.

Bargues M.D., Schofield C.J., Dujardin J.P. 2010. Classification and phylogeny of Triatominae. Chapter 6. *In:* Telleria J., Tibayrenc M. (Eds.), American Trypanosomiasis: Chagas disease. Elsevier Inc., MA, pp. 115-149.

Blandón-Naranjo M., Zuriaga M.A., Azofeifa G., Zeledón R., Bargues M.D. 2010. Molecular evidence of intraspecific variability in different habitat-related populations of *Triatoma dimidiata* (Hemiptera: Reduviidae) from Costa Rica. Parasitol. Res. 106:895-905.

Dorn P.L., Calderón C., Melgar S., Moguel B., Solorzano E., Dumonteil E., Rodas A., de la Rua N., Garnica R., Monroy C. 2009. Two distinct *Triatoma dimidiata* (Latreille, 1811) taxa are found in sympatry in Guatemala and Mexico. PloS Negl. Trop. Dis. 3:e393.

Dujardin J.P., Schofield J.C. 2004. Triatominae: systematics, morphology and population biology. In: Maudlin I., Holmes P.H., Miles M.A. (Ed.), The Trypanosomiases. CAB International, Wallingford, pp. 181-201.

Dujardin J.P., Schofield J.C., Panzera F. 2000. Les Vecteurs de la Maladie de Chagas. Recherches taxonomiques, biologiques et génétiques. Académie Royale des Sciences d'Outre Mer, Classe des Sciences Naturelles et Médicales, Brussels.

Marcilla A., Bargues M.D., Ramsey J., Magallon-Gastelum E., Salazar-Schettino P.M., Abad-Franch F., Dujardin J.P., Schofield C.J., Mas-Coma S. 2001. The ITS-2 of the nuclear rDNA as a molecular marker for populations, species and phylogenetic relationships in Triatominae (Hemiptera: Reduviidae), vectors of Chagas disease. Mol. Phylogen. Evol. 18:136-142.

Mas-Coma S., Bargues M.D. 2009. Populations, hybrids and the systematic concepts of species and subspecies in Chagas disease triatomine vectors inferred from nuclear ribosomal and mitochondrial DNA. Acta Trop. 110:112-136.

Panzera F., Ferrandis I., Ramsey J., Ordoñez R., Salazar-Schettino P.M., Cabrera M., Monroy M.C., Bargues M.D., Mas-Coma S., O'Connor J.E., Angulo V.M., Jaramillo N., Cordón-Rosales C., Gómez D., Pérez R. 2006. Chromosomal variation and genome size support existence of cryptic species of *Triatoma dimidiata* with different epidemiological importance as Chagas disease vectors. Trop. Med. Int. Health. 11:1092-1103.

Schofield C.J., Grijalva M.J., Diotaiuti L. 2009. Distribución de los vectores de la enfermedad de Chagas en países "no endémicos": la posibilidad de transmisión vectorial fuera de América Latina. Enf. Emerg. 11(suppl.1):20-27.

Schmunis G.A. 2004. Medical significance of American trypanosomiasis. *In*: Maudlin I., Holmes P.H., Miles M.A. (Eds.), The Trypanosomiases. CAB International, Wallingford, pp. 355-368.

WHO/HTM/NTD 2010. Working to overcome the global impact of neglected tropical diseases: first WHO report on neglected tropical diseases. World Health Organization, Geneva.

WHO 2012. A ccelerating work to overcome the global impact of neglected tropical diseases. A road map for implementation. World Health Organization, Geneva.

World Bank 1993. World Development Report 1993. Investing in Health. Oxford University Press, New York.

Zeledón R., Rojas J. 2006. Environmental management for the control of *Triatoma dimidiata* (Latreille, 1811), (Hemiptera: Reduviidae) in Costa Rica: a pilot project. Mem. Inst. Oswaldo Cruz 101:379-386.

Zeledón R., Rojas J.C., Urbina A., Cordero M., Gamboa S.H., Lorosa E.S., Alfaro S. 2008. Ecological control of *Triatoma dimidiata* (Latreille, 1811): five years later a Costa Rican pilot project. Mem. Inst. Oswaldo Cruz 103:619-621.

CONGENITAL CHAGAS DISEASE: DIAGNOSIS, TREATMENT AND CONTROL

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Trypanosoma cruzi infection and congenital Chagas disease

Chagas disease, caused by the kinetoplastid flagellate *T. cruzi*, is one of the major causes of cardiac failure in Latin America. This trypanosomiasis has become a global public health problem due to migrations from Latin America to non-endemic countries, particularly in United States, Europe, Japan and Australia. This parasite, infecting 8 to 10 million people, can be transmitted by vector bugs, orally, transfusion of infected blood or from mothers to their foetuses.

Congenital transmission occurs in endemic as well as non-endemic areas and from one generation to another. This pattern of transmission facilitates uncontrolled spread of the parasite infection for long periods of time. The transmission of congenital infection occurs in 1 to 12% *T. cruzi*-infected mothers (with an average around 4-6%). According to epidemiological data from Latin America, the estimated number of cases of congenital *T. cruzi* infection is > 15000 per year. The incidence of congenital cases in non-endemic areas is not known, although several reports attest to its occurrence (OPS, 2006; Carlier and Truyens, 2010).

Clinical manifestations of congenital Chagas disease

Congenital *T. cruzi* infection, though an acute infection, is frequently asymptomatic at birth (in 40 to 100% of cases). However, clinical manifestations of congenital Chagas disease can appear at birth or within days or weeks after birth. Non-specific signs (fever, low birth weight, prematurity, hepatosplenomegaly, pneumonitis…), as seen in other TORCH congenital infections, are generally observed. More severe clinical manifestations, such as meningoencephalitis, acute myocarditis and anasarca/fetal hydrops, can be also observed, leading to death if untreated, particularly in case of co-infection with HIV. Untreated congenital *T. cruzi* infection, whatever the neonatal morbidity, can develop into chronic chagasic myocardiopathy or digestive megaviscera later in life. Since congenital *T. cruzi* infection is frequently asymptomatic and there is no specific clinical marker, its detection must necessarily be based on laboratory diagnosis (Torrico et al., 2004; Carlier and Truyens, 2010).

Laboratory diagnosis of *T. cruzi* congenital infection

The laboratory diagnosis of congenital infection with *T. cruzi* involves the detection of infection in pregnant women and the confirmation of infection in newborns of positive mothers.

Detection of infection during pregnancy can be performed using two conventional serological tests (e.g., specific ELISA or indirect immunofluorescence assay – IFA). These diagnostic tests are generally available at low cost in most health-care facilities. Rapid diagnostic methods (such as inmunochromatographic, immunodot and immunofiltration tests) can also be used, but they need confirmation with standard serological tests.

Detection of congenital infection in neonates can be performed by detecting living parasites in umbilical cord blood or venous blood of the newborn (microscopic examination of buffy coat after blood centrifugation in capillary tubes; so-called microhematocrit test). These parasitological tests offer rapid and definitive diagnosis, but require skilled personnel and assured quality control, which may not be available in all health-care facilities. Polymerase chain reaction (PCR) is under evaluation and has not been validated yet for the diagnosis of congenital infection, although it might improve its early detection (Virreira et al., 2003).

Detection of congenital infection in old infants over 8 months (when maternal antibodies have disappeared) can be performed by detecting *T. cruzi*-specific antibodies using serological tests, as for mothers. However, their performance in infants over 8 months of age delays diagnosis and treatment. A negative serological result in infants below 8 months of age indicates an absence of congenital infection.

Detection of blood parasites at any time after birth or a positive *T. cruzi*-specific serology in infants over 8 months of age are the gold standards for the diagnosis of congenital Chagas disease (when previous transmission to infant by vectors and blood transfusion has been ruled out) (Carlier and Truyens, 2010; Carlier et al., 2011).

Treatment of neonates and infants infected with *T. cruzi*

Cases of congenital *T. cruzi* infection have to be treated as soon as the diagnosis has been confirmed. Although randomized comparative clinical trials have not been carried out, the experience of expert clinical groups in treating congenital *T. cruzi* infection indicates that: (i) both benznidazole and nifurtimox can be used to treat congenital cases; (ii) the recommended dose of benznidazole in infants, as in adults, is 5-7 mg/kg per day (doses of benznidazole up to 10 mg/kg per day can be used in neonates and infants aged < 1 year); (iii) the recommended doses of nifurtimox in neonates and infants are 10-15 mg/kg per day; (iv) such doses can be administered orally; precautions should to be taken to obtain appropriate dosage of active drug (benznidazole is now available in easily dispersible tablets of paediatric dosage of 12.5 mg); (v) the recommended duration of treatment is 60 days and should not be < 30 days. Treatment is generally successful and without the adverse reactions seen in adults if administered within the first year of life (Carlier and Truyens, 2010; Altcheh et al., 2011; Carlier et al., 2011).

Prevention of congenital transmission and control of *T. cruzi* congenital infection

For pregnant women who are already infected with *T. cruzi*, there is no specific or direct means of preventing congenital infection. Since the teratogenic risks of the available medicines (benznidazole and nifurtimox) are not well known and the risk of adverse reactions is high in adults, anti-parasitic treatment is not recommended during pregnancy.

For women who are not pregnant, prevention of congenital transmission is possible by treating those infected and controlling vectors and blood-transmission in disease endemic areas to reduce the risk of infection and the reservoir of infected women (Sosa-Estani et al., 2009).

The presently accepted and recommended WHO strategy for controlling *T. cruzi* congenital infection is based on: i) an antenatal screening during pregnancy to detect mothers who are infectious and at risk of transmitting infection to their foetuses (there is no way to identify, in advance, those mothers who will transmit the infection to their foetuses); ii) the detection of congenital infection based on laboratory diagnosis (neonatal parasitological screening in newborns of infected mothers and/or infant serological screening after 8 months of age), and iii) the systematic treatment of positive neonates. The cost/benefice of this control strategy has been evaluated and shown much more cheaper than the cumulative costs of managing chagasic patients during years (Carlier and Truyens, 2010; Carlier et al., 2011).

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References

Altcheh J., Moscatelli G., Moroni S., Garcia-Bournissen F., Freilij H. 2011. Adverse events after the use of benznidazole in infants and children with Chagas disease. Pediatrics 127:212-218.

Carlier Y., Torrico F., Sosa-Estani S., Russomando G., Luquetti A., Freilij H., Albaja V.P. 2011. Congenital chagas disease: recommendations for diagnosis, treatment and control of newborns, siblings and pregnant women. PloS Negl. Trop. Dis. 5:e1250.

Carlier Y., Truyens C. 2010. Maternal-fetal transmission of *Trypanosoma cruzi*. In: Telleria J, Tibayrenc M, editors. American trypanosomiasis – Chagas disease, One hundred years of research. UK, USA, Elsevier, pp. 539-581.

OPS. 2006. Estimación cuantitativa de la enfermedad de Chagas en las Americas (Quantitative Estimation of Chagas disease in the Americas). Washington DC, Pan American Health Organization (OPS/HDM/CD/425-06).

Sosa-Estani S., Cura E., Velazquez E., Yampotis C., Segura E.L. 2009. Etiological treatment of young women infected with *Trypanosoma cruzi*, and prevention of congenital transmission. Rev. Soc. Bras. Med .Trop. 42:484-487.

Torrico F., Alonso-Vega C., Suarez E., Rodriguez P., Torrico M.C., Dramaix M., Truyens C., Carlier Y. 2004. Maternal *Trypanosoma cruzi* infection, pregnancy outcome, morbidity, and mortality of congenitally infected and non-infected newborns in Bolivia. Am. J. Trop. Med.. Hyg. 70:201-209.

Virreira M., Torrico F., Truyens C., Alonso-Vega C., Solano M., Carlier Y., Svoboda M. 2003. Comparison of polymerase chain reaction methods for reliable and easy detection of congenital *Trypanosoma cruzi* infection. Am. J. Trop. Med. Hyg. 68:574-582.

BIODIVERSITY, POVERTY AND INCIDENCE OF CHAGAS DISEASE IN MEXICO

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ASPECTS OF RESEARCH WORK ON MALARIA IN THE FIELD

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It is of great relevance to produce in the laboratory a number of models to reproduce the experimental work needed to better understand malaria not only as a major infection, causing major mortality and morbidity, but also to better perceive the biology of the parasite in the hosts and its interactions. From new drugs to vaccines, knowledge demands good observations in strains of malaria parasites, especially *Plasmodium falciparum*. Drug resistance has had a major impact on the failures of malaria control and in the recent history of the denoted malaria eradication programs, drug failure has always been considered relevant.
With the income of molecular biology in the study of pathogens and infections, major steps in the learning of malaria were taken and from gene sequencing to epidemiology and much has been learnt mainly in relation to drug resistance markers and genetics. Vector studies and human genetics have also given more information on the dissemination of mutations or the appearance of different strains.
This talk will refer to a) the interest for animal or in vitro models for the study of malaria; b) how to transfer the data from the laboratory into the field; c) what are some of the major problems in malaria control that come from other scientific areas such as potential global warming, development or social studies; d) the role of networking in research and development. Not all laboratories in the field maintain up to date laboratories in this field, and expensive reagents or technical staff is also required to keep research in good function and therefore, technology transfer can be complex.
Our experience in Angola, São Tomé and Principe or Cabo Verde (where a pre-elimination malaria program is being discussed) will be presented as well data from these countries.

Key words: malaria, field studies, malaria control, diagnostics, drug resistance.

SEVERE FALCIPARUM AND VIVAX MALARIA: NEW THERAPEUTIC APPROACHES

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More than 200 million malaria cases were reported world-wide in 2010 (WHO, 2011). Outside Africa, the most widely distributed species is *Plasmodium vivax*. Antimalarial drug policies in endemic countries resemble each other, using Artemisinin-based combination therapies (ACTs) for uncomplicated *P. falciparum* and chloroquine + primaquine for *P. vivax* infections. ACTs has been and still is one of the main pillars, responsible for the reduction of malaria infections worldwide (Nosten et al., 2000; Barnes et al., 2005). In recent years, reports from the border between Thailand and Cambodia (Noedl et al., 2008; Dondorp at al., 2009) show resistance to artemisinin against *P. falciparum*. Although *in vitro* resistance to artemether-lumefantrine against *P. falciparum* is reported from South America (Jambou et al., 2005), so far there is no evidence for treatment failures. Chloroquine resistance against *P. vivax* was first reported in 1989 from Papua New Guinea (Rieckman et al., 1989). Since then various countries, including Myanmar, Turkey, Ethiopia, Vietnam, Indonesia, Korea and Madagascar reported treatment failures (Barnadas et al., 2008; Sutanto et al., 2010). Thailand reported its first chloroquine resistant *P. vivax* cases in 2011 (Rijken et al., 2011), while in India (Srivastava et al., 2008) chloroquine treatment seems still to be effective. Reports from Colombia, Brazil, and Guyana indicate chloroquine resistance appearing in numerous regions of South America (DeSantana et al., 2008). Until now only insufficient information on treatment outcomes with ACTs for the treatment against *P. vivax* is available. Diagnosis of malaria in remote areas is now frequently based on rapid diagnostic tests. These devices showed high sensitivity and specificity in many studies, but one major weakness is related to *Plasmodium vivax* diagnosis. One could imagine that an increasing number of patients presenting vivax infection will be diagnosed as “malaria” and will received ACTs. Climate changes may contribute to a redefinition of specific falciparum and vivax endemic areas; the efficacy of ACTs for the treatment of both parasites is an issue to be addressed.

Whatever the antimalarial drug used, patients suffering severe malaria still showed a high mortality rate. ACTs have drastically decreased the number of malaria cases, but the number of deaths is not affected in the same proportion. Reasons for poor outcome during severe malaria are complex, and the specific role of the antimalarial drug is probably lower than for uncomplicated cases. Access to a high quality health care, availability of intensive care unit and trained staff are scarce. Respiratory distress, repeated seizures, severe anemia are prognostic factors that are poorly taken into account in low income countries. Training and capacity building may help to improve the care to patients. However, rapidly active drugs able to decrease tissue and cells injuries caused by parasites infection may provide adjunctive effects in order to maintain patients alive during the time needed by antimalarial drugs to act against parasites. Some of these adjunctive drugs, including erythropoietin, have been tested in different vital emergency situations and have provided evidence for a benefit in term of survival and sequels.

Cerebral malaria is a complex disorder with many similarities to neurological stroke. During cerebral malaria, reduced cerebral blood flow is the pathological consequence of microcirculation obstruction. Cerebral ischemia or hypoxia is occurring as a consequence of infected red blood cells sequestration during the disease. During ischemic/hypoxic insult, a cascade of pathophysiological processes could result in apoptosis. Fas and FasL over expression were correlated with apoptosis occurrence. Ischemia induced widespread neuronal expression of Epo receptor and this may be a survival response in damaged areas.

Epo is a well known growth factor, and was more recently identified as a multipotent cytokine with pleiotropic effects. Systemic administration of exogenous Epo regulates cerebral blood flow, protects endothelial cells against oxydative stress and reduces inflammation by inhibiting pro-inflammatory cytokines. These effects are partly due to the inhibition of cell apoptosis.

Dimerizing of EpoR leads to autophosphorylation of the receptor associated Janus tyrosine kinase 2 and activation of distal signal transduction cascade: phosphatidylinositol-3-kinase (PI3-K), akt protein kinase, RAS mitogen-activated protein kinases, signal transducers and activators of transcription-5 (STAT-5) and NF-kB-dependant transcription. These mechanisms are contributing to attenuation of

apoptosis, and reduction of brain inflammation. Recombinant human erythropoietin has shown widespread efficacy in animal models of stroke. More recently, EPO treatment was shown to improve functional recovery, and enhance neurogenesis and angiogenesis after focal ischemia, suggesting a beneficial effect of EPO treatment on brain repair after stroke. Indeed, despite its large size, recombinant human EPO administered peripherally crosses the blood-brain barrier to protect against brain injury. Epo treatment can be initiated in the first 24 hours after initiation of the lesion.
Compounds having Epo'sneuroprotective properties but without haematological side effects have been developed, anda 11 amino acid peptide derived from the tertiary structure of Epo by the Warren Pharmaceutical. The tissue-protective activities of Epo are mimicked by small and nonerythropoietic peptides that stimulate a small portion of Epo's three-dimensional structure. This peptide exhibits Epo's trophic effects and increases cognitive functions in rodents.
Some cytoprotective concepts obtained during stroke were applied to parasitic diseases.
We demonstrated that the artesunate-erythropoietin drug combination led to clinical recovery 24 hours earlier for surviving mice, and to increase in the global survival rate compared to artesunatemonotherapy ($P < 0.01$). Wiese's study also demonstrated that recombinant erythropoietin increases survival in mice with CM in a dose and time dependent manner. While statins alone failed to prevent death of murine cerebral malaria, it could be speculated that they could act as an adjuvant therapy (Bienvenu, 2008). Mice were treated with a drug combination of artesunate and atorvastatin, and we observed a significant improvement in survival at day 13 postinfection compared to survival with atorvastatin alone ($P < 0.02$; log rank test, cumulative survival analysis).
During human cerebral malaria, immune interventions using anti-inflammatory drugs were used as adjuvant therapy: immunomodulators (anti-TNF), pentoxifylline, and dexamethasone, delay or prevent CM without affecting parasitemia in experimental systems, but no evidence was obtained in humans.
High levels of Epo are associated with protection against neurological sequelae in African children with cerebral malaria (Casals-Pascual, 2008). Plasma Epo (> 200 units/liter) was associated with > 80% reduction in the risk of developing neurological sequelae [adjusted odds ratio (OR) 0.18; 95% C.I. 0.05-0.93; P=0.041]. Admission with profound coma and convulsions after admission were also independently associated with neurological sequelae.
An open-labelled study including cerebral malaria children (Blantyre coma score below 3) was conducted in Mali (Picot, 2009). The objective was to assess the short-term safety (seven days) of erythropoietin at high doses combined to quinine. 35 patients with unrousable coma were included in the study. None of expected side effects of erythropoietin were observed during the seven days follow-up. No significant increase in the case fatality rate (7/35 patients) was observed compared to other studies with mortality rates ranging from 16 to 22% in similar endemic areas. These data provide the first evidence of the short-term safety of erythropoietin at high doses combined to quinine. A multicentre study is needed to assess the potential of Epo as an adjunctive therapy to increase the survival during cerebral malaria.
The major issue is now to provide access to these treatments for poor people. A huge amount of work is still needed to decrease the cost, to increase the availability and to improve the use in emergency conditions. Waiting for a vaccine is not ethical when half-millions of people are dying each year.

References

Barnadas C., Ratsimbasoa A., Tichit M., Bouchier C., Jahevitra M., Picot S., Ménard D. 2008. *Plasmodium vivax* resistance to chloroquine in Madagascar: clinical efficacy and polymorphisms in pvmdr1 and pvcrt-o genes. Antimicrob. Agents Chemother. 12:4233-40.

Barnes K.I., Durrheim D.N, Little F., Jackson A., Mehta U., Allen E., Dlamini S.S., Tsoka J., Bredenkamp B., Mthembu D.J., White N.J., Sharp B.L. 2005. Effect of artemether-lumefantrine policy and improved vector control on malaria burden in Kwa Zulu-Natal, South Africa. PloS Med. 11:e330.

Bienvenu A.L., Picot S. 2008. Statins alone are ineffective in cerebral malaria but potentiate artesunate. Antimicrob. Agents Chemother. 11:4203-4.

Bienvenu A.L., Ferrandiz J., Kaiser K., Latour C., Picot S. 2008 Artesunate-erythropoietin combination for murine cerebral malaria treatment. Acta Trop. 106(2):104-108.

Casals-Pascual C., Idro R., Picot S., Roberts D.J., Newton C.R. 2009. Can erythropoietin be used to prevent brain damage in cerebral malaria? Trends Parasitol. 25:30-6.

de Santana Filho F.S., Arcanjo A.R., Chehuan Y.M., Costa M.R., Martinez-Espinosa F.EJ. L

Vieira J.L., Barbosa M.G., Alecrim W.D., Alecrim M.G. 2007. Chloroquine-resistant *Plasmodium vivax*, Brazilian Amazon. Emerg. Infect. Dis. 13:1125-1126.

Dondorp A.M., Nosten F., Yi P., Debashish D., Aung P.P., Tarning J., Lwin K.M., Ariey F., Hanpithakpong W., Lee S. J., Ringwald P., Silamu K., ImwongM., Chotivanich K., Lim P., Herdman T., An S.S., Yeung S., Singhasivanon P., Day N.P.J., Lindegardh N., Socheat D., White N.J., 2009. Artemisinin resistance in *Plasmodium falciparum* malaria. N. Engl. J. Med. 361:455-67.

Jambou R., Legrand E., Niang M., Khim N., Lim P., Volney B., Ekala M.T., Bouchier C., Esterre P., Fandeur T., Mercereau-Puijalon O. 2005. Resistance of *Plasmodium falciparum* field isolates to *in vitro* artemether and point mutations of the SERCA-type PfATPase6. Lancet 366:1960-3.

Noedl H., Se Y., Schaecher K., Smith B.L., Socheat D., Fukuda M.M. 2008. Evidence of artemisinin-resistant malaria in Western Cambodia. N. Engl. J. Med. 359:2619-20.

Nosten F., van Vugt M., Price R., Luxemburger C., Thway K.L., Brockman A., McGready R., Kuile F., Looareesuwan S., White N.J. 2000. Effects of artesunate-mefloquine combination on incidence of *Plasmodium falciparum* malaria andmefloquine resistance in Western Thailand: a prospective study. Lancet 356:297-302.

Picot S., Bienvenu A.L., Konate S., Sissoko S., Barry A., Diarra E., Bamba K., Djimde A., Doumbo O. 2009. Epoietin beta-Quinine drug combination in children with cerebral malaria in Mali. Malar. J. 8:169.

Rieckmann K.H., Davis D.R., Hutton D.C. 1989. *Plasmodium vivax* resistance to chloroquine? Lancet 2:1183-1184.

Rijken M.J., Boel M.E., Russell B., Imwong M., Leimanis M.L., Phyo A.P., Muehlenbachs A., Lindegardh N., McGready R.., Rénia L., Snounou G., Singhasivanon P., Nosten F. 2011. Chloroquine resistant vivax malaria in a pregnant woman on the western border of Thailand. Malar. J. 10:113.

Srivastava H.C., Yadav R.S., Joshi H., Valecha N., Mallick P.K., Prajapati S.K., Dash A.P. 2008. Therapeutic responses of *Plasmodium vivax* and *P. falciparum* to chloroquine, in an area of Western India where *P. vivax* predominates. Ann. Trop. Med. Parasitol. 102:471-80.

Sutanto I., Endawati D., Ling L.H., Laihad F., Setiabudy R., Baird J.K. 2010. Evaluation of chloroquine therapy for vivax and falciparum malaria in Southern Sumatra, Western Indonesia. Malar. J. 9:52.

WHO Malaria Report, WHO 2011.

MOLECULAR EPIDEMIOLOGY OF *PLASMODIUM VIVAX* MALARIA IN DIFFERENT ENDEMIC SETTINGS

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At the beginning of the previous century, *Plasmodium vivax* was the dominant parasite in the temperate zones (including most of North America, Northern Europe, the Mediterranean perimeter, and China) and in many of the tropical zones areas (including Latin and Central America, and the Indian sub-continent) (Mendis et al., 2001). Currently, *P. vivax* accounts for about 10% of malaria cases in Africa and over half of all malaria cases outside Africa, with an estimated total of 90 million cases yearly (WHO World Malaria Report, 2010). However, over the last forty years, only a small proportion of the funds and scientific manpower devoted to malaria research have been directed at *P. vivax* studies. This relative neglect has been clearly justified by the fact that mortality directly attributed to malaria is almost exclusively due to *P. falciparum* infection, and the highest number of deaths occurs in Africa where *P. vivax* is relatively rare. Nowadays, some worrying concerns about *P. vivax* malaria control are recently arising: the emergence of drug resistance, especially chloroquine resistance (CQR) as well as the clinical reports of severe malaria due to *P. vivax* (Price et al., 2009) and the identification of *P. vivax* parasites in *Duffy-negative* patients (Menard, et al., 2010).
A research project on *P. vivax* malaria resurgence in the Community of Newly Independent States (NIS-Countries) of the former USSR was launched at the end of year 2000, with the aim of analysing different malaria epidemiological settings in three NIS-countries, namely Armenia, Azerbaijan and Uzbekistan by using modern molecular techniques applied to the parasitology. The project was funded by the European Commission (VIVAXNIS-*INCO COPERNICUS2 project contract ICA2-CT-2000-10046*) and was coordinated by the Istituto Superiore di Sanità in Rome. The main results achieved within five years of project activities have been the assessment of genetic variability of *P. vivax* isolates circulating in the studied areas by polymorphism analysis of *pvmsp1* and *pvcsp* genes, and the analysis of the extent of polymorphism of *pvdhfr* and *pvdhps* genes, two molecular markers for antifolate drug resistance in *P. vivax*. In the study conducted in the frame of VIVAXNIS project, we have found a high level of MSP-1 sequence variation, especially in parasite isolates from Azerbaijan, where several MSP-1 types (*Sal-1* and *Belem* types as well as different 3a-*recombinant* types according to the classification reported in Putaporntip et al., 1997) were observed despite the low level of malaria transmission present in the region (Leclerc et al., 2004). By comparing our results to those reported in the literature, we noticed that in a study conducted in Brazil Amazon region, where the level of malaria endemicity is considered low, the polymorphism of MSP-1 varies according to the different areas, and the isolates of *P. vivax* circulating in a given area seems to be homogenous, showing a low level of genetic diversity (Santos-Ciminera et al., 2007). In Iran, a comparative study on MSP-1 polymorphism conducted in the Northern (bordering Azerbaijan) and Southern malaria endemic areas, which differ in endemicity, showed a higher genetic diversity in the *vivax* populations circulating in the southern part of the country, where the degree of malaria transmission is higher than the northern part of the country (Zakeri et al., 2006). This is consistent with the general concept that the genetic diversity decreases together with the decrease of transmission level. However, this figure seems to be in contrast with the particular situation found in the Central Azerbaijan in our study.
Recently, Karunaweera et al. (2007) identified a novel sets of 14 highly polymorphic microsatellite markers (*mss)* characterized by repeat units of three or four nucleotides. These markers have been recently used to investigate the genetic variability and transmission dynamics of *P. vivax* isolates from Brazilian Amazonia, a low malaria endemic area (Ferreira et al., 2007). The study showed that *P. vivax* populations are more genetically diverse and frequently comprise multiple clone infections than sympatric *P. falciparum* isolates. The authors observed also that the genetic diversity in *P. vivax* populations under low-level transmission is not severely constrained by the low rates of effective meiotic recombination, with an expected negative impact on public health. Using 7 of this set of *mss*, we have recently conducted a genotyping study in different endemic areas, namely Armenia, Sudan, Madagascar, French Guyana and Iran. Despite to the low or very low transmission intensity in these regions, we found a noticeable level of genetic diversity (reported as *average virtual heterozygosity*

[He] value) in each of the studied plasmodial populations, confirming that the genetic variability of *P. vivax* populations is high irrespective to the relevant endemic setting.
The resistance of *P. vivax* to primaquine, quinine, proguanil, and pyrimethamine has been detected since 80's and at the end of 80's also the resistance to chloroquine (*Pv*CQR) has been detected in isolates from Papua New Guinea (Baird et al., 2009). *Pv*CQR is limited even considering the worldwide use of chloroquine and probably is based on a different mechanism as the one suggested for *P. falciparum* (*CRT* gene, related to *P. falciparum* CQ-resistance and recently characterized in *P. vivax*, seem not to be involved). Anyway, the molecular basis of CQR in *vivax* is not elucidated yet. The unavailability of standardized continuous culture methods for *P. vivax* has hampered so far cellular and molecular extensive studies on drug sensitivity. In order to overcome this problem, some alternative methods have been set up by researchers in the last years, but often these methods are labour intensive and need really experienced investigators as the method recently proposed by a research team from Mahidol University in Thailand (Udomsangpetch et al., 2008).
The combination of sulfadoxine /pyrimethamine (S/P) has been one of the most efficacious second line drugs to treat chloroquine-resistant malaria cases and it is still used in the Intermittent Preventive Treatments (*IPTs*), a malaria prevention strategy currently implemented in several endemic areas. However, S/P resistance is now widespread in Southeast Asia and South America and it is increasing within Africa. The relatively rapid emergence of antifolate-resistant in *P. vivax* had led some authors to speculate that this species may be intrinsically resistant to antifolate drugs. In the last years, some studies have shown that, also in *P. vivax* as it occurs in *P. falciparum*, the main mechanism of pyrimethamine resistance is linked to specific mutations in the *dhfr* gene of the parasite and that the level of resistance to pyrimethamine is directly related to the number of mutate codons present in *dhfr* gene in a given *vivax* isolate. On the contrary, the molecular basis of sulfadoxine resistance in *P. vivax* has been poorly documented so far and only recently the *P. vivax dhps* gene has been cloned and sequenced (Korsinczky et al., 2004). In that study, based on amino acid homology and on model of the secondary structure of the DHPS enzyme from *P. falciparum*, it was possible detecting amino acid residues that are probably related to sulfadoxine resistance. In particular, it was speculated that the presence of a V585 wild-type residue (equivalent of 613 position of *P. falciparum*) may be the key for the innate resistance of *P. vivax* to sulfadoxine. Moreover, the same authors noted that some *P. vivax* isolates are characterized by two amino acid changes, A383G and A553G, that would be associated with a diminution of affinity between the *P. vivax* DHPS and sulfadoxine. In two papers recently issued (Imwong et al., 2005; Menegon et al., 2006) a further characterization of *pvdhps* gene has been presented. In Imwong et al. (2005), a first and so far unique report of multiple mutations identified in both *pvdhfr* and *pvdhps* genes on *vivax* isolates from Thailand has been presented as well as a possible association of these identified haplotypes with S/P resistance. In Menegon et al. (2006), the analysis of the *P. vivax dhps* of the studied isolates showed size polymorphism that has not been reported before in *P. vivax* and that is not present in *P. falciparum dhps* (gene). This polymorphism in *P. vivax dhps* gene is due to the presence of a unique tandem repeat region present in the fragment amplified for mutation screening and it could be a useful tool to genotype the mutated isolates.
The results obtained in the frame of these studies provide the scientific community with new genetic features of *P. vivax* molecular epidemiology in different endemic settings and highlight the importance of studies entirely devoted to this dangerous human parasite. Considering the increasing number of travellers coming from *vivax* endemic areas to Europe and the related risk of re-emergence of *vivax* malaria in Europe (risk supported by the recent *vivax* outbreak in Greece), the implementation of specific methods to fight against this disease could also have an impact on social and economic development. In conclusion, the concept, widely accepted until recently, that by studying and controlling *P. falciparum* it is possible to study and control also *P. vivax*, must be abandoned for good. The need for resources to be allocated specifically to research projects focused only on *P. vivax* should represent the right strategy in the near future as well as a good dissemination of the research results by international meetings/workshops focussing only on *P. vivax.*

References

Baird J.K. 2009. Resistance to therapies for infection by *Plasmodium vivax*. Clin. Microbiol. Rev. 22:508-534.

Ferreira M.U., Karunaweera N.D., da Silva-Nunes M., Silva N.S., Hartl D.L., Wirth D.F., 2007. Population structure and transmission dynamics of *Plasmodium vivax* in rural Amazonia. J. Infect. Dis. 195:1218-26.

Imwong M., Pukrittayakamee S., Cheng Q., Moore C., Looareesuwan S., Snounou G., White N.J., Day N.P. 2005. Limited polymorphism in the dihydropteroate synthetase gene (dhps) of *Plasmodium vivax* isolates from Thailand. Antimicrob. Agents Chemother. 49:4393-5.

Karunaweera N.D., Ferreira M.U., Hartl D.L., Wirth D.F. 2007. Fourtheen polymorphic microsatellite DNA markers for the human malaria parasite *Plasmodium vivax.* Mol. Ecol. Notes.7:172-5.

Korsinczky M., Fischer K., Chen N., Baker J., Rieckmann K., Cheng Q. 2004. Sulfadoxine resistance in *Plasmodium vivax* is associated with a specific amino acid in dihydropteroate synthase at the putative sulfadoxine-binding site. Antimicrob. Agents Chemother. 48:2214-2222.

Leclerc M., Menegon M., Cligny A., Noyer J., Mammadov S., Aliyev N., Gasimov E., Majori G., Severini C. 2004. Genetic diversity of *Plasmodium vivax* isolates from Azerbaijan. Malaria J. 3:40.

Ménard D., Barnadas C., Bouchier C., Henry-Halldin C., Gray L.R., Ratsimbasoa A., Thonier V., Carod J.F., Domarle O., Colin Y., Bertrand O., Picot J., King C.L., Grimberg B.T., Mercereau-Puijalon O., Zimmerman P.A. 2010. *Plasmodium vivax* clinical malaria is commonly observed iDuffy-negative Malagasy people. Proc. Natl. Acad. Sci. USA. 30: 967-971.

Mendis K., Sina B.J., Marchesini P., Carter R. 2001. The neglected burden of *Plasmodium vivax* malaria. Am. J. Trop. Med. Hyg. 64: 97-106.

Menegon M., Majori G., Severini C., 2006. Genetic variations of the *Plasmodium vivax* dihydropteroate synthase gene. Acta Trop. 98:196-199.

Price R.N., Douglas N.M., Anstey N.M. 2009. New development in *Plasmodium vivax* malaria: severe disease and the rise of chloroquine resistance. Curr. Opin. Infect. Dis. 22:430-453

Putapornitp C., Onqwutiwes S., Tanabe K., Thaithong S., 1997. Interallelic recombination in the merozoite surface protein 1 (MSP-1) gene of *Plasmodium vivax* from Thai isolates., Mol. Biochem. Parasitol. 84:49-56.

Santos-Ciminera P.D., Alecrim M.G., Roberts D.R., Quinnan G.V. Jr. 2007. Molecular epidemiology of *Plasmodium vivax* in the State of Amazonas, Brazil. Acta Trop. 102:38-46.

Udomsangpetch R., Kaneko O., Chotivanich K., Sattabongkot J. 2008. Cultivation of *Plasmodium vivax*. Trends Parasitol. 24:85-8

World malaria report 2010. Geneva, World Health Organization, 2010. http://www.who.int/malaria/world_malaria_report_2010/worldmalariareport2010.pdf.

Zakeri S., Mehrizi A.A., Mamaghani S., Noorizadeh S., Snounou G., Djadid N.D. 2006. Population structure analysis of *Plasmodium vivax* in areas of Iran with different malaria endemicity. Am. J. Trop. Med. Hyg. 74:394-400.

NEONATAL PORCINE COCCIDIOSIS

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Since the 1970s cumulating evidence about the importance of porcine coccidiosis in intensive piglet production was published and today this infection is recognised as one of the most frequent causes of diarrhea in newborn piglets. A survey in Europe showed that the average farm prevalence of *I. suis* was around 70% (reviewed by Torres, 2004).
As a typical intestinal coccidian, *I. suis* is directly transmitted by ingestions of sporulated oocysts from the environment. Sporozoites leave the oocyst and invade the epithelium of the small intestine, primarily of the distal jejunum and the ileum, where asexual development (merogony) leads to cell destruction. At the final stage of the intestinal sexually differentiated stages are formed which fuse to a zygote and develop into an oocyst that is then released from its host cell and excreted with the faeces. In the environment sporulation is accomplished at optimum temperatures of 20-30 °C (for review see Worliczek and Joachim, 2011). These stages can survive in humid environment for several months and are highly resistant to many commonly used disinfectants (Straberg and Daugschies, 2007; Langkjaer and Roepstorff, 2008). The turnover time of *I. suis* is extremely fast, with a minimum prepatent period of four to five and a sporulation within 24 hours at optimum condition the whole life cycle is completed within five to seven days (Mundt et al., 2006).
Infection leads to the destruction of the intestinal lining and to enteritis and villous atrophy with non-haemorrhagic diarrhoea for 1-7 days, starting around the time of oocyst excretion (Mundt et al., 2006). Although experimental infection can reliably be established in naïve piglets, infected animals even in the same litter show very variable signs of disease (Mundt et al., 2006), reflecting a finding previously been described for natural infections (Martineau and del Castillo, 2000). The consequences of infection are poor weight gain or weight loss and consequent unthriftiness due to the reduction of the resorptive surface, which may last until after weaning (Aliaga-Leyton et al., 2011; Worliczek and Joachim, 2011). Despite the self-limiting nature of isosporosis, co-infections with bacterial pathogens, especially *Clostridium perfringens*, can increase mortality dramatically (Mengel et al., 2012). Even without secondary complications piglet isosporosis can cause considerable economic losses making treatment mandatory in high-productivity farms with clinical presentation (Maes et al., 2007; Scala et al., 2009; Kreiner et al., 2011).
The most important factor for the outcome of infection appears to be host age, and there is a pronounced age resistance in piglets older than three weeks of age (Worliczek et al., 2009). In younger animals disease (mainly characterised by pasty to watery diarrhea which can lead to heavy exsiccosis) can be induced after experimental infection (e.g., Mundt et al., 2006), and thus *I. suis* can be considered a primary pathogen, although the infection cannot be induced in older animals (Koudela and Kučerová, 1999; 2000). However, significant oocyst excretion can also be observed during the early post-weaning phase (Meyer et al., 1999; Aliaga-Leyton et al., 2011). Stress and a change in diet may be factors that favour shedding after reinfection and/or re-shedding due to an interference with the immune system (see below).
However, despite intensive research on the epidemiology, pathology sand treatment of porcine coccidiosis, details of its interaction with other intestinal microorganisms, the host's immune system and the physiology of the intestine are so far poorly described.
The rapid life cycle of *I. suis* makes it mandatory for effective control (a) to interrupt the life cycle before parasite multiplication in the intestines leads to clinically relevant tissue damage and (b) to prevent the production and excretion of oocysts which may serve as infective stages for the next generation of susceptible piglets and maintain infection in the herd. The narrow time frame for treatment can provide a considerable challenge for the management of the disease. Sulfonamides, still recommended in many textbooks, shows low potential for field use (Maes et al., 2007; Scala et al., 2009; Joachim and Mundt, 2011; Kreiner et al., 2011). The metaphylactic application of the triazinone toltrazuril on the other hand, registered in the EU for this purpose, provides good control of both oocyst excretion and diarrhea (Mundt et al., 2007); however, concerns have recently been raised about drug resistance as described for chicken coccidia (Stephan et al., 1997).

In order to be able to develop alternative control measures detailed knowledge on the immune responses to *I. suis*, on the pathogenesis and the most important parameters that influence the individual outcome of infection are necessary.
Current knowledge on the immunity to coccidial infections in mammals cannot readily be extrapolated to *I. suis* due to peculiarities in the porcine immune system, especially in the first weeks of age. Newborn piglets are agammaglobulinaemic and must receive colostrum to display any humoral protection. Peyer's Patches have to develop into organised lymphoid structures in order to harbour functional B cells. In addition to antibodies, lymphocytes are also transmitted to the piglets with the colostrum in the first two days of life. Similar to bovines, TcR-γδ-T cells are a prominent subpopulation of T-cells and the first to populate the gut of neonatal piglets. They represent the majority of T cells in the first two weeks of life, followed by T-helper cells in the second and cytotoxic T lymphocytes from the fifth week of life. From the sixth week of life the gut lymphocyte composition resembles that of an adult pig (Bailey et al., 2001; Stokes et al., 2004). It is reasonable to assume that this situation is key to the immune response to primary infections with *I. suis* in the first weeks of life, as TcR-γδ-T cells are significantly increased in the gut of infected piglets compared to age-matched uninfected controls (Worliczek et al., 2010a).
Interference of *I. suis* with the establishment of the intestinal microflora during that time is another point of discussion. An appropriate immune response to pathogens is highly dependent on a functional physiological bacterial community, and during the early phase of colonisation a disturbed microflora might contribute to a dysregulation of the immune response, possibly contributing to immunopathogenic effects in the mucosa and rendering the gut lining more susceptible to attachment and invasion of bacterial pathogens (Marques and Boneca, 2011; Mengel et al., 2012).
Latest research shows that antigen-specific cellular immune responses against *I. suis* exist, indicating the development of an immunological memory (Worliczek et al., 2010b). The specific production of antibodies in the piglets is currently under investigation, and a role of colostral cells and maternal antibodies is implemented in experimental infections of piglets from infected versus non-infected sows (unpublished results). Especially the investigation of protective effects of maternal factors is of interest with regard effective vaccination strategies for newborn animals.
In summary, the latest research on *I. suis* and porcine isosporosis has revealed putative mechanisms of pathogenesis, some features of the immune response to the parasite and possible new intervention strategies. Experimental models will have to be complemented by *in vitro* studies to further dissect the mechanisms of cell-protozoon interactions in the complex network between the pig host, the parasite, the accompanying microflora and environmental and genetic factors.

References

Aliaga-Leyton A., Friendship R., Dewey C.E., Todd C., Peregrine A.S. 2011. *Isospora suis* infection and its association with postweaning performance on three Southwestern Ontario swine farms. J. Swine Health Prod. 19:94-99.

Bailey M., Plunkett F.J., Rothkötter H.J., Vega-Lopez M.A., Haverson K., Stokes C.R. 2001. Regulation of mucosal immune responses in effector sites. Proc. Nutrition Soc. 60:427-435.

Joachim A., Mundt H.C. 2011. Efficacy of sulfonamides and Baycox® against *Isospora suis* in experimental infections of suckling piglets. Parasitol. Res. 109, 1653-1659.

Koudela B., Kučerová S. 2000. Immunity against *Isospora suis* in nursing piglets. Parasitol. Res. 86:861-863.

Koudela B., Kučerová S. 1999. Role of acquired immunity and natural age resistance on course of *Isospora suis* coccidiosis in nursing piglets. Vet. Parasitol. 82:93-99.

Kreiner T., Worliczek H.L., Tichy A., Joachim A. 2011. Influence of toltrazuril treatment on parasitological parameters and health performance of piglets in the field – an Austrian experience. Vet. Parasitol. 183:14-20.

Langkjaer M., Roepstorff A. 2008. Survival of *Isospora suis* oocysts under controlled environmental conditions. Vet. Parasitol. 152:186-193.

Maes D., Vyt P., Rabaeys P., Gevaert D. 2007. Effects of toltrazuril on the growth of piglets in herds without clinical isosporosis. Vet. J. 173:199-201.

Marques R., Boneca I.G. 2011. Expression and functional importance of innate immune receptors by intestinal epithelial cells. Cell. Mol. Life Sci. 68:3661-3673.

Martineau G.P., del Castillo J. 2000. Epidemiological, clinical and control investigations on field porcine coccidiosis: clinical, epidemiological and parasitological paradigms? Parasitol. Res. 86:834-837.

Mengel H., Krüger M., Krüger M.U., Westphal B., Swidsinski A., Schwarz S., Mundt H.C., Dittmar K., Daugschies A. 2012. Necrotic enteritis due to simultaneous infection with *Isospora suis* and clostridia in newborn piglets and its prevention by early treatment with toltrazuril. Parasitol. Res. 110:1347-1355.

Meyer C., Joachim A., Daugschies A. 1999. Occurrence of *Isospora suis* in larger piglet production units and on specialized piglet rearing farms. Vet. Parasitol. 82:277-284.

Mundt H.C., Joachim A., Becka M., Daugschies A., 2006. *Isospora suis*: an experimental model for mammalian intestinal coccidiosis. Parasitol. Res. 98:167-175.

Mundt H.C., Mundt-Wüstenberg S., Daugschies A., Joachim A. 2007. Efficacy of various anticoccidials against experimental porcine neonatal isosporosis. Parasitol. Res. 100:401-411.

Scala A., Demontis F., Varcasia A., Pipia A.P., Poglayen G., Ferrari N., Genchi M. 2009. Toltrazuril and sulphonamide treatment against naturally *Isospora suis* infected suckling piglets: is there an actual profit? Vet. Parasitol. 163:362-365.

Stephan B., Rommel M., Daugschies A., Haberkorn A. 1997. Studies of resistance to anticoccidials in *Eimeria* field isolates and pure *Eimeria* strains. Vet. Parasitol. 69:19-29.

Stokes C.R., Bailey M., Haverson K., Harris C., Jones P., Inman C., Pie S., Oswald I.P., Williams B.A., Akkermans A.D.L., Sowa E., Rothkötter H.J., Miller B.G. 2004. Postnatal development of intestinal immune system in piglets: implications for the process of weaning. Anim. Res. 53:325-334.

Straberg E., Daugschies A. 2007. Control of piglet coccidiosis by chemical disinfection with a cresol-based product, Neopredisan 135-1). Parasitol. Res. 101:599-604.

Torres A. 2004. Prevalence survey of *Isospora suis* in twelve Europe countries. Proceedings 18th International Pig Veterinary Society Congress, Hamburg, Germany, pp. 243-243.

Worliczek H.L., Joachim A. 2011. Neonatal porcine coccidiosis. In: Mehlhorn H. (Ed.), Progress in Parasitology. Springer, Heidelberg, pp. 79-91.

Worliczek H.L., Mundt H.C., Ruttkowski B., Joachim A. 2009. Age, not infection dose, determines the outcome of *Isospora suis* infections in suckling piglets. Parasitol. Res. 105(suppl. 1):157-162.

Worliczek H.L., Buggelsheim M., Alexandrowicz R., Witter K., Schmidt P., Gerner W., Saalmüller A., Joachim A. 2010a. Changes in lymphocyte populations in suckling piglets during primary infections with *Isospora suis*. Parasite Immunol. 32:232-244.

Worliczek H.L., Ruttkowski B., Joachim A., Saalmüller A., Gerner W. 2010b. Faeces, FACS, and functional assays – preparation of *Isospora suis* oocyst antigen and representative controls for immunoassays. Parasitology 137:1637-1643.

COMPARATIVE EPIDEMIOLOGY OF COCCIDIAN INFECTIONS OF MAMMALIAN LIVESTOCK AND CONSEQUENCES FOR THEIR CONTROL

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In young mammalian livestock, intestinal coccidiosis plays a considerable role as it does in poultry farming. For various reasons, awareness of the significance of coccidial disease and knowledge of and methods for assessing coccidiosis are limited. This article will look at experience with the occurrence of coccidiosis in piglets, calves and lambs, its course and its identification, and will aim to describe a logical path through this complex subject matter.

Taxonomy and development

Coccidiosis in livestock is caused by pathogenic species of the genus *Eimeria* in cattle and sheep and by *Isospora suis* in swine. Typical features of both genera are their pronounced host specificity and a reproductive cycle (asexual and sexual development) that takes place in the target tissue of a single host. Coccidia have a considerable reproductive potential. Oocysts are exceptionally resilient to various external factors, i.e. they persist for months, if humidity and temperature are favourable, as a potential source of infection in the stable or on pasture and can even survive periods of frost. The different species differ considerably with respect to certain biological features. The main aspects here are the length of the prepatent period, the primary site of reproduction and, in conjunction with the tissue lesions, the nature and time of occurrence of clinical symptoms.

Pathomorphology of coccidiosis

The proliferation of the various developmental stages of coccidia causes typical lesions in the host tissue (intestinal mucosa). Clinical symptoms may appear during schizogony as a result of tissue damage (piglet, *I. suis*; calf, *E. alabamensis*) or not until the end of the prepatent period. Since intestinal coccidiosis is a self-limiting infection, spontaneous healing with restoration of morphological and functional integrity occurs at the end of the patent period in all forms of the disease (Daugschies et al., 2002; Daugschies and Najdrowski, 2005). Species such as *E. bovis a*nd *E. zuernii* cause particularly severe damage as they reproduce deep in the wall of the intestine or in the intestinal crypts.

The pathomorphological changes lead primarily to diarrhea which varies. In any case, the intestinal lesions lead to impairment of digestive function, which manifests as reduced digestion of nutrients and reduced nutrient absorption (Daugschies et al., 1998; Alzieu et al., 1999). The protective function of the intestinal mucosa is also severely impaired. This frequently leads to secondary bacterial infections on the damaged mucosa which can affect the pathomorphology of the disease and the clinical picture (Mundt et al., 2003).

Infection with coccidia

Susceptible animals always become infected by ingesting sporulated oocysts from a contaminated environment, i.e., coccidiosis is a contamination-borne infection. It is generally only young animals which are susceptible. Once they have survived an infection, they usually develop immunity. This is not sterile immunity, i.e. immunized animals are protected against the disease but can still excrete oocysts. Moreover immunity may still be breached (Daugschies et al., 1986; Bohrmann, 1991; Taylor and Catchpole, 1994; Svensson et al., 1996; Faber et al., 2002). Cross-immunity does not develop. Piglets rapidly develop resistance with age; initial infections have no clinical manifestations from as early as 4 weeks of age although oocyst excretion can be considerable.

Coccidiosis is always the sum of numerous factors determined by the parasites, the host and the environment. However, the species of coccidia mentioned here are always primary pathogens, i.e. it is not vital for other factors to co-exist if susceptible animals are infected with a certain dose of infective oocysts.

It is known that the strain virulence of some of the coccidia species that occur in poultry (*E. brunetti*, *E. tenella*, *E. maxima*, *E. necatrix*) can vary considerably and it is probable that this also applies to mammalian coccidia, however, respective studies have not been published so far.

Course of the infection

The course of an infection within a group of animals depends on various factors. Specifically in terms of the parasite, the determining factor is whether a certain infectious dose can be ingested at a certain time. The variability of the course of coccidiosis, measured primarily by oocyst excretion, is striking even under experimental conditions with identical infection parameters. This variability has been demonstrated clearly in infections with *I. suis* and *E. zuernii* which have been studied more closely.

The conditions under which animals are infected in the field differ from those that prevail in experimental infections. The global prevalence of the coccidia in mammalian livestock is very high and it can be assumed that each individual animal will acquire infection sooner or later (Gräfner et al., 1985; Fox, 1985; Barutzki et al., 1990; Mundt et al., 2005b). If exposure is low, most infections will remain subclinical and will thus remain undetected. In regions and farms/herds with a high density of susceptible animals higher infection pressure increases the risk of infection and subsequent disease. There are, however, considerable differences between animal species.

Coccidiosis basically takes a similar course in piglet-rearing facilities. It is currently assumed that small numbers of *I. suis* oocysts persist in the farrowing pens, and that these infect individual piglets immediately after birth, thus initiating the first reproductive cycle. This only causes a small amount of damage, and it is unusual to see sick piglets in the first week after birth. However, there is a sharp rise in oocyst contamination at the end of the first week; the pressure of infection increases considerably and clinical coccidiosis is seen in the second and third weeks after birth. If the contamination with *I. suis* is higher directly after birth the infection is likely to interfere with bacterial colonization. Under certain conditions severe *Clostridium perfringens* infections with even a high mortality may result (Mundt et al., 2008; Mengel et al., 2012).

The conditions prevailing for ruminants are different. The frequently high density of animals and the limited possibilities for ensuring hygiene in sheep-rearing make it extremely likely those fully susceptible lambs will be born into a highly contaminated environment and may fall ill at an early stage. They may also become ill later on when older animals are transferred to a highly contaminated environment (e.g., when they are turned out to pasture). The course of bovine coccidiosis can also beextremely variable depending on management conditions and the type of production.

On problem farms, coccidiosis manifests clinically or can lead to financial losses even without causing clinical symptoms, e.g., failure to gain weight (Fitzgerald, 1980; Bürger, 1983; Gräfner et al., 1985; Fox, 1985; Gjerde and Helle, 1991; Alzieu et al., 1999; Bach et al., 2003; Maes et al., 2007).

The prevalence of oocyst excretion and to a limited degree the intensity as well, correlates positively with the clinical picture. A prevalence of around 60 to 70% in a cattle herd and a high number of *E. bovis* or *E. zuernii* in the faeces is indicative of a high pressure of infection. If this pressure is low, the prevalence tends to be around 20 to 30%, with fewer than 1000 or 500 pathogenic opg being excreted by individual animals.

Relevant infections in lambs are caused by the pathogenic species *E. crandallis* and *E. ovinoidalis*. Animals become infected in a heavily contaminated environment (stable or pasture) immediately after birth or shortly after they have been introduced to the contaminated environment. The infection is very extensive, as is the degree of oocyst excretion.

Diagnosis

Diagnosis of coccidial infections or clinical coccidiosis must address a number of questions. The usual approach is to examine oocyst excretion in the faeces (semiquantitatively or quantitatively) and to differentiate the coccidial species (pathogenic and non-pathogenic species).

The identification of coccidial oocysts in the faeces or in the bedding shows the general prevalence on a farm or in a section of a stable; if the pathogens are differentiated, this approach shows the prevalence of a certain species. Correlating oocyst excretion with existing clinical symptoms, or to characterize an infection on the basis of oocyst excretion in a group of animals over time is difficult.

Correlation between parasitological and clinical picture

Oocyst excretion basically correlates well with the clinical picture. This can be demonstrated clearly under defined experimental conditions in an infection model. Under natural infection conditions the correlation between the clinical and the parasitological picture is better the more animals in a herd or group are affected (extent) and the more intense the infection is. However, oocyst excretion is very variable and the extent of excretion does not always reflect the severity of the clinical disease in the respective animal. The relationship between the diagnosis of the pathogen and the degree of clinical disease may be difficult to assess in cases of less severe infection and other factors (infectious and

non-infectious) that contribute to the development of coccidial diarrhea or induce enteritis irrespective of coccidian may complicate correct conclusions.

Hygiene and control

Since coccidiosis always develops as a result of infection from a contaminated environment, it appears reasonable to strive for control by improved hygiene measures. In practice, however, sufficient decontamination of the environment (e.g., pastures, housing on deep litter) is often not a realistic option. Although hygiene measures alone are not sufficient to obtain control in herds where coccidiosis causes health problems they should always be considered as an important aspect of successful coccidiosis control. Even when attention to hygiene is rigorous, as in piglet rearing, clinical coccidiosis may still develop. This is mainly due to the nature of the parasites and the fact that just a few oocysts are all it takes to initiate the development of an infection and to rapidly increase the infection pressure to a critical level. Given the common housing conditions in ruminant management, the hygiene measures that can realistically be applied are very limited and areas once contaminated with oocysts (e.g., pastures, stables with deep litter) will remain so for weeks or months.

Whenever chemotherapy is necessary it should be conducted at an appropriate time during the development of the parasite before the onset of clinical signs. In other words only treatments during the prepatent period prevent damage and economic losses to a high degree.

References

Alzieu J.P., Mage C., Maes L., De Mûelenaere C. 1999. Economic benefits of prophylaxis with diclazuril against subclinical coccidiosis in lambs reared indoors. Vet. Rec. 144(16):442-4.

Bach U., Kalthoff V., Mundt H.-C., Popp A., Rinke M., Daugschies A., Luttge B. 2003. Parasitological and morphological findings in porcine isosporosis after treatment with symmetrical triazintriones. Parasitol Res. 91(1):27-33.

Barutzki D., Marquardt S., Gothe R. 1990. *Eimeria* infections of sheep in Northwest Germany.Vet. Parasitol. 37:79-82.

Bohrmann R. 1991. Treatment with toltrazuril in natural outbreak of coccidiosis in calves. Dtsch. Tierärztl. Wochenschr. 98:343-345.

Bürger H.-J. 1983. *Eimeria*-Infektionen beim Rind. Berl. Münch. Tierärztl. Wochenschr. 96:350-357.

Catchpole J., GREPORY M.W. 1985. Pathogenicity of the coccidium *Eimeria crandallis* in laboratory lambs. Parasitol. 91:45-52.

Daugschies A., BÖSE R., MARX J., TEICH K., FRIEDHOFF K.T. 2002. Development and application of a standardized assay for chemical disinfection of coccidia oocysts. Vet. Parasitol. 103:299-308.

Daugschies A., Bürger H.-J., AkimarU M. 1998. Apparent digestibility of nutrients and nitrogen balance during experimental infection of calves with *Eimeria bovis*. Vet. Parasitol. 77:93-102.

Daugschies A., Najdrowski M. 2005. Eimeriosis in cattle: current understanding. J. Vet. Med. B. 56:417-427.

Daugschies AM. Akimaru M., Bürger H.-J. 1986. Experimentelle *Eimeria bovis*-Infektionen beim Kalb: 1. Parasitologische und klinische Befunde. Dtsch. Tierärztl. Wschr. 93:393-397.

Faber J.E., KOLLMANN D., HEISE A., BAUER C., FAILING K., BÜRGER H.J., ZAHNER H. 2002. *Eimeria* infections in cows in the periparturient phase and their calves: oocyst excretion and levels of specific serum and colostrum antibodies. Vet. Parasitol. 106:1-17.

Fitzgerald P.R. 1980. The economic impact of coccidiosis in domestic animals. Adv. Vet. Sci. Comp. Med. 24:121-143.

Fox J.E. 1985. Coccidiosis in cattle. Mod. Vet. Pract. 66:113-116.

Gjerde B., HELLE O. 1991. Chemoprophylaxis of coccidiosis in lambs with a single oral dose of toltrazuril. Vet. Parasitol. 38(2-3):97-107.

Gräfner G., Graubmann H.-D., Schwartz K., Hiepe T., Kron A. 1985. Weitere untersuchungen zu vorkommen, epizootiologie und bekämpfung der *Eimeria*-kokzidiose des rindes unter den bedingungen der intensiven stallhaltung. Mh. Vet. Med. 40:41-44 (In German).

Gregory M.W., Catchpole J. 1987. Ovine coccidiosis: Pathology of *Eimeria ovinoidalis* infection. Int. J. Parasitol. 17(6):1099-1111.

Hooshmand-Rad P., Svensson CA., Uggla A. 1994. Experimental *Eimeria alabamensis* infection in calves. Vet. Parasitol. 53:23-32.

Maes D., Vyt P., Rabaeys P., Gevaert D. 2007. Effects of toltrazuril on the growth of piglets in herds without clinical isosporosis. Vet. J. 173(1):197-9.

Mengel H., Krüger M., Krüger M.U., Westphal B., Swidsinski A., Schwarz S., Mundt H.-C. Dittmar K., Daugschies A. 2012. Necrotic enteritis due to simultaneous infection with *Isospora suis* and clostridia in newborn piglets and its prevention by early treatment with toltrazuril Parasitol. Res. 110:1347-1355.

Mundt H.-C., Krüger M., Westphal B., Mengel H., Krüger M.U., Dittmar K., Kuhnert Y., Daugschies A.2008. Investigations into the synergic effect of postnatal experimental infection with *Isospora suis* and natural infection with *Clostridium perfringens β 2* for the development of necrotic enteritis in piglets. 20th IPVS, June 22-25, Durban, South Africa.

Mundt H.-C., Bangoura B., Rinke M., Rosenbruch M., Daugschies A. 2005a. Pathology and treatment of *Eimeria zuernii* coccidiosis in calves: investigations in an infection model. Parasitol. Int. 54(4):223-30.

Mundt H.-C., Cohnen A., Daugschies A., Joachim A.H., Prosl H.R., Schmäschke R., Westphal B. 2005b. Occurrence of *Isospora suis* in Germany, Switzerland and Austria. J. Vet. Med. B 52, 93-97.

Mundt H.-C., Daugschies A., Joachim A., Wüstenberg S. 2003. Piglet coccidiosis update. Pig Progress 2003:23-24.

Mundt H.-C., Joachim A., Becka M., Daugschies A. 2006. *Isospora suis*: an experimental model for mammalian intestinal coccidiosis. Parasitol Res. 98(2):167-75.

Mundt H.-C., Uebe F., Rinke M., Daugschies A. 2004. Histopathological study of the intestinal mucosa of calves after experimental infection with *Eimeria bovis* – Comparison between untreated calves and calves treated with toltrazuril (Baycox 5%, Oral Suspension) at different times. 23rd World Buiatrics Congress, 11-16 July, Quebec, Canada.

Stockdale P.H.G. 1977. The pathogenesis of the lesions produced by *Eimeria zuernii* in calves. Can. J. Comp. Med. 41(3):338-344.

Svensson C., Olofsson H., Uggla A. 1996. Immunisation of calves against *Eimeria alabamensis* coccidiosis. Appl. Parasitol. 37:209-216.

Taylor A., Catchpole J. 1994. Review article: Coccidiosis of domestic ruminants. Appl. Parasitol. 35:73-86.

THE FLOTAC STRATEGY: NEW APPROACH FOR THE DIAGNOSIS OF HUMAN AND VETERINARY PARASITES

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Accurate diagnosis of parasitic infections is of pivotal importance for both individual patient management and population-based studies, such as drug efficacy trials and surveillance of parasitic disease control and elimination programs, in both human and veterinary public health. After the development and validation of the FLOTAC techniques, i.e. new multivalent techniques for qualitative and quantitative copromicroscopic diagnosis of parasites in animals and humans, the so-called FLOTAC strategy is proposed for a standardized parasitological diagnosis, from the sampling to the laboratory analysis. For these purposes, new devices, which are part of the FLOTAC family of materials, are now available, namely Fill-FLOTAC and Mini-FLOTAC. Fill-FLOTAC is a disposable sampling kit. It consists of a collector and a filter and facilitates the performance of the first five consecutive steps of the FLOTAC techniques: (i) collection (including weighing), (ii) transfer of the fixative, (iii) homogenization, (iv) filtration and (v) transfer into tubes. Mini-FLOTAC is a new apparatus, a further development and simplification of FLOTAC without requiring any centrifugation step, which comprises two physical components, namely the base and the reading disc. There are two 1-ml flotation chambers, which are designed for optimal examination of faecal sample suspensions in each flotation chamber (total volume = 2 ml) and which permits a maximum magnification of × 400. Mini-FLOTAC can be used for performing the three Mini-FLOTAC techniques (basic, dual and double), which are variants of a single technique but have different applications. The seven operating steps of the Mini-FLOTAC techniques are: (1) weigh the faecal sample (2 g for dogs, cats and humans + 2 ml of formalin 5%; 10 g for herbivores); (2) add the flotation solution (FS) using a dilution ratio of: (a) 1:20 for dogs, cats and humans with an analytic sensitivity of 10 parasitic elements per grams (PEG = eggs, larvae, oocysts and cysts); (b) 1:10 for herbivores (analytic sensitivity = 5 PEG); (3) homogenize; (4) filter; (5) fill the two Mini-FLOTAC flotation chambers; (6) wait 10 min; (7) translate and examine under a microscope. Some preliminary studies conducted to compare the sensitivity and efficiency of different copromicroscopic techniques for detecting and counting parasitic elements from different animal species, including humans, suggest that the Mini-FLOTAC techniques are rapid and promising methods and can be used in place of the FLOTAC techniques, the “Gold standard”, in laboratories where the centrifugation step cannot be performed.

TICKS AND TICK-BORNE DISEASE OF DOGS IN THE UK

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Ticks are clinically-important blood-feeding ectoparasites that cause damage and disease in their hosts as they feed, either directly through blood-loss, the transfer of saliva and injection of neurotoxins or indirectly through the transmission of an extensive range of bacteria, viruses and protozoa (Otranto and Wall, 2008). Changes in tick distributions and the prevalence of tick borne disease within Europe have been reported over the last 10-20 years (Gray et al., 2009; Jameson and Medlock, 2011). The most widespread species, *Ixodes ricinus*, is thought to have extended its range in Europe at the northerly limits of its distribution (Lindgren et al., 2000) and, associated with this spread, new foci of tick-borne encephalitis virus have been reported (Lindgren and Gustafson, 2001). The distribution of the European meadow tick, *Dermacentor reticulatus*, an important vector of canine babesiosis in Europe, is also believed to have extended northwards and populations have become established in Poland, Belgium, Germany, the Netherlands and in southern England (Beugnet, 2009; Jameson and Medlock, 2011). *Anaplasma platys* anaplasmosis has been recorded in France and *A. phagocytophilum* anaplasmosis in cattle, horses, dogs and cats in northern Europe (Beugnet, 2009). A clear trend for the northwards expansion of the brown dog tick, *Rhipicephalus sanguineus* has also been observed; this species is of particular importance as a vector of babesiosis, ehrlichiosis and hepatozoonosis (Dantas-Torres, 2010). Climate change may be responsibe for part of this, but changes in habitat management and host availability may also be important.

Average temperatures have increased significantly over the last 50 years and it is predicted that temperatures may increase by a further 0.4 °C within the next two decades and between 1-4 °C in the next 100 years (IPCC, 2007). Such changes will inevitably affect the seasonal activity of different tick life-cycle stages and alter the latitudinal and altitudinal limits to their distribution. In northern Europe future climate warming, with longer summer seasons and warmer and wetter spring or autumn, might be expected to promote higher challenge and longer periods of tick exposure particularly extending the spring and autumn windows in which ticks are active and questing. Tick mortality may be lower given milder winters, but higher in hotter drier summers. Seasonal activity patterns may therefore become more strongly bimodal, particularly increasing the period of activity and feeding in the autumn. Changing climate may also affect habitat structure and the the population dynamics and survival rates of vertebrate hosts and these effects may interact, altering the complex transmission cycles. Given the complexity of the climate/vector/host/pathogen interactions it is extremely difficult to make confident predictions about future tick-borne disease epidemiology. It is also difficult to differentiate the impact of climate-mediated changes on tick distributions from underlying changes in host abundance, in particular deer populations, which are also believed to have expanded in many areas.

Conservation strategies which encourage the greater integration of green-spaces into the urban and peri-urban environments and management for wildlife and biodiversity, also facilitate an increase in abundance of hosts such as small mammals and deer. This is likely to lead to an increase in the abundance and distribution of ticks and an increased exposure of people and companion animals to ticks in environments such as gardens and parks. Changes may also be associated indirectly with social and economic trends, often mediated by climate, which influence outdoor activity, exposure and the risk of being bitten (Godfrey and Randolph, 2011).

A wide variety of tick species are established in the UK (Table 1), most of which are specialist parasites of birds or small mammals. The most abundant generalist, *Ixodes ricinus*, is already very widely distributed, so there is relatively little scope for further expansion of its range, other than perhaps in terms of altitudinal increase. Nevertheless, its abundance was reported to have increased at 73% of locations surveyed (Scharlemann et al., 2008) and serologically confirmed cases of Lyme disease in humans, which is transmitted by *I. ricinus*, have increased 5-fold between 2000 and 2009 (Defra, 2009). Of particular interest is *Dermacentor reticulatus* which is thought to have been introduced into the UK and existed in an isolated population in west Wales for many years. However, the species has now apparently expanded its range and populations have been found recently in southeast and southwest England (Medlock and Jameson, 2010).

The recent removal of compulsory tick treatment for companion animals entering the UK from continental Europe is a further factor which has raised fears about the potential for the introduction of new tick species or new tick-borne pathogens. Of particular concern is the brown dog tick, *R. sanguineus*. This species is found worldwide, but in Europe it has a predominantly Mediterranean distribution. Although isolated introduced cases have been found sporadically in the UK it has not yet become established. Notably, it has been removed from 'treated' pets at entry into the UK ports, indicating that the acaricide treatment of imported dogs has not been completely effective (Jameson and Medlock, 2011). Current UK climate conditions are not thought to be appropriate to allow the outdoor overwinter survival of this species. Nevertheless, the tick can survive in protected microclimates, such as residential houses, and could potentially survive within kennels. Under appropriate conditions, it can complete its life cycle in only a few months and can therefore increase in abundance extremely quickly. If *R. sanguineus* were to become established in the UK, it may then be easier for novel introduced pathogens to establish and circulate, because the entire life-cycle of this tick species may be associated with the dog in an enclosed environment.

The highlighted concerns over the potential impacts of changing climate and increased global movement of people and companion animals on the distribution of ticks highlight the need for an accurate understanding of existing prevalence patterns, without which future changes cannot be detected. In recent studies, the distribution and prevalence of ticks infesting domestic dogs in Great Britain were examined (Smith et al., 2011). A total of 173 veterinary practices were recruited to monitor tick attachment to dogs in their local area between March and October 2009. Practices selected five dogs at random each week from those brought to the surgery and undertook a thorough, standardised examination for ticks. Each veterinary practice participated for three months before being replaced. Any ticks identified were collected and a sample sent to the investigators for identification along with a clinical history. A total of 3534 dogs were examined; 810 dogs were found to be carrying at least one tick. *Ixodes ricinus* was identified in 72.1% cases, *Ixodes hexagonus* in 21.7% and *Ixodes canisuga* in 5.6% cases. Five samples of *Dermacentor reticulatus* were also received, adding to the growing evidence that an established population of *D. reticulatus* now exists in southern England. Almost all the ticks found were adults. No ticks were detected by 19% of the veterinary practices, 50% of the veterinary practices reported that at least 14.9% of the dogs seen were infested and 14.6% reported that more than 50% of the dogs inspected carried ticks. A number of risk factors were associated with the likelihood of tick attachment on dogs. Gundog, terrier and pastoral breed groups were more likely to carry ticks, as were non-neutered dogs. Dogs with shorter hair were less likely to have ticks, and dogs were most likely to carry a tick in June. The ticks collected were examined for the presence of *Borrelia burgdorferi* s.l. *Babesia* and *Anaplasma* using PCR (Smith et al., 2012). Overall, *Borrelia* was detected in 17/739 (2.3%) of ticks tested, *Babesia* spp. in 78/742 (10.5%) of ticks tested and *Anaplasma* spp. in 253/675 (37.5%) of the ticks tested. Only one of the dogs carrying an infected tick had travelled outside the UK. The data suggest that the prevalences of *Borrelia*, *Babesia* and *Anaplasma* in the UK tick population are considerably higher than most recent estimates indicate.

The effective control and management of ticks and tick-borne disease requires a comprehensive understanding of the biology and ecology of the ticks, the characteristics of the pathogens they transmit, the mechanisms of infection transmission and also the array of ecological and environmental factors which influence the interactions between them. Unfortunately, our understanding of such complex systems is, in many cases, far from complete.

Table 1. The species, distribution, primary host and vector status of tick species currently established in the UK

Species	Distribution	Host	Vector Status
Ixodes arbicola (tree hole tick)	Mostly England	Great tit/blue tit	
Ixodes caledonicus (Northern bird tick)	N. England/Scotland	Pigeons/corvids	
Ixodes frontalis (Passerine tick)	England	Ground feeding passerines	Avian tick-related haemorrhagic syndrome
Ixodes lividus (Sand Martin tick)	England	Sand Martin	
Ixodes rothschildi (Puffin tick)	SW England/SW Wales	Puffin	
Ixodes uniclavatus (Cormorant tick)	British coastal	Seabirds	
Ixodes acuminatus (Southern rodent tick)	Coastal SW England	Small rodents	
Ixodes apronophorus (Marsh tick)	SE England/ East Anglia	Water voal	
Ixodes canisuga (dog/fox tick)	Across Britain	Badger/fox/dogs	
Ixodes ventalloi (Rabbit tick)	Scillies/Lundy/Gower	Rabbit	
Ixodes trianguliceps (Shrew tick)	Across Britain	Small mammals	*Anaplasma phagocytophilum*
Ixodes vespertilionis (Long-legged bat tick)	SW England/N Wales	Horseshoe bats	
Ixodes hexagonus (Hedgehog tick)	Across Britain	Hedgehog/dogs/cats	*Borrelia burgdorferi*
Ixodes ricinus (Deer/Sheep tick)	Across Britain	Deer/sheep, dogs, cats, birds, etc.	*Borrelia burgdorferi* *Anaplasma phagocytophilum* *Babesia divergens/B. microti*, Louping ill (*Rickettsia helvetica)*
Dermacentor reticulatus (Marsh tick)	N. Wales/Devon/Essex	Dog/sheep/cattle/horses	Rickettsia *roultii*
Haemaphysalis punctata (Coastal red tick)	SE England /Wales	Birds/sheep/cattle/humans	
Argas reflexus (Pigeon tick)	England	Domestic pigeons	
Carios maratimus (Marine argasid)	British coastal	Seabirds	
Carios verspertilionis (Blyborough tick)		Bats	

References

Dantas-Torres F. 2010. Biology and ecology of the brown dog tick, *Rhipicephalus sanguineus*. Parasit. Vectors 3:26.

Defra F. 2009. Zoonosis Report. http://www.defra.gov.uk/publications/files/pb13571-zoonoses 2009-110125.pdf.

Godfrey E.R., Randolph S.E. 2011. Economic downturn results in tick-borne disease upsurge. Parasit. Vectors 4:35.

Gray J.S., Dautel H., Estrada-Pena A., Kahl O., Lindgren E. 2009. Effects of climate change on ticks and tick-borne diseases in Europe. Interdiscip. Perspect. Infect. Dis. 2009:593232.

Intergovernmental Panel on Climate Change, 2007. IPCC Fourth Assessment Report (AR4)-Climate Change 2007: Synthesis Report. Geneva, Switzerland.

Jameson L.J., Medlock J.M. 2011. Tick surveillance in Great Britain. Vector Borne Zoonotic Dis. 11:403-412.

Lindgren E., Talleklint L., Polfeldt T. 2000. Impact of climatic change on the northern latitude limit and population density of the disease-transmitting European tick *Ixodes ricinus*. Environ. Health Persp. 108:119-123.

Lindgren E., Gustafson R. 2001. Tick-borne encephalitis in Sweden and climate change. Lancet 358:16-18.

Medlock J.M., Jameson L.J. 2010. Ecological approaches to informing public health policy and risk assessments on emerging vector-borne zoonoses. Emerg. Health Threat. J. doi: 10.3134/entj.10.002

Otranto D., Wall R. 2008. New strategies for the control of arthropod vectors of disease in dogs and cats. Med. Vet. Entomol. 22:219-302.

Scharlemann J.P.W., Johnson P.J., Smith A.A., Macdonald D.W., Randolph S.E. 2008. Trends in ixodid tick abundance and distribution in Great Britain. Med. Vet. Entomol. 22:238-247.

Smith F., Ballantyne R., Morgan E., Wall R. 2011.The incidence of tick infestation on domestic dogs in the UK. Med. Vet. Entomol. 25:377-384.

Smith F.D., Ballantyne R., Morgan E., Wall R. 2012. Estimating Lyme disease risk using pet dogs as sentinels. Comp. Immunol. Microb. 35:163-167.

SUSTAINABLE CONTROL OF GASTRO INTESTINAL NEMATODES; USE OF MEDITERRANEAN BIOACTIVE PLANT

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The routine use of commercial chemical anthelmintics has for many years been the basis of control programs for gastrointestinal nematodes. However, the rapid development and wide distribution of anthelmintic resistance in nematode populations combined with consumers concern for chemical residues in animal products has renewed interest in promoting more integrated and sustainable approaches of control. Such approaches should combine a) knowledge on how to manipulate parasite's life cycle characteristics, the climatic conditions, the specific husbandry characteristics b) the potential of using bioactive plants 2) breeding projects aiming to increase resilience to parasites and 3) strategic use of anthelmintics.
Among those the possible exploitation of bioactive forages, rich in condensed tannins, with anthelmintic properties, by incorporation in the diet of sheep or goats, seems a promising option to reduce the reliance on chemical molecules. This is even more critical under Mediterranean conditions, where the breeding of small ruminants usually relies on the exploitation of rangelands which are covered with a variety of plants usually rich in plant secondary metabolites. Our results so far include the activity of different plants specifically the lentisk tree (*Pistacia lentiscus*), the kermes oak tree (*Quercus coccifera*) and the carob tree (*Ceratonia siliqua*). The *in vitro* anthelmintic effect of the above was measured by the Larval Migration Inhibition Assay on *Haemonchus contortus.* The results indicated the presence of anthelmintic activity for all plant extracts confirming the main role of tannins, after adding an inhibitor of tannins, for the lentisk and the carob extracts. Furthermore, in *in vivo* experiments the consumption of the plants by lambs experimentally infected with *H. contortus* and *Trichostrongylus colubriformis*, was associated with significant decreases of egg excretion, and overall related with decreased worm fecundity. Significant reductions of *T. colubriformis* populations were also observed by using a carob flour rich diet.

SCHISTOSOMIASIS CONTROL IN AFRICA: HOPES AND NEEDS

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Schistosomiasis remains a major public health problem across Africa and one of the most important of the neglected tropical diseases (NTDs). Estimates suggest that there may be as many as 201.5 million cases of schistosomiasis in Africa representing over 90% of all known cases. The two main species responsible for infection are *Schistosoma haematobium* and *S. mansoni* that cause urogenital and intestinal schistosomiasis respectively. Both species have wide and often overlapping distributions reflecting in part the distribution of their intermediate snail hosts (*Bulinus* and *Biomphalaria* spp.). There is still much to be done in terms of morbidity control across large areas of Africa. An increasing number of endemic countries have initiated national NTD control progammes and with donor support, particularly through the Schistosomiasis Control Initiative and USAID, are implementing treatment programmes. The main interventions for schistosomiasis control, in addition to preventive chemotherapy using praziquantel, are snail control, sanitation, safe water supplies and behaviour change strategies. Unfortunately relative few studies, or indeed control programmes, relating to schistosomiasis in Africa have been carried out using additional interventions in recent years. Here we will consider studies relating to schistosomiasis elimination that have recently commenced on the islands of Zanzibar. The recent commitment by Merck Serono to increase substantially donations of praziquantel, together with the new WHO strategy for schistosomiasis control, is set to change the schistosomiasis disease landscape over the coming decade. As countries increase their efforts towards alleviating the burden of this debilitating disease it is important to consider whether schistosomiasis elimination is an achievable goal. There are good examples of where elimination has already been achieved by the integration of preventive chemotherapy with the tools of transmission control. In this paper we will consider some of the key questions that need to be considered as schistosomiasis control programmes continue to expand across Africa.

DYNAMIC ORGANIZATION OF THE TEGUMENT OF SCHISTOSOME PARASITES: RELEVANCE FOR VACCINE DESIGN

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Schistosomes infect over 200 million people world-wide and are responsible for significant human morbidity. These parasites inhabit the blood system of their mammalian hosts. The success of human blood flukes as inhabitants of the host bloodstream can be attributed, at least in part, to their pre-adapted tegument. The tegument is a remarkable cellular entity. Composed of a single syncytial cytoplasm and supported by numerous insunken nucleated cell bodies, the tegument of schistosomes is a highly interactive and dynamic layer. The tegument is maintained by coordinated and orchestrated activity of numerous molecules and organelles that transport materials through the layer. Upon entry into the human host, schistosome cercariae transform their surface membrane from a single layered membrane adorned with an extensive glycocalyx into a double membrane structure that is host adapted. Extensive proteomic analyses of the tegument of schistosomes reveal a series of membrane-resident molecules that are predicted to sit in the apical membrane complex of the tegument (Braschi and Wilson, 2006; Mulvenna et al., 2010). Some of these molecules have been tested in vaccination studies against experimental schistosomiasis, including S. mansoni tetraspanin-2 (SmTSP2) and Sm29 (Tran et al., 2006; Cardoso et al., 2008). Putatively resistant people in endemic regions show strong protective antibody responses to these proteins in sera (Cardoso et al., 2006; Tran et al., 2006). RNAi suppression of SmTSP2 in early invasive stages of *S. mansoni* implicate this molecule in apical membrane turnover within the human host (Tran et al., 2010). Survival of schistosomes within the hosts is dependent in part on efficient establishment and maintenance of the tegumentary surface of the parasite.

In order to understand the roles that surface-related proteins of schistosomes have in tegument maintenance, we have conducted a comprehensive analysis of the “interactome” of schistosome surface proteins. For this we have prepared tegument proteins for blue-native gel electrophoresis (BN-PAGE) (Wittig and Schagger, 2008) of pre-formed protein complexes from a range of detergent extracts of schistosome tegument. Further in order to obtain a global view of surface interactions, parasites have been incubated in the presence of membrane-impermeable (BS3- bis (sulfosuccinimidyl) suberate) and permeable (DMS- dimethylsuberimidate) cross-linkers (Chang et al., 2004), their tegument compartment released and protein complexes analyzed using tandem mass spectroscopy after fractionation using SDS-PAGE. By comparison of protein mobility between a reference, non-crosslinked gel, and crosslinked gels a picture of the global ‘interactome’ is beginning to emerge. Finally, immuno-affinity experiments using antibodies for selected proteins on crosslinked samples have been used to verify results from shotgun experiments, confirming key interactions and definitively identifying proteins involved in interaction networks with key proteins such as SmTSP2 and Sm29.

In parallel, we have adapted cryo-preparation methods to prepare cryo-preserved samples of adult schistosomes for detailed immunocytochemistry. This method, incorporating high-pressure freezing and cryosubstitution in uranyl-acetate/methanol solutions (Jones et al., 2008; Jabbar et al., 2010). The method has enabled us to define with precision sub-cellular and organellar location of tegumentary proteins of schistosomes.

Here we present data from our interactome and localization studies. The results will provide better insights into the complex interactions of this intriguing cell layer and enable us to model more effectively the cell biology of the layer during invasion and development of these important parasites. These data may be important in understanding how vaccine targets function in the tegument during parasite development and illuminate how and when they may be susceptible to host immune attack.

References

Braschi S., Wilson R.A., 2006. Proteins exposed at the adult schistosome surface revealed by biotinylation. Mol. Cell Prot. 5:347-356.

Cardoso F.C., Macedo G.C., Gava E., Kitten G.T., Mati V.L., de Melo A.L., Caliari M.V., Almeida G.T., Venancio T.M., Verjovski-Almeida S., 2008. *Schistosoma mansoni* tegument protein Sm29 is able to induce a Th1-type of immune response and protection against parasite infection. PloS Negl. Trop. Dis. 2:e308.

Cardoso F.C., Pacifico R.N., Mortara R.A., Oliveira S.C., 2006. Human antibody responses of patients living in endemic areas for schistosomiasis to the tegumental protein Sm29 identified through genomic studies. Clin. Exp. Immunol. 144:382-391.

Chang Z.Z., Kuchar J., Hausinger R.P., 2004. Chemical Cross-linking and Mass Spectrometric Identification of Sites of Interaction for UreD, UreF, and Urease. J. Biol. Chem. 279:15305-15313.

Jabbar A., Kyngdon C.T., Gauci C.G., Walduck A.K., McCowan C., Jones M.K., Beveridge I., Lightowlers M.W., 2010. Localisation of three host-protective oncospheral antigens of *Taenia ovis*. Int. J. Parasitol. 40:579-589.

Jones M.K., Bong S.H., Green K.M., Holmes P., Duke M., Loukas A., McManus D.P., 2008 Correlative and dynamic imaging of the hatching biology of *Schistosoma japonicum* from eggs prepared by high pressure freezing. PloS Negl. Trop. Dis 2:e334.

Mulvenna J., Moertel L., Jones M.K., Nawaratna S., Lovas E.M., Gobert G.N., Colgrave M., Jones A., Loukas A., McManus D.P., 2010. Exposed proteins of the *Schistosoma japonicum* tegument. Int. J. Parasitol. 40:543-554.

Tran M.H., Freitas T.C., Cooper L., Gaze S., Gatton M., Jones M.K., Lovas E., Pearce E.J., Loukas A., 2010. Suppression of mRNAs encoding tegument tetraspanins from *Schistosoma mansoni* results in impaired tegument turnover. PloS Path. 6:e1000840.

Tran M.H., Pearson M.S., Bethony J.M., Smyth D.J., Jones M.K., Duke M., Don T.A., McManus D.P., Correa-Oliveira R., Loukas A., 2006. Tetraspanins on the surface of *Schistosoma mansoni* are protective antigens against schistosomiasis. Nat. Med. 12:835-840.

Wittig I., Schagger H., 2008. Features and applications of blue-native and clear-native electrophoresis. Proteomics 8:3974-3990.

COMPATIBILITY POLYMORPHISM IN THE INTERACTION BETWEEN *BIOMPHALARIA GLABRATA* AND *SCHISTOSOMA MANSONI*: FROM POPULATIONS TO MOLECULAR MECHANISMS

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Abstract
Coevolutionary dynamics in host-parasite interactions potentially lead to an arms race that result in compatibility polymorphism. The mechanisms underlying compatibility have remained largely unknown in the interactions between the snail *Biomphalaria glabrata* and *Schistosoma mansoni*, one of the agents of human schistosomiasis. This review presents a combination of data obtained from field and laboratory studies arguing in favour of a matching phenotype model to explain compatibility polymorphism. Investigations focused on the molecular determinants of compatibility have revealed two repertoires of polymorphic and/or diversified molecules that have been shown to interact: the parasite antigens *S. mansoni* Polymorphic Mucins and the *B. glabrata* Fibrinogen-related Proteins immune receptors. We hypothesize their interactions define the compatible/incompatible status of a specific snail/schistosome combination. This line of thought suggests concrete approaches amenable to testing in field-oriented studies attempting to control schistosomiasis by disrupting schistosome-snail compatibility.

BIOMPHALARIA GLABRATA INNATE IMMUNITY: SPECIFICITY AND MOLECULAR BASIS OF IMMUNE PRIMING RESPONSE AGAINST *SCHISTOSOMA MANSONI*

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Background
Biomphalaria snails are important invertebrates due to their role in the transmission of schistosomiasis, the second most widespread human parasitic disease after malaria (Chitsulo et al., 2004). Schistosomiasis or bilharzia is a tropical parasitic disease affecting 200 million humans in 74 countries, causing 200000 deaths annually (WHO, 2002). It is the second most important tropical disease in terms of morbidity after malaria. Schistosomiasis is caused by flatworms of the genus *Schistosoma* (Plathyhelminth, Digenea) (Chitsulo et al., 2004; Gryseels et al., 2006). Infection occurs in water by the free-living larval stages (cercaria for the human definitive host and miracidia for the mollusc intermediate host). To fight *S. mansoni* vaccines are not yet available and chemotherapy consists of a single drug, praziquantel for which resistance cases have been observed (Doenhoff et al., 2002; Melman et al., 2009). Consequently, significant attention has been paid to freshwater snails because of their medical and epidemiological importance as intermediate hosts for *Schistosoma* parasites. Thus a better understanding of the immunobiological interactions and of the compatibility (susceptibility/resistance status) between the invertebrate host *Biomphalaria glabrata* and its parasite *Schistosoma mansoni* could be of valuable importance in the discovery of new ways to prevent and/or control schistosomiasis diseases by limiting the parasite in the field.
Here we investigate a peculiar immune process identified in the *Biomphalaria/Schistosoma* model, the so call “immune priming”. Indeed previous study has described a time-dependent “acquired resistance” (immune priming) in *B. glabrata* snail against a second exposure to the same strain of *S. mansoni* (Sire et al., 1998).
However, the question of whether immune priming or memory is the privilege of vertebrate animals is controversial and remains under intense questioning (Kurtz, 2005; Sadd and Schmid-Hempel, 2006; Hauton and Smith, 2007; Vazquez et al., 2009). Several works provided evidences that invertebrate immunity could possess higher specificity levels. In the last decade, several transcriptomic approaches developed in different invertebrate species have revealed large and individual repertoires of putative immune receptors that could represent the molecular support of this specific recognition (SRCR or Sp185/333 of sea urchin; (Pancer, 2000)), in insects (DsCAM of *Drosophila melanogaster* and *Anopheles gambiae*; (Watson et al., 2005; Dong et al., 2006)) and in molluscs (FREPs of *Biomphalaria glabrata* (Zhang et al., 2004)). Moreover, we show recently that FREPs were involved in immune complexes with diversified antigens of the *B. glabrata* specific trematode pathogen *Schistosoma mansoni* (Mone et al., 2010).
If invertebrates have at one’s disposal this ability to recognize specifically antigens, they might also possess specific immune mechanisms that allow specifically recognizing and destroying a pathogen after a first exposure. This hypothesis was tested in the present work and several evidences for such specific secondary immune responses were reported. Underlying molecular mechanisms were investigated.
Methodology/Principal Findings
We investigate the specificity of this priming by comparing the infection success of *B. glabrata* snails submitted to successive infections using homologous and heterologous combinations. Secondly, we develop a histological approach to investigate the putative role of parasite development and migration events in the time dependant “acquired resistance” previously evidenced (Sire et al., 1998). Thirdly, we investigate the mechanisms involved in priming using snail exposure to irradiate miracidia and traumatic injuries. Fourthly, we investigate the molecular basis of immune priming using a bi-dimensional comparative proteomics approach (2D Gel) of snail plasma compartment. This approach permits to identify lectin family members as the main molecules differentially expressed following

priming. Finally, we followed lectin gene expression using real time quantitative PCR in primed snails following homologous and heterologous challenges.

Conclusions/Significance

We developed a combination of experiments aiming to confirm the existence of immune priming in this model and to characterize the specificity and molecular mechanisms involved in. We succeed for the first time to demonstrate that immune priming exists in our model and we identify part of the molecular determinants involved in this phenomenon. Characterizing the molecular determinants involved in the immuno-biological interaction between *Biomphalaria glabrata* and *Schistosoma mansoni* would be a valuable tool for identifying potential resistance genes that would be used for schistosomiasis vector mediation in the vector, *Biomphalaria glabrata* by introducing resistance genes into susceptible vector populations.

References

Chitsulo L., Loverde P., Engels D. 2004. Schistosomiasis. Nat. Rev. Microbiol. 2:12-13.

Doenhoff M.J., Kusel J.R., Coles G.C., Cioli, D. 2002. Resistance of *Schistosoma mansoni* to praziquantel: is there a problem? T. Roy. Soc. Trop. Med. H. 96:465-469.

Dong Y., Taylor H.E., Dimopoulos G. 2006. AgDscam, a hypervariable immunoglobulin domain-containing receptor of the *Anopheles gambiae* innate immune system. PloS Biol. 4:e229.

Gryseels B., Polman K., Clerinx J., Kestens L. 2006. Human schistosomiasis. Lancet. 368:1106-1118.

Hauton C., Smith V.J. 2007. Adaptive immunity in invertebrates: a straw house without a mechanistic foundation. Bioessays 29:1138-1146.

Kurtz J. 2005. Specific memory within innate immune systems. Trends Immunol. 26:186-192.

Melman S.D., Steinauer M.L., Cunningham C., Kubatko L.S., Mwangi I.N., Wynn N.B., Mutuku M.W., Karanja D.M., Colley D.G., Black C.L., Secor W.E., Mkoji G.M., Loker E.S. 2009. Reduced susceptibility to praziquantel among naturally occurring Kenyan isolates of *Schistosoma mansoni*. PloS Negl. Trop. Dis. 3:e504.

Mone Y., Gourbal B., Duval D., Du Pasquier L., Kieffer-Jaquinod S., Mitta G. 2010. A large repertoire of parasite epitopes matched by a large repertoire of host immune receptors in an invertebrate host/parasite model. PloS Negl. Trop. Dis. 4:e813.

Pancer Z. 2000. Dynamic expression of multiple scavenger receptor cysteine-rich genes in coelomocytes of the purple sea urchin. P. Natl. Acad. Sci. USA. 97:13156-13161.

Sadd B.M., Schmid-Hempel P. 2006. Insect immunity shows specificity in protection upon secondary pathogen exposure. Curr. Biol. 16:1206-1210.

Sire C., Rognon A., Theron A. 1998. Failure of *Schistosoma mansoni* to reinfect Biomphalaria glabrata snails: acquired humoral resistance or intra-specific larval antagonism? Parasitology 117:117-122.

Vazquez L., Alpuche J., Maldonado G., Agundis C., Pereyra-Morales A., Zenteno E. 2009. Review: Immunity mechanisms in crustaceans. Innate Immun. 15:179-188.

Watson F.L., Puttmann-Holgado R., Thomas F., Lamar D.L., Hughes M., Kondo M., Rebel V.I., Schmucker D. 2005. Extensive diversity of Ig-superfamily proteins in the immune system of insects. Science 309:1874-1878.

WHO 2002. TDR Strategic Direction for Research: Schistosomiasis. World Health Organization Information.

Zhang S.M., Adema C.M., Kepler T.B., Loker E.S. 2004. Diversification of Ig superfamily genes in an invertebrate. Science 305:251-254.

URINARY SCHISTOSOMIASIS IN IRAN: A DECADE AFTER INTERRUPTION OF TRANSMISSION

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Urinary schistosomiasis has been extensively studied over the past six decades in Iran. This infection used to be an endemic disease in southwestern of the country. Unlike other endemic countries of the region, transmission of the infection in Iran was confined to the western of Khuzestan Province bordering with Iraq. Prior to beginning of control measures in 1959 the numbers of infected individuals were estimated to be about 40000-50000. The integrated measures including active case detection, chemotherapy, molluscicide, health education, and environmental engineering efforts, caused a gradual, but significant reduction in number of cases; ultimately the number of egg passer patients reached to zero level in 2001. Alongside the above mentioned program, rapid development of infrastructures and improvement of life standards were the key elements towards our success. However elimination of disease requires to be confirmed by much more sensitive methods like Serological detection of urinary schistosomiasis using ELISA technique in juvenile born after 2001, and PCR detection of *S. haematobium* DNA in snail intermediate host, *Bulinus truncates*. Regarding current transmission of the infection in neighbouring Iraq and unauthorized traffic of visitors crossing the borders, the risk of resurgence of disease in prone areas of Khuzestan remains a major concern for health authorities in the country. This requires implementation of appropriate monitoring measures to prevent reintroduction of the urinary schistosomiasis in south western Iran.

SCHISTOSOMIASIS IN EUROPE

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Introduction

Schistosomiasis is a chronic and debilitating infection caused by schistosomatid blood flukes and is of considerable socio-economic importance affecting 200 million people in developing tropical and subtropical countries, with particularly high prevalence in Africa and Asia (Webster et al., 2006; Steinauer et al., 2008). However, in spite of the close proximity of Europe to Asia and Africa, little is known of the presence of agents of medical and veterinary schistosomiasis in European countries, nor of the potential risk of the disease as an emerging socio-economic problem in Europe (Casimiro et al., 2006). There is a long history of imported cases of schistosomiasis with infections presenting in human patients either returning from foreign travel or from immigrants from outside Europe (Grobusch et al., 2003), this is by far the most common cause of infection in humans but there are records of natural infection of urinary schistosomiasis from Portugal and other localities in southern Europe (Casimiro et al., 2006). This is also true of most of the animal cases, with infected cattle being transported from endemic regions as a result of the international meat trade (Arfaa et al., 1967; Wright, 1980; Mone et al., 1999). However, this article aims to discuss the issues of schistosomatid species endemic (or invasive) to Europe including the presence of established populations of *Schistosoma* and the associated condition of cercarial dermatitis.

Agents of schistosomiasis in Europe

Schistosomiasis is caused by parasites of the genus *Schistosoma*, and on most accounts the majority of human cases are imported infections of intestinal schistosomiasis caused by *Schistosoma mansoni* and *S. japonicum*, and urinary schistosomiasis caused by *S. haematobium* (Grobusch et al., 2003; Leder, 2009). *Schistosoma bovis*, the primary cause of cattle schistosomiasis, is also considered to be imported although historically it has been found in isolated foci throughout several countries around the Mediterranean (Wright, 1980; Mone et al., 1999). More recently natural foci of *S. turkestanica* (syn. *Orinetobilharzia turkestanicum*) have been identified in Hungary but its full host range in this region is not yet known (Majoros et al., 2010). It is also important to note that both *S. bovis* and *S. turkestanica* are known causative agents of the inflammatory skin condition cercarial dermatitis in humans, as are several species of non-*Schistosoma* schistosomatids which are primarily parasites of water fowl especially ducks, geese and swans (Kolářová, 2007). The bird schistosomatids have long been incriminated as agents of human cercarial dermatitis in Europe with *Austrobilharzia*, *Gigantobilharzia* and *Bilharziella* illustrated to cause human infection. However, the main cause of cercarial dermatitis in Europe is considered to be species from the genus *Trichobilharzia*, one of the most species rich genera in the Schistosomatidae (Kolářová, 2007).

Cercarial dermatitis

Cercarial dermatitis, also known as swimmers itch, is caused by the inflammatory response of schistosomatid cercariae penetrating the skin of non specific hosts (Kolářová, 2007). The disease presents as strong maculo-papulovesicular skin eruption leading to severe itching. There is also an association with pulmonary and neurological problems, especially with repeated exposure to infective cercariae. The disease is considered to be seasonal and outbreaks coincide with spring and summer months in temperate environments which have the highest release rates of cercariae, peaking snail populations and increased numbers of people indulging in water sports (Kolářová, 2007). The vast majority of European cercarial dermatitis cases are considered to be caused by bird schistosomes particularly species of *Trichobilharzia*, although as discussed previously other species from other genera can cause cercarial dermatitis including *Schistosoma* (Kolářová, 2007). There is a long history of records of swimmers itch throughout Europe with agents of the disease being described in the UK (Matheson, 1930) and France (Brumpt, 1931) before the Second World War. However, bird schistosomatids are know known throughout Europe and incriminated with outbreaks of swimmers itch in France (Ferté et al., 2005), Austria (Hörweg et al., 2006), Germany (Loy and Haas, 2001), Iceland (Skírnisson and Kolářová, 2005), Czech Republic (Kolářová et al., 1997), and the Netherlands

(Schets et al., 2008). In fact, in some European countries swimmers itch is now considered to be of substantial public health concern (Dorevitch et al., 2012).

Endemic causes of schistosomiasis

The ability of schistosomes to establish within a specific locality is predetermined by the occurrence of a specific intermediate snail host. Arfaa et al., (1967) illustrated that the Portuguese snail species *Bulinus contortus* was susceptible to Iranian isolates of *S. haematobium* and *S. bovis*. Both parasite species have been shown to be endemic to Europe with *S. haematobium* causing natural infections in the Algarve region of Portugal in the first half of the 20th century (Gracio, 1981) and *S. bovis* being found in several countries of southern Europe, especially around the Mediterranean basin (Wright, 1980; Mone et al. 1999). Although in recent decades there have been no reported cases of natural infections of *S. haematobium* in Portugal, and fewer cases of *S. bovis* infections throughout the Mediterranean. However, there is a presence of susceptible snail hosts such as *B. contortus* and *B. truncatus* and suitable climatic conditions which could allow successful reestablishment and transmission of both parasites in Southern Europe (Arfaa et al., 1967; Wright, 1980; Casimiro et al., 2006).

However, the diversity and distribution of trematodes throughout Europe is poorly understood and there is little knowledge of the structure of parasite communities in European countries. This was particularly illustrated in a study by Majoros et al., (2010) describing for the first time the occurrence of natural foci of *S. turkestanica* in the Gemenc Forest regions of Hungary, also known to have a history of cercerial dermatitis which was locally termed “water mange” (Majoros et al., 2010). The parasite was utilising red deer as the definitive host, being transmitted by the snail species *Radix auricularia*, and represented the first mammalian schistosome to be described in Central/Eastern Europe (Majoros et al., 2010). It is currently unclear if *S. turkestanica* is a potential emerging agent of zoonotic schistosomiasis in Europe (Majoros et al., 2010). The occurrence of *S. turkestanica* in Hungary was initially considered to be the result of a recent biological invasion from Asia as the parasite primarily infects ungulates and rodents in countries such as China, Russia, Iran and Turkey (Wang et al., 2009). However, recent studies on the molecular phylogeography of *S. turkestanica* indicate a distinct Hungarian (Eastern European) lineage, and molecular clock data suggest that this lineage diverged away from the Asian populations 100000 – 200000 years ago (Lawton and Majoros, unpublished). This also coincides with invasion of red deer into Europe from Iran and North Africa during inter glacial periods of the ice age (Ludt et al., 2004; Lawton and Majoros unpublished), if this is true then *S. turkestanica* should be considered as a truly Europe endemic schistosome and is likely to be found in other Balkan countries.

Schistosoma turkestanica represents an agent of schistosomiasis that is not restricted to tropical and sub tropical regions as described classically and is a true indication of the adaptability of schistosomes. The occurrence of *Schistosoma* in Hungary also illustrates the little known role of Europe in the evolution of schistosomes, as the current excepted theories are an “Out of Asia and into Africa” scenario (Lawton et al., 2011). It may now be plausible to consider an “Out of Asia and into Africa and Europe” scenario where populations of species that gave rise to the African schistosomes may have also diverged into certain parts of Europe. Conversely, it could be hypothesised that the schistosomes that gave rise to the ancestors of the African species invaded Africa, as they became isolated, due to geographical processes, the Asian populations of schistosome species went through another radiation which resulted in the invasion of Europe. However, only through further investigation of schistosome species and the identification of further European infection foci would a complete understanding of the establishment and diversification of these parasites in Europe be provided.

Final remarks

The presence of zoonotic agents of schistosomiasis in Europe has as yet brought little concern despite the public health issues considered to be caused by cercarial dermatitis. In fact, the lack of knowledge of the occurrence of populations of infected snails and trematode diversity within Europe makes it not only difficult to estimate endemicity of schistosomatid parasites but also the potential long term risk that they may cause (Casimiro et al., 2006). Both *S. bovis* and *S. turkestanica* are of veterinary importance and are already considered to be of economic concern in Africa (Aradaib et al., 1995) and Asia (Wang et al., 2009) respectively. *Schistosoma bovis* is already known to have historically caused problems around the Mediterranean but as yet the full extent of *S. turkestanica* as a veterinary agent of schistosomiasis in Eastern Europe is unclear. This is also true for non-schistosomatid agents of swimmers itch as new previously unknown foci are continually being identified (Dorevitch et al., 2012). Schistosomiasis and cercarial dermatitis are climate sensitive

diseases, and with continual changes in the European climate as well changes in peoples association with fresh water, the potential presence of the disease should be a real concern (Casimiro et al., 2006; Mas-Coma et al., 2009). As stated previously the establishment of schistosomiasis is predetermined by the occurrence of snail hosts. Both *Bulinus* and *Radix* have a long history in Europe but *Biomphalaria tenagophila*, an intermediate host of the medically important *S. mansoni,* has also been found to have invaded Romania providing potential foci the establishment of medical schistosomiasis (Majoros et al., 2008). Schistosomes are considerably adaptable and able to establish in the temperate zones of Europe, it is therefore only through continual monitoring, increased sampling and greater awareness of both trematode and snail diversity will it be possible to understand the distribution, occurrence and risk of endemic schistosomisis and its invasion from elsewhere.

References

Aradaib I.R., Abdelmageed E.M., Hassan S.A., Riemann H.P. 1995. A review on the diagnosis infection in cattle of *Schistosoma bovis*: current status and future prospects. Cienc. Rural. 25:493-498.

Arfaa F., Massoud J., Chu K.Y. 1967. Susceptibility of Portuguese *Bulinus contortus* to Iranian strains of *Schistosoma haematobium* and *S. bovis*. Bull. World Health Organ. 37:165-6.

Brumpt E. 1931. *Cercaria ocellata* déterminant la dermatite des nageurs, provient d'une bilharzie des canards. C. R. Acad. Sci. (Paris) 193:612-614.

Casimiro E., Calheiros J., Santos F.D., Kovats S. 2006. National assessment of human health effects of climate change in Portugal: approach and key findings. Environ. Health Perspect. 114:1950-6.

Dorevitch S., Pratap P., Wroblewski M., Hryhorczuk D.O., Li H., Liu L.C., Scheff PA. 2012. Health risks of limited-contact water recreation. Environ. Health Perspect. 120:192-7.

Ferté H., Depaquit J., Carré S., Villena I., Léger N. 2005. Presence of *Trichobilharzia szidati* in *Lymnaea stagnalis* and *T. franki* in *Radix auricullariain* north-eastern France: molecular evidence. Parasitol Res. 95:150-154.

Grácio M.A.A. 1981. A importância da malacologia em parasitologia médica . J Soc Cienc Med Lisb 2:119-140 ([in Portuguese).

Grobusch M.P., Mühlberger N., Jelinek T., Bisoffi Z., Corachán M., Harms G., Matteelli A., Fry G., Hatz C., Gjørup I., Schmid M.L., Knobloch J., Puente S., Bronner U., Kapaun A., Clerinx J., Nielsen L.N., Fleischer K., Beran J., da Cunha S., Schulze M., Myrvang B., Hellgren U. 2003. Imported schistosomiasis in Europe: sentinel surveillance data from TropNetEurop. J. Travel Med. 10:164-9.

Hörweg C., Sattmann H., and Auer H. 2006. Cercarial dermatitis in Austria: Questionnaires as useful tools to estimate risk factors? Wien. Klin. Wochenschr. 118 :77- 80.

Kolárová L. 2007. Schistosomes causing cercarial dermatitis: a mini-review of current trends in systematics and of host specificity and pathogenicity. Folia Parasitol. (Praha). 54(2):81-7

Kolárová L., Horák P., Sitko J. 1997. Cercarial dermatitis in focus: schistosomes in the Czech Republic. Helminthologia 34:127-139

Skírnisson K., Kolarova L. 2005. Swimmer's itch in Landmannalaugar, Iceland. Laeknabladid. 91(10):729-36.

Lawton S.P., Hirai H., Ironside J.E., Johnston D.A., Rollinson D. 2011. Genomes and geography: Genomic insights into the evolution and phylogeography of the genus *Schistosoma.* Parasit. Vectors. 4:131.

Lawton S.P., Majoros G. (Unpublished). DNA bar coding reveals distinct European lineage of the zoonotic parasite *Schistosoma turkestanica* (syn. *Orientobilharzia turkestanicum* (Skrjabin, 1913)).

Loy C., Hass C. 2001. Prevalence of cercariae from *Lymnaea stagnalis* snails in a pond system in Southern Germany. Parasitol. Res, 87:878- 882.

Ludt C.J., Schroeder W., Rottmann O., Kuehn R. 2004. Mitochondrial DNA phylogeography of red deer (*Cervus elaphus*). Mol Phylogenet Evol. 31: 1064-83

Majoros G., Dán A., Erdélyi K. 2010. A natural focus of the blood fluke *Orientobilharzia turkestanica* (Skrjabin, 1913) (Trematoda: Schistosomatidae) in red deer (*Cervus elaphus*) in Hungary. Vet Parasitol. 170(3-4):218-23.

Majoros G., Fehér Z., Deli T., Földvári G. 2008. Establishment of *Biomphalaria tenagophila* Snails in Europe. Emerging Infectious Diseases. 14:(11)1812-1813.

Mas-Coma S., Valero M.A., Bargues M.D. 2009. Climate change effects on trematodiases, with emphasis on zoonotic fascioliasis and schistosomiasis. Vet. Parastiol. 163:264-280.

Matheson S. 1930. Notes on *Cercaria elvae* Miller as the probable cause of an outbreak of dermatitis in Cardiff. Trans. R. Soc. Trop. Med. Hyg. 23:421.

Moné H., Mouahid G., Morand S. 1999. The distribution of *Schistosoma bovis* Sonsino, 1876 in relation to intermediate host mollusc-parasite relationships. Adv Parasitol. 44:99-138.

Schets F.M., Lodder W.J., van Duynhoven Y.T., de Roda Husman A.M. 2008. Cercarial dermatitis in the Netherlands caused by *Trichobilharzia* spp. J. Water Health. 6:187-95.

Steinauer M.L., Hanelt B., Mwangi I.N., Maina G.M., Agola L.E., Kinuthia J.M., Mutuku M.W., Mungai B.N., Wilson W.D., Mkoji G.M., Loker E.S. 2008. Introgressive hybridisation of human and rodent schistosome parasites in Western Kenya. Mol. Ecol. 17. 5062-5074.

Wang C.R., Chen J., Zhao J.P., Chen A.H., Zhai Y.Q., Li L., Zhu X.Q. 2009. *Orientobilharzia* species: neglected parasitic zoonotic agents. Acta Trop. 109(3):171-5.

Webster B.L., Southgate V.R, Littlewood T.J. 2006. A revision of the interrelationships of *Schistosoma* including the recently described *Schistosoma guineensis*. Int. J. Parasitol. 36:947-955.

Wright C.A. 1980. Biology and systematics of the molluscan hosts of Schistosomiasis in the Mediterranean basin in perspective. Parassitologia. 22(3):257-62.

TRANSMISSION MECHANISMS OF THE HEXACANTH LARVAE OF TAENIIDS OF MEDICAL AND VETERINARY SIGNIFICANCE

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Fully-developed hexacanth larvae (oncospheres) of taeniid cestodes are armed with three pairs of hooks and exhibit a distinct bilateral symmetry in their hook-muscle system and the arrangement of other cellular elements of their body. The following three structures of hexacanths play an important role in their transmission to the first host: [1] the hook-muscle system; [2] the penetration glands; and [3] the nerve cells. The **hook-muscle system** of all taeniids is similar to that of *Echinococcus granulosus* (see: Świderski and Eckert 1978; Świderski 1983), with the muscle cells of each hook organised into three systems: (1) a protraction system for hook extension, (2) an abduction system for drawing the hooks together towards the median plane of bilateral symmetry, and (3) a retraction system for withdrawing the hooks into the body (Świderski, 1983; 1988). Interconnections observed between different muscle fibres provide a structural basis for a coordinated, synchronised action. These hooks play an important role in penetrating the host tissue and thus initiating the mechanism of invasion. Two types of **penetration glands** were observed in hexacanths of *Echinococcus* spp. and in *Taenia ovis***,** type 1 (PG1), with a quadri-nucleate syncytium containing two lateral pairs of cell bodies interconnected by narrow cytoplasmic bridges, and type 2 (PG2), also with a quadri-nucleate syncytium (Świderski, 1982; 1988, Jabbar et al., 2010a; 2010b). In *T. ovis*, a third type, a uni-nucleate median mesophoric gland cell, also occurs (Jabar et al., 2010b). Penetration glands are mainly involved in the secretion of histolytic enzymes which cause the lysis and destruction of host tissues. However, immuno-cytochemical studies have shown that they are also an important stimulus for host-protecting antigens, and consequently their secretory products may have the potential to play an important role in the field of vaccine development. The complex hook and body movements observed during hexacanth activation, hatching and host tissue penetration are evidence of coordinated activities that are likely mediated by some kind of neurotransmission**.** The presence of typical **nerve cells,** with characteristic dense-cored vesicles, has been described for *Echinococcus* spp. (Świderski, 1982; 1994; 1997). The aims of studying hexacanth nerve cells of taeniids are twofold: one of scientific interest, i.e., a better knowledge of the infective eggs of this unique cestode family; and the other of potential veterinary-medical importance, i.e., the prevention of infection by the production of non-viable eggs, thus controlling or eradicating the parasite by disrupting its life-cycle. In targeting the nerve cells, it is hoped to find new chemotherapeutic agents that can act specifically on some neuronal transmitter or receptors of the infective larva, thus preventing activation, hatching and host penetration. The latter can be achieved by blocking the synchronised movements of the larval hooks via the hook-muscle system and/or limiting the secretory activity of the penetration glands and thus obstructing enzyme secretion.

References

Jabbar A., Crawford S., Młocicki D., Świderski Z., Conn D.B., Jones M.K., Beveridge I., Lightowlers M.W. 2010b. Ultrastructural reconstruction of *Taenia ovis* oncospheres from serial sections. Int. J. Parasitol. 40:1419-1431.

Jabbar A., Świderski Z., Młocicki D., Beveridge I., Lightowlers M.W. 2010a. The ultrastructure of taeniid cestode oncospheres and localization of host-protective antigens. Parasitology 137:521-535.

Świderski Z. 1982. *Echinococcus granulosus:* ultrastructure of the glandular regions of the infective oncosphere. In: Electron Microscopy 1982 (Ed. J.B. Le Poole and E. Zeitler). Proc. 10th Internat. Congress of Electron Microscopy, 17-24 August, 1982, Hamburg, 3:511-512.

Świderski Z. 1983. *Echinococcus granulosus:* hook-muscle system and cellular organisation of infective oncospheres. Int. J. Parasitol. 13:289-299.

Świderski Z. 1988. Comparative ultrastructure of taeniid eggs. In: Electron Microscopy, 1986 (Ed. V. Mangclaviraj). Proc. 6th Asia-Pacific Congress and Workshop on Electron Microscopy, July 26-August 4, 1988, Bangkok, Thailand, 1:697-698.

Świderski Z. 1994. *Echinococcus multilocularis:* ultrastructure of the oncospheral nerve cells. In: Electron Microscopy-1994: ICEM 13. Paris (Ed. B. Jouffrey and C. Colleix). Proc.13th International Congress on Electron Microscopy, Paris, 17-22 July, 1994, 3B:1425-1426.

Świderski Z. 1997. Ultrastructure of the oncospheral nerve cells in *Echinococcus granulosus* and *E. multilocularis*. In: Archivos Internationales de la Hidatidosis. (Ed. A. Menezes da Silva).Vol. 32. Proc. 18th Internatational Congress of Hydatidology, Lisboa, Portugal, pp. 126-128.

Świderski Z., Eckert J. 1978. Light and electron microscope studies on the muscle and hook systems of infective oncospheres of *Echinococcus granulosus.* Proc. 4th ICOPA (Ed. W. Ślusarski), ICOPA-IV, 19-26 August 1978, Warsaw, Poland, Short Communications, Section C: Parasitic Infections of Economic and Social Importance, vol. C, pp. 131-132.

MIGRATORY ROUTES OF HELMINTHS THROUGHOUT ORGANS OF INCOMPATIBLE HOST

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The importance of particular parasite species on the host state of health is usually deduced from organ/tissue disorders developing at final site where parasite adults or larvae locate. Prior to settle at specific final site, however, many helminthic parasites of vertebrates (including man) naturally migrate through various host organs. Under certain circumstances, therefore, serious pathologies can develop also due transient presence of migratory parasites in organs/tissues. In compatible definitive hosts, this situation may arise during prepatent phase of definitive host infection when larvae or immature worms migrate through the host body until they reach final location where they mature. In incompatible hosts parasites are not able to realize complete migration route and dye during various phases of infection without maturing; in humans this situation may arise during zoonotic infections.

Zoonotic diseases were reported mainly from tropical and subtropical countries in which their presence seems to be common. However, recent data show that a number of human zoonotic infections increases also in colder climates (Jenkins et al., 2011). When humans become infected by animal helminths, the causative agent of health disorders are parasite larvae. Pathogenesis of the zoonoses caused by the parasites which reside for their whole life-span in a particular organ where they can develop for many years are well known (e.g., hydatidosis). However, the infections caused by larvae migrating to and from various organs can be overlooked due to the fact that symptoms reflecting the actual presence of parasites and associated pathologies are temporary and disappear after few days. For example, this situation may arise during infections of humans by bird schistosomes (e.g., *Trichobilharzia* sp.) the life span of which in mammals usually does not exceed 14 days. Under certain circumstances, however, serious organ/tissue disorders can also develop. Severe pathologies are associated with infections by larvae the life span of exceeds months in incompatible hosts. During long-term survival (even years) the larvae can reside in a particular organ or can migrate to and from various organs during their whole life in incompatible host (e.g., *Toxocara* sp.). The severity of the diseases is influenced by many factors, among which the size of inoculum, duration of infection and the host immune status are the most important (Good et al., 2001).

Parasite products (e.g., antigens or toxic metabolites) are responsible for an inflammatory reaction at the site of parasite location; severe reactions can develop also around dead worms as a result of large amount of antigens released. Pathologies may also develop due by mechanical destruction of the tissue by immature worms (Kolářová, 2001). The immune response can be responsible for destroy of parasites, however, also additional factors, such as parasite nutrition requirements, can lead to a death and further elimination of infectious agent.

Human zoonotic infections can be acquired orally or percutaneously. When parasites are able to escape from the digestive tract or skin, the following migratory routes are species specific and the character of pathways reflects parasite and host biology. Within a species, parasites may exhibit different behaviour in various strains of host (e.g., Hamilton et al., 2006).

From the digestive tract or skin, most of parasites start their migration by entering to the venous/lymphatic vessels through which are transported to various visceral organs. However, larvae of some helminthic species prefer to enter into the nervous system and migrate further to the brain.

Transport through the venous system represents probably, the most frequent migratory route of worms. In incompatible hosts, after entering into the venous system of digestive tract or skin, parasites *via vena cava inferior* reach right heart → lungs → left heart → into systemic circulation which is considered to be responsible for their transport to organs similar as in compatible hosts. The distribution via the lymphatic is combined by entering into the larger lymphatic vessels, and later, through the lymph nodes parasites reach the larger veins and continue similarly as in previous case.

One of the most best example on explanation how parasites utilize dissemination through systemic circulation were obtained by studies on *Toxocara* sp. Larvae of the parasite migrate in this manner in compatible (dog, cats) as well as incompatible (rodents, humans) hosts (Magnaval et al., 2001). The studies on mice primarily infected by *Toxocara* showed that larvae are able to migrate into visceral organs (liver, lungs), and also to additional organs such as the eye and CNS under certain

circumstances. The probability of the eye and CNS involvement increases during strong infections by the nematode eggs or larvae and with progress of the infection (Hamilton et al., 2006; Camparoto et al., 2008). Till present, there are, however, ambiguities related to manner of passage through the lungs. It is known that only larvae which are able to enter the small lung capillaries which reach 8 μm diameter can pass through the organ. And this is not the case of *Toxocara* larvae the size of which is 400-450 x 15-20 μm. For *Baylisascaris,* it is suggested that big larvae (about 1.4 mm x 62 μm at 14 days) rupture pulmonary capillaries, enter pulmonary veins, return to the left heart, and gain access to the systemic circulation by which they can be distributed to various organs including the CNS (Kazacos and Boyce, 1989). This might be true also for *Toxocara* larvae; however, recent results obtained from studies on the experimentally infected rodents showed that also other migratory can be used by the parasites.

Using small imaging system (SAIS) our studies on behaviour of *Toxocara canis* larvae labelled with carboxyfluorescein succimidyl ester showed that many larvae burrowed to the alveolar wall or were found free in the alveoli. The accompanying histological examination showed intra- as well as extravascular location of the larvae not only in the lungs, but also in the liver and other organs. Our study also revealed simultaneous presence of *T. canis* larvae in the liver, lungs, brain and eye (Kolbeková et al., 2011). Similarly as for Abo-Shehada (1984/85), the results indicated that beside migration through the vessels, the parasites are able to migrate through tissues and the body cavity. According to Pecinali et al. (2005) this type of migration might be facilitated by the presence of larger fenestration and/or a discontinuous capillary system in the host organs.

The migratory route to the host CNS is also not known in detail. The studies on mice infected primarily by *T. canis* showed that invasion of larvae to the organ depend on the infection dose and time course of the primary infection. The higher size of inoculum is given the higher probability of larval invasion into the brain exists and with progress of infection increasing accumulation of larvae in the mouse brain can be noted (Good et al., 2001; Hamilton et al., 2006).

In contrary to *Baylisascaris*, the invasion of *T. canis* to the human CNS is considered to be unusual (Schantz, 1989). Instead of *Baylisascaris* larvae of which are not inherently neurotropic, various data suggest that the CNS involvement results from extensive somatic migration of the parasites; it is supposed that larvae reach from the systemic circulation various organs including the CNS and most likely they access the brain by penetrating cerebral blood vessels (Gavin et al., 2005). In *Toxocara* this type of enter into the brain tissues was not confirmed yet (Kolbeková et al., 2011).

It seems, that during invasion into the brain the *Toxocara* larvae have to cross blood brain barrier (BBB) but the mechanism which make it possible is uknown in detail. There are three ways how the pathogens may across BBB: trancelullar, paracellullar, and/or by means of infected pathogens (Katchanov and Nawa, 2010). It can be supposed that *Toxocara* larvae can cross BBB trans- or paracellularly similarly as it was reported for *Angiostrongylus* (Lee et al., 2006). This hypothesis can be supported by recent studies on neurotoxocarosis in experimentally infected mice which show that the presence of larvae in the CNS is associated with biochemical and immunopathologicalogical changes which develop during the BBB disruption (e.g., Hamilton et al., 2008; Liao et al., 2008; Othman et al., 2010).

Final count of *Toxocara* larvae in the brain is also influenced by the host immune status (Othman et al., 2010). In humans and mice repeatedly infected by the parasites it is believed that the migratory larvae are trapped in the liver soon after escape from the intestine by the host immune response which was stimulated by previous exposures to the parasites (Abo-Shehada et al., 1991). Contrary to primary infections, therefore, the spectrum of affected organs is considered to be lower than during reinfections. However, our study on *T. canis* reinfections revealed that administration of the larvae into mice soon after the primary infection can have opposite effect. Using SAIS, our study revealed greatly accelerated migration of the reinfecting parasites administered 3 weeks post primary infection to the host CNS and eyes; moreover in all challenged mice, reinfecting larvae prevailed in the resident parasite population (Kolbeková et al., 2011). In accordance with Sugane and Oshima (1983) it seems, therefore, that only mature immune response can protect against the CNS involvement.

The studies on behaviour of bird schistosomes of visceral *Trichobilharzia* sp. in mammals also showed that schistosomula can escape from the skin to internal organs. It is believed, that this escape can be stimulated by some chemo-atractive compounds which are recognised by the parasites. Soon after the transformation, schistosomula of *Trichobilharzia szidati* respond to chemical signals in mouse skin (L-arginine, L-arginine-containing peptides, and D-glucose (Grabe and Haas, 2004).

Studies on various species of the bird visceral schistosomes of *Trichobilharzia*, *Gigantobilharzia*, *Bilharziella* and *Ornithobilharzia* (for a review see Horák et al., 2002) revealed in experimentally infected mammals that the parasites are able to migrate similarly as for *Toxocara* under some circumstances. Schistosomula of visceral *Trichobilharzia* species were found in the lungs and, moreover, they were also detected in other organs such as liver, kidney, heart and intestine of various mammals (Haas and Pietsch, 1991; Chanová et al., 2007).

The studies of Chanová et al. (2007) on mice infected by *T. szidati* revealed schistosomula in the lungs of primoinfected mice between 2-4 day p.i.; the parasites were located extravascularly in the alveolar walls. No migratory pattern similar to that in the lungs of avian hosts (migration from the blood vessels → blood capillaries → pulmonary tissue → penetration into the free air space of the lungs) was noted by authors in mice.

Immature *Trichobilharzia* were found to survive for several days and even weeks in noncompatible hosts and cause damage in the host tissues (Chanová et al., 2007). The most severe injuries in visceral organs developed were detected in primarily infected mammals in which the parasites were able to escape from the skin due to weak immune reaction; reinfections led to strong inflammatory reaction which was able to trap and eliminate the parasites in the host skin (for a review see Horák et al., 2002).

Data on behaviour of strongly neurotropic parasites in incompatible hosts are scanty. The studies on *Angiostrongylus cantonensis*, the definitive host of which are rats, showed that following alimentary infection of incompatible hosts the larvae can migrate through soft tissues to the peripheral and central nervous system (Hung and Chen, 1988). The CNS can be, however, also invaded by parasites which enter into host through skin. The studies on bird schistosome of *Trichobilharzia regenti*, which mature in the nasal area of birds, showed that after infection of mammals cercariae are able to perform the transformation to schistosomula in the host skin and soon after they are able to migrate through peripheral nerves into the CNS (Hrádková and Horák, 2002). During migration through the CNS the parasites feed on the host nervous tissue (Lichtenbergová et al., 2011). In immunocompetent mice the parasite life span is about 14 days, however, in immunosupressed host the parastes can survive even for 2 months (Kouřilová et al., 2004). In incompatible vertebrates, both angiostrongyloidosis and trichobilharziosis regenti can lead to the severe CNS disorders. Whereas *A. cantonensis* is known causative agent of neurological symptoms in humans, the pathogenesis of the infections caused by *T. regenti* is still uknown.

In conclusion, recent studies on experimentally infected incompatible vertebrate host bring data which may explain the pathogenesis of human zoonotic infections. Considering the frequent exposures of humans to zoonotic agents underline the necessity to intensify the studies on behaviour of parasites in incompatible hosts and, therefore, explain clinical aspects related to zoonotic infections in vertebrates.

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References

Abo-Shehada M.N., Herbert I.V. 1984/85. The migration of larval *Toxocara canis* II. Post-intestinal migration in primary infection. Vet. Parasitol. 17:75-83.

Camparoto M.L., Fulan B., Colli C.M., Paludo M.L., Falavigna-Guilherme A.L., Fernandez 2008. Initial stage of development and migratory behaviour of *Toxocara canis* larvae in BALB/c mouse experimental model. Genet. Mol. Res. 7:444-450.

Chanová M., Vuong S., Horák P. 2007. *Trichobilharzia szidati*: The lung phase of migration within avian and mammalian hosts. Parasitol. Res. 100:1243-1247.

Gavin P.J., Kazacos K.R., Shulman S.T. 2005. Baylisascaris. Clin. Microb. Rev. 18:703-718.

Grabe K, Haas, W. 2004. Navigation within host tissues: *Schistosoma mansoni* and *Trichobilharzia ocellata* schistosomula respond to chemical gradients. Int. J. Parasitol. 34:927-934.

Good B., Holland C.V., Stafford P. 2001. The influence of inoculum size and time post-infection on the number and position of *Toxocara canis* larvae recovered from the brains of outbread CD1 mice. J. Helminth. 75:175-181.

Haas W., Pietsch U. 1991. Migration of *Trichobilharzia ocellata* schistosomula in the duck and in the abnormal murine host. Parasitol. Res. 77:642-644.

Hamilton C.M., Brandes S., Holland C.V, Pinelli E. 2008. Cytokine expression in the brains of *Toxocara canis*-infected mice. Parasite Immunol. 30:181-185.

Hamilton C.M, Stafford P., Pinelli E., Holland C.V. 2006. A murine model for cerebral toxocarosis: characterization of host susceptibility and behaviour. Parasitology 132:791-801.

Horák P., Kolářová L., Adema C.M. 2002. Biology of the schistosome genus *Trichobilharzia*. Adv. Parasitol. 52:155-233.

Hrádková K., Horák P. 2002. Neurotropic behaviour of *Trichobilharzia regenti* in duck and mice. J. Helminthol. 76:137-141.

Hung T.-P., Chen E.R. 1988. Angiostrongyliasis (*Angiostrongylus cantonensis*). *In*: Vinken P.J., Bruyn G., W., Klawans H.L. (Eds.), Handbook of Clinical Neurology Science, Amsterdam.

Jenkis E.J., Schurere J.M., Gesy K.M. 2011. Old problem on playng field: Helminth zoonoses transmitted among dog, wildlife, and people in changing northern climate. Vet. Parasitol. 182:54-69.

Kazacos K.R., Boyce W.M. 1989. *Baylisascaris* larva migrans. J. Am. Vet. Med. Assoc. 195:894-903.

Kolářová L. 2001. Central nervous system as a target of helminth migration in humans. Helminthologia 38:237-241.

Kolbeková P., Větvička D., Svoboda J., Skírnison K., Leissová M., Syrůček M., Marečková H., Kolářová L. 2011. *Toxocara canis* larvae reinfecting BALB/c mice exhibit accelerated speed of migration to the host CNS. Parasitol. Res. 109:1267-1278.

Kouřilová P., Syrůček M, Kolářová L. 2004. The severity of mouse pathologies caused by the bird schistosome *Trichobilharzia regenti in* relation to host immune status. Parasitol. Res. 93:534-542.

Liao C.W., Cho W.L., Kao T.C., Se K.E., Lin Y.H., Fan C.K. 2008. Blood-brain barrier impairment with enhanced SP, NK-1R, GFAP and Claudin-5 expression in experimental cerebral toxocariasis. Parasite Immunol. 30:525-534.

Lichtenbergová L, Lassmann H., Jones M.K., Kolářová L., Horák P. 2011. *Trichobilharzia regenti*: Host immune response in the pathogenesis of neuroinfection in mice. Exp. Parasitol. 128:328-335.

Magnaval J.F., Glickman L.T., Dorchies P., Morassin B. 2001. Highlights of human toxocariasis. Korean J. Parasitol. 39:1-11.

Othman A.A., Abdel-Aleem G.A., Saied E.M., Mayah W.W., Elatrash A.M. 2010. Biochemical and immunopathological changes in experimental neurotoxocariasis. Mol. Biochem. Parasitol. 172:1-8.

Pecinali N.R., Gomes R.N., Amendoeira F.C., Pereira Bastos A.C.M., Martins M.J.Q.A., Pegado C.S., Pereira Bastos O.M., Bozza P.T., Castro-Faria-Neto H.C. 2005. Influence of murine *Toxocara* infection on plasma and bronchoalveolar lavage fluid eosinophil numbers and its correlation with cytokine levels. Vet. Parasitol. 134:121-130.

Schantz P.M. 1989. *Toxocara* larva migrans now. Am. J. Trop. Med. Hyg. 41:21-34.

Sugane K., Oshima T. 1983. Trapping of large numbers of larvae in the livers of *Toxocara canis* reinfected mice. J. Helminth. 57:95-99.

PRESENT CHALLENGES AND FORCES TO ADDRESS HUMAN PARASITIC DISEASES IN EUROPE

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In Europe, parasitic diseases have emerged, are emerging or will emerge because of a constant increase of factors of emergence: increase of international travel, modification of food or sexual habits, immunosuppression, social upheavals, climate changes, increase of wild life. Several examples are good illustrations: cryptosporidiosis and disseminated toxoplasmosis in immunocompromised patients, trichinellosis outbreaks in former Yugoslavia and Romania, opistorchiasis in Italy, echinococcosis moving westwards, malaria in Greece…In Europe, there is a consistent batch of national institutions and formal or informal networks of parasitologists able to cope with these emerging parasitosis (Dupouy-Camet et al., 2009). European research in Parasitology has a very high impact all over the world as highlighted by a recent analysis of publications on Parasitology. In most countries of the EU, national reference labs and these labs have collaborative actions coordinated by the European Union Reference Laboratory for Parasites (EURLP), based in Rome. Each institution is reporting yearly data on some selected zoonotic parasitic disease (trichinellosis, toxoplasmosis, echinococcosis, cysticercosis…) to the ECDC and EFSA to constitute yearly reports in which some selected parasitosis are included. Non-profit organisations such as the European federation of parasitologists (EFP) and the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) are also contributing to promote and to support the exchange of knowledge and coordination of research related to parasitic organisms. The European Multicolloquiums of Parasitology organized every 4 years are places where parasitologists can exchange their experience on the diagnosis, treatment and prevention of parasite-related diseases. In some occurrences, some outbreaks sharing a common source can have a European extent (trichinellosis) and therefore alerts are of utmost importance. These alerts can be done through the official channels but also through rapid electronic publication by Eurosurveillance. The EFP proposes to constitute a “think tank” of European specialists able to quickly answer to any query from European health authorities during such emerging parasitic problems.

References

Dupouy-Camet J., Olesen O.F., Dei-Cas E., Loiseau P.M., Mas-Coma S. 2009. Perspectives for Parasitology and Parasitology networks in Europe. Trends Parasitol. 25(7):293-295.

THE EUROPEAN UNION REFERENCE LABORATORY FOR PARASITES

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The European Union Reference Laboratory for Parasites (EURLP) has been appointed in 2006 by the DG SANCO of the European Commission at the Department of Infectious, Parasitic and Immunomediated Diseases of the Istituto Superiore di Sanità (Rome, Italy; www.iss.it/crlp/index.php). The EURLP team consists of nine scientists and eight technicians. The EURLP performs research, diagnosis, surveillance, training and control activities for the network of the 37 National Reference Laboratories (NRLs) of MS and associated countries, the EU Commission, and third countries on helminthic (e.g., *Trichinella* spp., *Anisakis* spp., *Pseudoterranova* spp., *Echinococcus* spp., *Opisthorchis* spp., and *Taenia* spp.) and protozoan (e.g., *Cryptosporidium* spp., *Giardia duodenalis*, *Toxoplasma gondii*, *Dientamoeba fragilis*) zoonoses. The Unit is accredited according to the ISO/IEC 17025 international standard. The EURLP acts also as proficiency test provider and its accreditation according to the ISO/IEC 17043 international standard will be reached in the year. The EURLP is also a provider of reference strains of parasites and their proteins and nucleic acids, of antigens and reference sera of both animal and human origin. Every year, scientists and technicians of European and third country laboratories visit the EURLP for training, diagnosis and research activities. The EURLP acts also for the DG SANCO, as validation body of apparatuses and diagnostic kits in the field of foodborne parasites. The EURLP organizes an annual workshop for the NRLs and associated countries, to discuss issues related to foodborne parasitic diseases in Europe.

THE ESCMID STUDY GROUP FOR CLINICAL PARASITOLOGY

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The ESCMID (European Society for Clinical Microbiology and Infectious Diseases) study group for clinical Parasitology started as study group on Toxoplasmosis (ESGT) in 2003 and has been expanded to the field of Clinical Parasitology (ESGCP) in 2007. The European Society of Clinical Microbiology and Infectious Diseases is a non-profit organization whose mission is to improve the diagnosis, treatment and prevention of infection-related diseases. This is achieved by promoting and supporting research, education, training, and good medical practice.
The ESGCP aims to exchange information on prevalence of parasitic infections in different parts of Europe and on clinical policies and practices within Europe. The study group stimulates the comparisation of diagnostic performances of different assays including quality control and the publication of this information. By organizing international courses the study group works on the improvement of the level of knowledge in clinical parasitology and stimulates interaction between parasitologists in Europe. The study group devotes itself to the promotion of research and education in diagnosis and therapy.
The ESGCP started sub groups targeting 5 different topics: malaria, intestinal parasites, leishmaniasis, echinococcosis and toxoplasmosis. These subgroups are currently working on research proposals and other forms of collaboration. The study group started coordinated actions in clinical parasitology. The malaria subgroup was invited to participate in an ECDC expert meeting on *Plasmodium vivax* transmission risk for Europe. This could serve as a starting point of future collaboration between ESCMID and ECDC on emerging and vector borne diseases. This sub group also writes a position paper on the Management of Imported Malaria in Europe, in collaboration with experts in the field of malaria from 13 different European countries.
For more information see: http://www.escmid.org/research_projects/study_groups/esgcp/.

AIMS AND ACTIONS OF THE EUROPEAN VETERINARY PARASITOLOGY COLLEGE

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EUROPEAN WORKING GROUPS ON HUMAN TOXOPLASMOSIS SINCE THE 90'S TILL PRESENT

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Human toxoplasmosis (congenital and in immuno-compromised patients) is a topic of interest for medicine doctors, scientists and veterinarians. Improving the knowledge of this disease at the European level appeared in the 1990's. Since then, several European networks have been built and have successfully worked on this topic. Numerous meetings and studies have been realized, leading to numerous papers and a significant increase of knowledge on toxoplasmosis. Data were obtained at the European level, with some controversies and a lot of interesting discussions. It is necessary for the future to maintain and enhance the collaborations associating European teams under the auspices of the leading structures in the field of Parasitology in Europe.

A EUROPEAN THINK TANK COORDINATING ACTIONS IN THE MANAGEMENT OF EMERGING PARASITOSES

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In Europe, parasitic diseases did not appear to have sufficient importance in the last period of the last century as for health officers to keep them among the priorities for wide initiatives as those implemented for instance for viral diseases. However, climate and global changes, the latter mainly referring to anthropogenic modifications of the environment and to increasing travelling facilities for organisms (humans, livestock, pets, plants, etc.), are giving rise to new unexpected situations. Present and potential phenomena of emergence/re-emergence of parasitic diseases, including both those endemic in Europe and others risking to be introduced into Europe from other continents, oblige to consider the convenience to re-assess the responding capacities of Europe to different kinds of situations, including from unexpected outbreaks up to drug resistance appearance, through transmission changes and geographical spread of parasitic diseases. There are many examples within protozoan and helminthic diseases, including from monoxenous (direct transmission) to heteroxenous (indirect transmission) parasites and from zoonotic to vector-borne diseases, having shown changes in their characteristics in the European continent in the last decade. All indicates that such climate and global change influences on parasitic diseases will continue in the near future. This scenario recommends the need for a wide re-analysis about present European availabilities, to strengthen coordination links and cooperation tasks between the different multidisciplinary actors involved, and to mainly improve the capacity for rapid response. The European Federation of Parasitologists (EFP), in close relationship with the European Centre for Disease Prevention and Control (ECDC, Stockholm), appears to be the most convenient "umbrella" under which such coordination actions between the numerous multidisciplinary actors may be implemented in the easiest and more pragmatic, efficient and fast way. An “EFP Parasitic Disease Quick Intervention Capacity Expert Committee” would be key in the implementation of the task force needed to be ready to face all type of unexpected future health challenge situations.

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BIODIVERSITY OF LUNG NEMATODES – PROTOSTRONGYLIDAE LEIPER, 1926 (EMEND. BOEV ET SCHULTZ, 1950) - OF ANIMALS IN SOME EASTERN EUROPEAN COUNTRIES: ARMENIA, BULGARIA, POLAND, RUSSIA

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INTRODUCTION. Prtotstrongylids belong to nematodes of suborder *Strongylata* and family Protostrongylidae, they are causative agents of several parasitic diseases of animals. Protostrongylidoses are widely distributed in the world and cause significant loss to livestock sector. The damage is consisting from decrease of productivity of animals as well as from mortality of animals, especially lambs.

Protostrongylidae life cycles. Protostrogylid nematods are biohelminthes and required intermediate hosts during their life cycle. Numerous species of snails and slugs can act as intermediate hosts for protostrongylid larvae (Boev, 1975; Damianov and Likharev, 1975; Akramovsky, 1976; Sysoev and Schileiko, 2009).

The lung protostrongylid nematodes are egg-laying nematodes. The larvae hatched from eggs laid in lungs, move to the oral cavity during animal' cough and swallowed. Then they pass through digestive tract without any changes and become thrown out to the environment with excrements.

In the environment first-stage larvae of protostrongylid parasites moves out from feces of a mammalian definitive host, and during the contact with snail, penetrate its foot, were molting twice and morphing into infective stage. Definitive hosts become infected after eating grass at pastures contaminated by invasive protostrongylids larvae or infected with nematodes' larvae snails, which is possible in case of contacting small-sized or young snails.

Infected larvae of protostrongylids in the animals' organism perforate the intestinal wall (usually large intestine) and penetrate mesenteric lymphatic nodes, then with blood stream they are brought into lungs, where moult third and then fourth time and become mature.

MATERIALS AND METHODS. Methods commonly applied in parasitology were used, i.e:

- dissection of lungs of domestic and wild ruminants to collect nematodes with subsequent laboratory analysis for species identification;
- monthly coprolarvoscopic studies of animals;
- examination of intermediate hosts – land snails through their dissection to establish facts of their infection with *Protostrongylidae* larvae.

RESULTS AND DISCUSSIONS. According to the authors' and literature data animals from 5 families were studied helminthologically, as follows: Cervidae (7 species), Bovidae (8), Canidae (1), Hyaenidae (1) and Leporidae (1). In total 18 species were studied.

The highest number of species was studied at the territory of Russian Federation (15 species), then in Bulgaria (6 species), Poland (6 species), Armenia (4 species). Domestic sheep and goats in all those regions were studied as well (Table 1).

The diversity of protostrongylids has been analyzed according to their hosts' species. A mixed infection with various Protostrongylidae species was mostly registered in Cervidae and Bovidae. The greatest numbers of these parasite species were found in domestic sheep and goats, 12 and 8 respectively. In the studied regions – Russia, Armenia, Bulgaria and Poland – 28 species of Protostrongilidae have been found, which makes almost half of the world's protostrongylid fauna. (Demiaszkiewicz et al., 1999; Panayotova-Pencheva, 2006; Movsesyan et al., 2009; 2010; Panayotova-Pencheva, 2011). The diversity of Protostrongylidae fauna includes 26 species in Russia, 9 in Bulgaria, 9 in Poland and 6 in Armenia. The species, common for all these regions, is *Muellerius capillaris*, while *Protostrongylus hobmaeri* had been registered in Russia, Armenia and Bulgaria (Table 2).

Generally, in world fauna 57 total species of mammals are hosts of *Protostrongylidae*. A world's fauna

of *Protostrongylidae* currently includes about 60 species.
The study of sheep' infection by protostrongylids was carried out in three age groups (lambs, young and adults) in all landscape zones in Armenia, as follows: dry subtropical (535-770 meters above sea level), semi-deserts (600-1200 m), mountain steppes (1200-1800 m) and mountain meadow-steppes (1800-2300 m).

Table 1. A list of definitive hosts species of Protostrongylidae from studied regions

Animal species	Russia	Armenia	Bulgaria	Poland	*Protostrongylidae* species
Order *Artiodactyla* Suborder *Ruminantia* Family Cervidae					
Musk deer – *Moschus moschiferus*	+	-	-	-	*Protostrongylus moschi; Pneumocaulus kadenazii;*
Fallow deer – *Dama dama*	+	-	-	+	*V. sagittatus*
Dappler deer – *Cervus nippon*	+	-	-	-	*E. cervi*
Red deer – *C. elaphus*	+	-	+	+	*V. sagittatus; E. cervi*
Siberian stag – *C. elaphus sibiricus*	+	-	-	-	*V. sagittatus; V. tuvae; E. cervi*
European roe deer – *Capreolus capreolus*	-	-		-	*M. capillaris; V. capreoli; Skrjabinocaulus sofievi*
Moose – *Alces alces*	+	-	-	+	*E.cervi; E. alces; V.capreoli*
Family Bovidae					
Goral – *Nemorhaedus goral*	+	-	-	-	*Protostrongylus andrejevi*
Chamois – *Rupicapra rupicapra*	+	-	+	-	*P. hobmaieri; N. linearis; M. capillaris; P. rupicaprae; M.tenuispiculatus*
Bezoar goat – *Capra aegagrus*	+	+	-	-	*C. ocreatus; P. rufescens; M. capillaris; P. muraschkinzewi*
Argali – *Ovis ammon*	+	-	-	-	*P. davtiani; P. hobmaieri; P. raillieti; S. leuckarti; C. ocreatus*
European mouflon – *Ovis musimon/ Mouflon musimon*	-	-	+	+	*P. davtiani; P. raillieti; P. hobmaieri; P. rufescens; M. capillaris, V. capreoli*
Armenian mouflon – *Ovis ophion armeniana*	-	+	-	-	*P. davtiani; C. ocreatus; P. muraschkinzewi*
Domestic sheep – *Ovis aries*	+	+	+	+	*P.brevispiculum; S.orloffi; P.hobmaieri; P. raillieti; P. muraschkinzewi; P.kochi; N. linearis; V. pneumonicus; M. capillaris; C. ocreatus, S. leuckarti; P. davtiani*
Domestic goat – *Capra hircus*	+	+	+	+	*S. orloffi; P.hobmaieri; P. raillieti; P.muraschkinzewi; N. linearis; V. pneumonicus; M. capillaris; C. ocreatus; P. rufescens; P. davtiani*
Order *Carnivora* Family Canidae					
Fox – *Vulpes vulpes*	+	-	-	-	*Skrjabinocaulus antoni*
Family Hyaenidae					
Manul – *Felis manul*	+	-	-	-	*S. antoni*
Order *Lagomorpha* Family Leporidae					
Brown hare – *Lepus europeus*	+	-	-	-	*P. tauricus*
Total species 18	**15**	**4**	**6**	**6**	

In all of the studied landscapes infection by protostrtogylids (by *Mullerius, Cystocaulus, and Protosntrongylus*) was revealed. The average disease extensity in lambs was 20.1%, in young animals – 26.8%, and in adults – 31.1%. The level of infection was different in different landscapes. The infection level of animals in lowlands (dry subtropics, semi-deserts) was much lower than those in mountain landscapes (mountain steppes, mountain meadow-steppes). The lowest level of infection

was registered in lambs of current year, the highest – at adults with intermediate state in young animals close to infection level of adults.

Table 2. A list of *Protostrongilidae* species in studied regions

Species	Russia	Armenia	Bulgaria	Poland
Cystocaulus nigrescens	+	+	-	
C. ocreatus	+	-	+	+
Elaphostrongylus alces	+			+
E. cervi	+		-	+
E.sp.	+			+
Muellerius capillaris	+	+	+	+
M. tenuispiculatus	+		+	+
Neostrongylus linearis	+	-	+	+
Spiculocaulus leuckarti	+	-	-	
S. orloffi	+	-	-	
Skrjabinocaulus sofievi	+	-	-	
S. antoni	+	-	-	
Pneumocaulus kadenazii	+	-	-	
Protostrongylus andrejevi	+	-	-	
P. brevispiculum	+	-	+	
P. davtiani	+	+	-	
P. hobmaieri	+	+	+	
P. kochi	+	+	-	
P. moschi	+	-	-	
P. muraschkinzewi	+	+	-	
P. raillieti	+	-	-	
P. rufescens	+	-	+	+
P. rupicaprae	+	-	+	
P. tauricus	+	-	-	
Varestrongylus capreoli	+	-	-	+
V. pneumonicus	+			
V. sagittatus	+	-	+	+
V. tuvae	+	-	-	
TOTAL 28 species	**26 species**	**6 species**	**9 species**	**9 species**

Besides investigation of definitive hosts, the intermediate hosts' (snails *Helicella derbentina*, *Napaeopsis hohenackeri, Helix lucorum, Chondrula tridens, Hesseola solidior, Succinea putris, Pupilla muscorum, Vitrinoides monticola, Deroceras caucasicum)* infestation with protostrongylids was studied as well.

During the life activity of land snails (feeding, movement, etc.) in natural ecosystems their meeting with protostrongylids larvae localized in excrements of animals or escaped from them to the grazing lands is unavoidable. If among these snails there are species of intermediate hosts they become infected by larvae and involved into parasites life cycles. So, snails are playing an important role in forming the biodiversity of protostrongylids and in dissemination of lung helminthoses.

Based on our study of more than 7000 snails of abovementioned species the extensity of their infection with prothostrongylids larvae varies from 0.4 to 12.0%, with intensity of the infection of 1-63 larvae per snail. The prevalence of the infection among snails varies depending of season and landscape belt. In spring the prevalence of the infection was lower than in summer and autumn with peak at the end of summer – beginning of autumn. In mountain landscapes snails' infestation level was higher (2.6-23.0%) than in lowlands (0.2-7.0%) (Boykhchyan, 2008; Movsesyan et al., 2009).

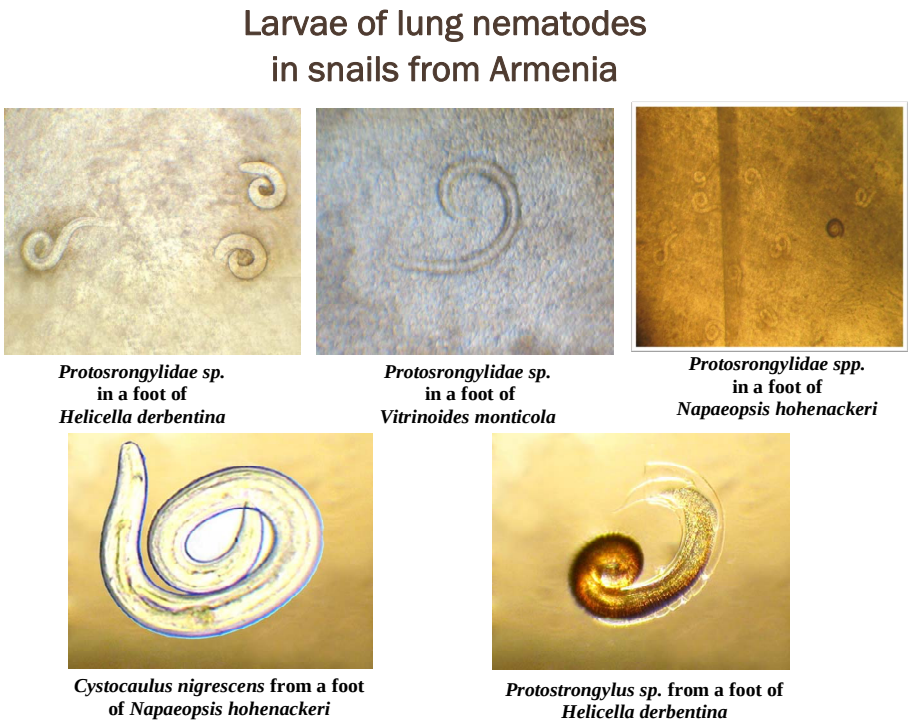

Figure 1. Larvae of protostrongylids in the body of snails

In regions studied by our team 77 species of land snails intermediate hosts of *Protostrongylidae* were registered, including 50 species in Russia, 39 in Armenia, 20 in Bulgaria and 12 in Poland. In the end it should be noted that study of biodiversity of Protostrongylidae and animal diseases caused by them in current transforming natural conditions, in connection with growing anthropogenic pressure and global climate changes is in particular interest from both the fundamental point of view and the practical usage during organization of fighting such helminthoses.

SUMMARY

A world's fauna of *Protostrongylidae* currently includes about 60 species. In the studied areas 26 species of protostrongylids were registered in Russia, 10 species in Bulgaria, 9 species in Poland and 6 species in Armenia. Animals from 5 families were studied helminthologically, as follows: Cervidae (7 species), Bovidae (8), Canidae (1), Hyaenidae (1) and Leporidae (1). The highest number of species was studied at the territory of Russian Federation (15 species), then in Bulgaria (7 species), Poland (6 species), Armenia (4 species). Domestic sheep and goats in all mentioned regions were investigated. Alongside with Protostrongylidae nematodes fauna our team have studied their intermediate hosts – land snails. In total 77 species of snails were registered, including 50 species in Russia, 39 in Armenia, 20 in Bulgaria and 12 in Poland. The most species of recorded snails are common for all these regions.

References

Akramovsky N.N. 1976. Fauna of Armenia: Snails. Armenian Academy of Sciences Press, Yerevan (in Russian).

Boev S.N. 1975. Protostrongylidae. In: K. Ryzhikov (Ed.) Essentials of nematology, Vol. 25. Nauka Press, Moscow, pp. 268.

Boyakhchyan E. 2008. The prevalence of sheep with protostrongylids in Armenia. Russian Parasitol. J. 3:5-12.

Damianov S.G., Likharev I.M., 1975. *Gastropoda terrestrial*. Fauna of Bulgaria, Vol.4, BAS Press, Sofia, pp. 426.

Demiaszkiewicz A.W., Drozdz J., Lachowicz J. 1999. The occurrence of lung nematodes in red deer in the Bialowieza forest. J. Med. Vet. 55:519-520.

Movsesyan S.O., Boyakhchian G.A., Arutyunova L.D., Chubaryan F.A., Petrosyan R.A., Nikogosyan M.A. 2009. Protostrongylides (Protostrongylidae*)* and caused by them helminthosis of sheep in Armenia. Russian Parasitol. J. 4:10-29.

Movsesyan S.O., Boyakhchian G.A., Chubarian F.A., Petrosian R.A., Nikogosian M.A., Arutyunova L.D., Panayotova-Pencheva M.S., Bankov J., Demiaszkiewicz A.W., Malczewski A. 2010. The role of snails in formation of biodiversity of pulmonary nematodes (Protostrongylidae) of animals. Russian Parasitol. J. 3:43-60.

Panayotova-Pencheva M.S. 2006. New records of protostrongylid lungworm from wild ruminants in Bulgaria. Vet. Med.-Czech. 51(10):477-484.

Panayotova-Pencheva M. 2011. Species composition and morphology of protostrongylids (Nematoda: Protostrongylidae) in ruminants from Bulgaria. Parasitol. Res. 109(4):1015-1020.

Sysoev A., Schileiko A. 2009. Land snails and slugs of Russia and adjacent countries. Pensoft Press, Sofia-Moscow, pp. 312.

CESTODE DIVERSITY: HOW MUCH DO WE KNOW?

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Tapeworms constitute the second largest class within *Platyhelminthes* and their extensive variety has been studied for almost three Centuries during which the known diversity of the group has regularly increased. Presently it is commonly admitted that 5 or 6000 species are known. However the real diversity of tapeworms remains difficult to appreciate globally and is even largely unknown at some levels.
Whilst the higher structure of the group is progressively unravelled, with at least 3 new orders defined in the last few years. The situation is much less clear for lower taxonomic levels and remains particularly confused at the specific level: new species are constantly added to our database; however some important revisions also show that the supposed diversity of many groups is, sometimes to a large extent, overestimated through a systematic taxonomic inflation process. Furthermore the lack of recent revisionary works prevents the assessment of some among the most species-rich groups of cestodes.
In the last few years a worldwide concerted effort from the community of cestodes systematicians allowed for a surge of taxonomic publications in the field and for the discovery of numerous new taxa. Taking this into account in this contribution, I will try to suggest where most of the remaining unknown diversity might be found, to offer an overview of the group diversity at several levels from species to the higher lineages. Examples from birds and fishes will be looked at in more details and estimates of the known – and unknown! – diversity of cestodes will be suggested.

INFLUENCE OF CLIMATE CHANGE ON PARASITIC INFECTIONS

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Objectives and methods. To give an overview over climate change in Europe with some regional examples and the knowledge we have on its influence on the prevalence of parasitic infections with the use of review of literature
Results. In the northern part of Europe, the Arctic there will be three times higher temperatures than in the rest of the world due to climate change according to moderate modeling. We have a lot to learn from what is happening in the circumpolar area for adaptation strategies. Parasitic infections as *Leishmania*, *Opistorchis*, *Echinococcosis multilocularis* and *alveolaris* are expanding along with *Giardia intestinalis* as some examples. The impact will differ regionally. In Eastern Europe a change in climate will result in higher temperatures and drier conditions. In western parts of Europe the precipitation will increase and there will be a rise in sea-level. Spring will arrive earlier. In southern parts of Europe the summers will be drier and there will be hot spells. Also, there will be an increase in unpredictable weather events with storms and flooding.
Conclusion. The health of humans and of animals will change in a changed landscape. This is particularly clear for infectious diseases including parasitic diseases. Using modeling the incidence and prevalence of infectious diseases can be calculated and foreseen. Given the dynamics and the complexity of climate/sensitive infectious disease, particularly those transmitted by mosquitoes or rodents there will be a change in incidence and prevalence of infectious diseases in Europe within the next coming decades. Europe needs to develop and sustain surveillance and early warning systems with a regional focus.

ECTOPARASITE DIVERSITY: HOST SPECIES, HOST COMMUNITIES AND GEOGRAPHY

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The search for patterns of biodiversity across locations and through time and the explanation of these patterns is one of the most popular themes in ecology. Understanding of biodiversity patterns in application to parasites is especially important because parasites play important roles in the regulation of populations and communities of their hosts and because this understanding is crucial for successful control of diseases that hit humans as well as wild and domestic animals. In addition, parasites are living organisms and are de facto part of biodiversity and parasitism is possibly more common than any other feeding strategy. When a biodiversity study concerns parasites, at least one extremely important difference between parasites and free-living organisms should be taken into account. Any parasitic species is characterized by 'dual location'. On the one hand, a 'location' for a parasite is a host species it exploits, whereas, on the other hand, it is geographic location when a host (and, consequently, a parasite) occurs. Using an advantage of our recent studies on fleas and mesostigmate mites, we will review the observed patterns of species richness and diversity of these two taxa associated with characteristics of individuals, populations and communities of their hosts as well as some of the major biogeographic patterns of their diversity.

THE *TRICHINELLA* GENOME: WHAT CAN IT TELL US AND HOW CAN WE APPLY IT?

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Understanding protein evolution is important for deciphering processes that drive species diversity and adaptation. In 2004, a large scale genome sequencing effort was begun to better understand metazoan evolution. Among the organisms to be studied was*Trichinella spiralis*, a nematode associated with early radiation of the phylum and the first to be sequenced from a basal lineage within the Nematoda. The *T. spiralis* draft genome encodes nearly 16000 proteins, spans 64 million base pairs and was derived from a 35-fold coverage using whole-genome shotgun and hierarchal map-assisted sequencing. Herein, we explore the relationship between changes in protein families and protein domains over the course of metazoan evolution and the relationship between these changes the ability of parasites to adapt to their environments. Change, as defined by birth/death and duplication/deletion events within protein families and domains, was analyzed using proteomes of 9 metazoan including data from *T. spiralis* and two outgroup species. Among the three major metazoan groups i.e., vertebrates, arthropods, and nematodes, both birth and death events reflected species adaptation and diversity. The rates of birth/death events in protein families and domains varied largely among the different lineages. Herein we will discuss new insights into protein evolution and its bearing on metazoan evolution.

GENETIC VARIATION AS A KEY TO UNDERSTANDING THE ORIGINS OF *TRICHINELLA* SPECIES, THE HISTORY AND STRUCTURE OF THEIR POPULATIONS, AND THE NUMBER OF INDEPENDENT INFECTIONS THAT A GIVEN ANIMAL IS LIKELY TO SUSTAIN

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By examining the extent and distribution of genetic variation among and within species in the genus *Trichinella,* several important insights have recently been derived concerning their origins, history of dissemination, and ongoing patterns of transmission. For example, genetic variation was a key in determining that the genus is comprised of several distinct species, an in reconstructing the timing and order of their evolutionary diversification. The extent and pattern of variation in mitochondrial and microsatellite markers was assayed for zoonotic parasites in the genus *Trichinella* in order to gain insight into past patterns of dissemination as well as features of ongoing transmission. Although species in this genus typically occupy limited geographic foci, one (*Trichinella spiralis*) is exceptional for its broad distribution and genetic homogeneity. Introduction to the Americas likely attended the expansion of European agriculture during or since the Colonial period. Patterns of introgression have been documented where distinct parasite lineages co-occur. Finally, natural infections have been observed to generally comprise full sibling cohorts; indicating that host immunity usually (but not always) precludes re-infection after a host has become infected by a successful mating pair. Molecular markers hold great promise in elucidating features of endemic and epidemic transmission.

TOXOPLASMA STRAINS AND HUMAN TOXOPLASMOSIS

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Epidemiological arguments for a role of *Toxoplasma gondii* strain in the outcome of human toxoplasmosis came from geographical differences in the clinical expression of toxoplasmosis. For instance, in the EMSCOT study comparing two cohorts of children with congenital toxoplasmosis, independently of treatment, Brazilian children developed ocular lesions earlier than European children: at 1 year of age, a retinochoroiditis was present in 50% of Brazilian children compared to 10% of European children (Gilbert et al., 2008). They had more frequently multiple and larger lesions, that were more likely to affect the posterior pole, and hence to lead to visual impairment. Other papers reported a higher rate of ocular toxoplasmosis in African born patients (Gilbert et al., 1999). Although these papers do not include genotyping of strains, the authors suggested that this increased frequency and severity of toxoplasmosis is due to exposure to more virulent strains of *Toxoplasma*. Do we have genotyping results in favour of this hypothesis?
The population structure of *Toxoplasma* should no longer be considered as a relatively simple population structure with 3 main clonal types, designated as type 1, 2 and 3, with a low divergence within each of the 3 main lineages, and between the lineages. The analysis with multilocus markers of strains from a wider geographic and host range showed an abundance of “atypical” genotypes that did not fit the conventional classification of three major lineages mainly described in USA and France. It revealed a more complicated population structure and this complexity is an obstacle for those who try to find a link between strains and human toxoplasmosis. Recently, in an effort to cluster these genotypes into groups of related strains that can be useful for clinical and epidemiological studies, 956 isolates collected from around the world were genotyped using three independent sets of polymorphic DNA markers, sampling 30 loci distributed across all nuclear chromosomes as well as the plastid genome (Su et al., 2012). Clustering methods organized the marked genetic diversity of 138 unique genotypes defined by PCR-RFLP into 15 haplogroups (HG) that collectively define six major clades. Although these methods are informative on a global level, the result must still be interpreted with caution as strains that appear genetically similar here may not be genealogically the same due to the limited nature of sampling and the variability of inheritance among progeny of any given genetic cross. The global population structure of *Toxoplasma gondii* is largely influenced by geographic distribution and by ecological aspects driving parasite transmission (anthropized versus wild population) (Mercier et al., 2010; 2011). In Europe and North America, haplogroups 2 and 3 predominate, although other haplogroups (notably HG 12) are described in North America. South America is a hotspot of diversity with highly divergent strains in its Amazonian part (“*Amazonian*” strains distributed over HG 5 and 10) and many other haplogroups, more or less present in the sample under study. Africa and Asia were more recently explored: these preliminary studies seem to show a lower diversity than in South America. Some major haplogroups (HG 6, 14) are shared between Africa and South America (Mercier et al., 2010).
The pathogenicity observed during a *Toxoplasma* infection depends on host species. It is well known that type I strains are highly pathogenic in mice, less than 10 parasites being able to kill all inoculated mice whereas it is non pathogenic for rats or sheep. On the contrary, the so called non-virulent type II is able to kill susceptible animals such as squirrel monkeys or marsupials. Little is known about pathogenicity of the other genotypes. In mice, all the levels of pathogenicity are encountered according to the genes these strains have inherited. But what happens in other species? Furthermore, within a same species, the host genetic background is involved in the outcome of infection. In humans, severity of congenital toxoplasmosis have been found associated with genes affecting immune response, such as genes coding for pathogen receptors (Jamieson et al., 2010; Witola et al., 2011). Overall the host immune system plays a major role: type II is not pathogenic in

immunocompetent adults, but may lead to the death of immunodeficient patients or of the most immature foetuses. We must keep in mind the fact that *Toxoplasma* is mainly an opportunistic parasite and that parasite genotype is just one factor among others influencing outcome of human toxoplasmosis. As a consequence, to evaluate its influence, genotyping of strains must be accompanied with detailed epidemiological and clinical data, including those already known to influence the outcome of human toxoplasmosis (period of maternal infection during gestation for congenital toxoplasmosis, or the aetiology and the level of immunodeficiency for immunocompromised patients).

In France, thanks to a network of medical parasitologists sending strains together with clinical and epidemiological data to the National Reference Centre, more than 900 *Toxoplasma* isolates originating from human cases of toxoplasmosis were genotyped. Due to the epidemiology of strains in this country, 84% of them belong to the clonal lineage, type 2, and 4% to type 3. This proportion would be different in another country. Nearly all the remaining isolates, different from type 2 or 3, and also a significant part of type 3 isolates, were acquired directly or indirectly (imported meat consumption) outside Europe. We have no evidence that strains infecting humans are different from those circulating in the environment in a given area.

The role of host immune system is obvious in immunocompromised persons. The conclusion of a study on 85 patients (HIV and non-HIV immunodeficient patients) was that the genotype of the infecting strain (type 2 versus non-type 2) had no influence on clinical manifestation (cerebral or extracerebral) or clinical outcome (Ajzenberg et al., 2009), indicating that immune status, rather than strain is responsible for virulence expression in these patients. The genotype of *Toxoplasma* strains was only linked to the presumed geographical origin of infection: immunocompromised patients commonly reactivate a type 2 strain if acquired in Europe and an atypical strain if acquired in sub-Saharan African countries, French overseas departments and South America.

In congenital toxoplasmosis, there is a significant difference in the outcome of congenital toxoplasmosis either due to type 2 or 3 strains compared to other strains. A large majority of cases of congenital toxoplasmosis (73%) due to type 2 and 3 are asymptomatic cases, whereas 52% of cases due to other strains are responsible for severe cases (unpublished data). Furthermore, severe cases due to type 2 were observed as classically described after early maternal infection, whereas those due to other strains were mainly observed after late maternal infection (Ajzenberg et al., 2002). We still do not have enough isolates different from type 2 or 3 to analyse the role of newly described haplogroups. However, we observed that the proportion of severe cases is higher when strains are characterized by a majority of type 1 or atypical alleles (“Amazonian”, “African” or Brazilian strains). These atypical strains were also responsible of re-infection in an immunocompetent pregnant woman leading to a congenital transmission.

In ocular toxoplasmosis, the role of strain was emphasized due to geographical differences in its prevalence: in Brazil, it is much higher than everywhere especially in the southern city of Erechim where up to 17.7% of 1042 adults were found to have retinal scars clinically related to toxoplasmosis. But in a country like France where type 2 is highly predominant, the majority of acquired ocular toxoplasmosis is due to type 2 strains (Fekkar et al., 2010).

In immunocompetent patients, severe toxoplasmoses with multi-organ failure were linked to highly divergent strains acquired from the Amazonian rain forest, belonging to haplogroups 5 and 10 (Carme et al., 2009). Occasional reports of such severe cases due to atypical strains were observed in other countries, sometimes after consumption of food imported from the American continent (Pomares et al., 2011).

The next step will be to precise the role of the newly described haplogroups and of the genes considered as virulence genes in the mouse model. This will necessitate studies on genotypes associated with clinically documented cases of human toxoplasmosis from South America, Asia or Africa.

References

Ajzenberg D., Cogné N., Paris L., Bessières M.H., Thulliez P., Filisetti D., Pelloux H., Marty P., Dardé M.L. 2002. Genotype of 86 *Toxoplasma gondii* isolates associated with human congenital toxoplasmosis, and correlation with clinical findings. J. Infect. Dis. 186:684-689.

Ajzenberg D., Yera H., Marty P., Paris L., Dalle F., Menotti J., Aubert D., Franck J., Bessières M.H., Quinio D., Pelloux H., Delhaes L., Desbois N., Thulliez P., Robert-Gangneux F., Kauffmann-Lacroix C., Pujol S., Rabodonirina M., Bougnoux M.E., Cuisenier B., Duhamel C., Duong T.H., Filisetti D., Flori P., Gay-Andrieu F., Pratlong F., Nevez G., Totet A., Carme B., Bonnabau H., Dardé M.L., Villena I. 2009. Genotype of 88 *Toxoplasma gondii* isolates associated with toxoplasmosis in immunocompromised patients and correlation with clinical findings. J. Infect. Dis. 199:1155-1167.

Carme B., Demar M., Ajzenberg D., Dardé M.L. 2009. Severe acquired toxoplasmosis caused by wild cycle of *Toxoplasma gondii*, French Guiana. Emerg. Infect. Dis. 15:656-658.

Fekkar A., Ajzenberg D., Bodaghi B., Touafek F., Le Hoang P., Delmas J., Robert P.Y., Dardé M.L., Mazier D., Paris L. 2011. Direct genotyping of *Toxoplasma gondii* in ocular fluid samples from 20 patients with ocular toxoplasmosis: predominance of type II in France. J. Clin. Microbiol. 49:1513-1517.

Gilbert R.E., Dunn D.T., Lightman S., Murray P.I., Pavesio C.E., Gormley P.D., Masters J., Parker S.P., Stanford M.R. 1999. Incidence of symptomatic toxoplasma eye disease: aetiology and public health implications. Epidemiol. Infect. 123:283-299.

Gilbert R.E., Freeman K., Lago E.G., Bahia-Oliveira L.M., Tan H.K., Wallon M., Buffolano W., Stanford M.R., Petersen E., European Multicentre Study on Congenital Toxoplasmosis (EMSCOT). 2008. Ocular sequelae of congenital toxoplasmosis in Brazil compared with Europe. PloS Negl. Trop. Dis. 2:e277.

Jamieson S.E., Peixoto-Rangel A.L., Hargrave A.C., Roubaix L.A., Mui E.J., Boulter N.R., Miller E.N., Fuller S.J., Wiley J.S., Castellucci L., Boyer K., Peixe R.G., Kirisits M.J., Elias L.de S, Coyne J.J., Correa-Oliveira R., Sautter M., Smith N.C., Lees M.P., Swisher C.N., Heydemann P., Noble A.G., Patel D., Bardo D., Burrowes D., McLone D., Roizen N., Withers S., Bahia-Oliveira L.M., McLeod R., Blackwell J.M. 2010. Evidence for associations between the purinergic receptor P2X(7) (P2RX7) and toxoplasmosis. Genes Immun. 11:374-383.

Mercier A., Ajzenberg D., Devillard S., Demar M.P., de Thoisy B., Bonnabau H., Collinet F., Boukhari R., Blanchet D., Simon S., Carme B., Dardé M.L. 2011. Human impact on genetic diversity of *Toxoplasma gondii*: example of the anthropized environment from French Guiana. Infect. Genet. Evol. 11:1378-1387.

Mercier A., Devillard S., Ngoubangoye B., Bonnabau H., Bañuls A.L., Durand P., Salle B., Ajzenberg D., Dardé M.L. 2010. Additional haplogroups of *Toxoplasma gondii* out of Africa: population structure and mouse-virulence of strains from Gabon. PloS Negl. Trop. Dis. 4:e876.

Pomares C., Ajzenberg D., Bornard L., Bernardin G., Hasseine L., Darde M.L., Marty P. 2011. Toxoplasmosis and horse meat, France. Emerg. Infect. Dis. 17:1327-1328.

Su C., Khan A., Zhou P., Majumdar D., Ajzenberg D., Dardé M.L., Zhu X.Q., Ajioka J.W., Rosenthal B.M., Dubey J.P., Sibley L.D. 2012. Globally diverse *Toxoplasma gondii* isolates comprise six major clades originating from a small number of distinct ancestral lineages. Proc. Natl. Acad. Sci. USA. 109:5844-5849.

Witola W.H., Mui E., Hargrave A., Liu S., Hypolite M., Montpetit A., Cavailles P., Bisanz C., Cesbron-Delauw M.F., Fournié G.J., McLeod R. 2011. NALP1 influences susceptibility to human congenital toxoplasmosis, proinflammatory cytokine response, and fate of *Toxoplasma gondii*-infected monocytic cells. Infect. Immun. 79:756-66.

TOXOPLASMOSIS IN IMMUNOCOMPROMISED PATIENTS: WHAT ARE THE KEY POINTS IN 2012?

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Introduction

Toxoplasma gondii is an opportunistic ubiquitous protozoan parasite responsible for congenital infections and infections in immunocompromised patients. It is a major opportunistic pathogen in AIDS patients and other immunocompromised patients such as haematological and solid organ transplant ones. Since the discovery of the parasite in 1908 numerous works and publications have been devoted to its pathophysiology. Understanding the complex interactions between the parasite and its host is of paramount importance to better prevent, diagnose and treat toxoplasmosis, particularly in AIDS patients (Maubon et al., 2008).

Epidemiology

The epidemiology of toxoplasmosis in AIDS patients depends on epidemiology of AIDS and toxoplasmosis, on the treatment of HIV infection and on prevention of toxoplasmic reactivations. Since the implementation of HAART the incidence of toxoplasmic encephalitis in AIDS patients has dramatically decreased. In other immunocompromised patients, toxoplasmosis represents a serious problem in both solid organ transplants (mainly heart transplant) and in hematopoietic stem cells transplant. In this later group, the parasite is mainly transmitted by the transplanted organ, while reactivation is the most common mechanism in haematological patients (Derouin and Pelloux, 2008).

Clinical features

Cerebral toxoplasmosis is the most common clinical feature in AIDS patients. Its clinical diagnosis is often easier than the diagnosis of disseminated toxoplasmosis in haematological patients. It seems that no clear clinical modification appeared after HAART in AIDS patients.

Diagnosis

The diagnosis of toxoplasmosis in immunocompromised patients is based on clinical diagnosis, imaging, serology, cultures and PCR. In AIDS patients, clinical diagnosis associated to imaging and evolutions under treatment allows to diagnose most cases. The specific Toxoplasma specific serology is useful only to determine the risk of reactivations. The diagnosis of disseminated toxoplasmosis in haematological patients is more complex and involves specialised techniques. These are mainly specific serological techniques and PCR. They allow to directly diagnose the disease or to indicate the risk of transmission and/or reactivation, depending on the patients (Fricker-Hidalgo et al., 2009)

Treatment

The treatment of toxoplasmosis is based on drugs that have been described long ago (in the 70's). None of them is perfect, due to potential adverse effects, and none of them can destroy cysts. The most commonly used for prophylaxis (primary and secondary) are pyrimethamine, sulfamides, and cotrimoxazole. The discovery of new efficient drugs, active against cysts, is urgently needed. Some promising results have been obtained with new therapeutic targets such as epigenetic ones (Bougdour et al., 2009)

Conclusion

In conclusion, lot of work has been devoted to toxoplasmosis in immunocompromised patients. Some major improvement occurred, mainly in the treatment of the underlying disease or the diagnosis. However a lot remains to be done to perfectly prevent, diagnose and treat this disease.

References

Bougdour A., Maubon D., Baldacci P., Bastien O., Ortet P., Barale J.C., Pelloux H., Menard R., Hakimi M.A. Drug-inhibition of HDAC3 and epigenetic control of differentiation in Apicomplexa parasites. 2009. J. Exp. Med. 206:953-966.

Derouin F., Pelloux H. On behalf of the ESCMID Study Group on Clinical Parasitology. 2008. Prevention of toxoplasmosis in transplant patients. Clin. Microbiol. Infect. 14:1089-1101.

Fricker-Hidalgo H., Bulabois C.E., Brenier-Pinchart M.P., Hamidfar R., Garban F., Brion J.P., Timsit J.F., Cahn J.Y., Pelloux H. 2009. Diagnosis of toxoplasmosis after allogenic stem cell transplantation: results of DNA detection and serological techniques on 70 patients. Clin. Infect. Dis. 48:9-15.

Maubon D., Ajzenberg D., Brenier-Pinchart M.P., Darde M.L., Pelloux H. 2008. What are the respective host and parasite contributions to toxoplasmosis? Trends Parasitol. 24 299-303.

CLINICAL PRESENTATION OF CONGENITAL TOXOPLASMOSIS: OUR EXPERIENCE AND REVIEW OF LITERATURE

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The toxoplasmosis during pregnancy is a major concern of public health because of the risk for maternal fetal transmission and severe sequelae for the fetuses and the neonates and represents an important aspect of medicine due to its deep social and inside family implications. Physicians may be confronted with a number of issues regarding toxoplasmosis, related to clinical presentation, laboratory testing and prevention. The congenital toxoplasmosis (CT) offers a picture of fetopathy along with cephalic signs and can mimic disease caused by herpes simplex virus, cytomegalovirus, and rubella virus. Signs in the classic triad suggesting CT are: chorioretinitis, hydrocephalus, and intracranial calcifications. However, other clinical manifestations also are associated with CT in infancy and later in life. Possible signs and symptoms of CT suggested by others authors are microcephaly, microophtalmy, convulsions, deafness, visual impairment, mental retardation, spasticity and palsies, learning disabilities, abnormal spinal fluid, splenomegaly, hepatomegaly, lymphadenopathy, maculopapular rash, growth retardation, anemia, thrombocytopenia (Montoya and Remington, 2000; Jones et al., 2003). Because clinical manifestations may be nonspecific, CT must be considered in a large variety of presentations. Most neonates with CT are asymptomatic, as determined by routine newborn tests: antibody studies on cord blood and cerebrospinal fluid, CT and MRI scan of the brain, neurological exams, standard eye exam, and toxoplasmosis test (Jones et al., 2001). We made a study in which 253 children with signs and symptoms of CT and 68 mothers were tested for the presence of specific antibodies by indirect IF and ELISA. The studied children were neonates (63.8%) or infants (36.2%). The maternal antibodies were tested in order to identify the acute infection during pregnancy. From all tested mothers, 57.3% had an apparently normal pregnancy and 42.7% had abortion and pathologic pregnancies (stillbirth, hemorrhage, and premature birth). From all pregnant women, 78% presented the acute infection during the first months of pregnancy, 11.8% in the second semester and 10.3% in the third semester. Our data suggest that 78% of signs and symptoms of CT are the consequence of an infection acquired in the first months of pregnancy, when the fetal embryogenesis takes place; 23.8% of the neonates developed brain and nervous system damage, in the first three months of life and 97.6% presented neurological disorder which included: spasticity/seizures (11%), microcephaly (17.8%), hydrocephalus (8.3%), microophtalmy (1.4%), mental retardation (71.3%), palsies (6.9%), cranial nerves paralysis (7%), deafness, cerebellar (1.4%), frontal-parietal atrophy (1.4%) cerebellar syndrome (1.4%) or other neurological, psychiatric or behavioral problems (9.6%). Studies show that toxoplasmosis may affect behavior and may be a causative or contributory factor in various psychiatric disorders (Wilson et al., 1980; Carter and Frank, 1986; Jones et al., 2003). The mechanism for this change is not completely understood, but there is evidence that toxoplasma encodes one dopamine-synthesizing enzyme and raises dopamine levels, in the human brain (Petersen, 2007). Infants more often have milder disease, with hepatosplenomegaly and lymphadenopathy in the first two months of life (Jones et al., 2003). Only a part of studiated children presented anemia (24.7%), thrombocytopenia (1.4%), jaundice (1.4%) and heart malformations (2.7%). Ocular disease (blindness or visual disability), eye lesions may occur even later in life. Ophthalmologic disorders were observed in our study in 36.6% of children.

These studies demonstrate the necessity of active scientific surveillance of pregnant women and of the newborn children. They emphasize the need of national programs for the control of toxoplasmosis both in pregnant women and in newborn children. Most infants have a subclinical infection, with no overt disease at birth, impeding prompt initiation of therapy in almost all cases, though it is vital to prevent future sequelae of the disease. Consequently, unless pre-natal serological screening is institutionalized, allowing for the diagnosis of the mother's infection, we will continue missing the diagnosis of congenital toxoplasmosis in many children, who may later present sequelae (ocular and/or neurological) of the disease.

References

Carter A.O., Frank J.W. 1986. Congenital toxoplasmosis: epidemiologic features and control. CMAJ. 135:618-23.

Jones J., Lopez A., Wilson M., 2003. Centers for disease control and prevention, Atlanta, Georgia, Am. Fam. Physician. 67(10):2131-2138.

Jones J.L., Kruszon-Moran D., Wilson M., McQuillan G., Navin T., McAuley J.B. 2011. *Toxoplasma gondii* infection in the United States: seroprevalence and risk factors. Am. J. Epidemiol. 154:357-65.

Montoya J.G., Remington J.S. 2000. *Toxoplasma gondii* In: Mandell G.L., Douglas R.G., Bennett J.E., Dolin R., eds. Mandell, Douglas, and Bennett's Principles and practice of infectious diseases. 5th Ed. Philadelphia: Churchill Livingstone, pp. 2858-88.

Petersen E. 2007. Toxoplasmosis. Semin. Fetal Neonatal Med. 12(3):214-223.

Wilson C.B., Remington J.S., Stagno S., Reynolds D.W. 1980. Development of adverse sequelae in children born with subclinical congenital *Toxoplasma* infection, Pediatrics 66:767-74.

UPDATES IN *TOXOPLASMA GONDII* INFECTION IN PREGNANCY AND NEONATES

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Development of new assays for the detection of immunity to *T. gondii* and optimization of pre-existing ones, leads to almost continuous change of criteria for interpreting their results (NCCLS, 2004; Montoya and Remington, 2008).
Implementation of screening programs for toxoplasmic infection in women of fertile age was preceded by studies for the appreciation of contamination level in the area in all countries unrolling this type of follow-up (Wallon et al., 1994). We also unrolled such an activity through collaboration with veterinarian doctors within a national funded program (TOXANOM).
Antitoxoplasmic serology in pregnant women is made in the majority of laboratories in our country by determination of specific IgG and IgM. In our study we found a prevalence of toxoplasmic infection in women of fertile age of 39%, an annual risk of contamination of 0.67% for non-immune females and a statistic significant association between rural area and contamination with *T. gondii* (Costache et al., 2008).
Lobby among women of fertile age for performing this analysis is carried out in our country by general practitioners, gynecologists and laboratory medicine doctors specialized in parasitology. The appropriate assays and clinical interpretation of the results obtained after testing should be made and given to the patients (pregnant women or parents of the newborn) by professionals who are specialized and permanently up-dated with the knowledge in this field. Unfortunately, there is still much outdated interpretation of serological profile, particularly among clinicians, considering the presence of specific antitoxoplasmic IgM as an absolute indicator of an acute infection. The IgM persistence phenomenon (residual Ig M) is very well known nowadays and has been studied in large groups of population abroad (Sensini, 2006). In our studies we also found it to be common (CI: 2-34%) in the female population with positive serology (IgG positive, IgM positive at least one and a half year after the first test) and has no pathological relevance. A correct clinical interpretation should relay in these cases on the dynamic follow-up, considering as starting point assays performed before pregnancy or during the first weeks, and if IgG and IgM values remain about the same two successive examinations we may conclude that they are residual. However, in the majority of cases we are faced with interpretation of a single assay, performed during pregnancy, and in this situation we have to broaden the spectrum of analysis by performing IgG affinity and/or IgA (Costache et al., 2010). Interpretation of IgG affinity/avidity test is also problematic. It has to be considered that a low affinity may not give a correct interpretation as a single parameter because it may persist in some cases for at least 6 moths, but when summed to other results it can make the difference between chronic infection and recently infection and it may ensure a good evolution of pregnancy with no risk for the transmission of the infection to the child (Dunn, 1999; Iqbal, 2007).
As a consequence of all the reasons presented above, a small number of women were followed for the appreciation of antitoxoplasmic immunity out-side our study, a statistically larger percentage did not undergo this procedure ever, even if they had given birth to children with congenital abnormalities or have had pregnancy loss (abortion) in antecedent. A quarter of mothers do not want to be tested, a fifth of this group being very young females (18-25 years of age) when the highest percentage of positive serology was present (21-30 years old age interval) (Costache et al., 2010).
If the mother was not tested, neither before, nor during the pregnancy, situation we found in our study in more than half of mothers in neonatal department, neonatal diagnosis of congenital toxoplasmosis may be established by the presence of specific IgA in symptomatic newborns in the presence of a negative serology for specific IgM, and IgA monthly testing for a period of one year in newborns with latent forms born from mothers with positive serology tested after birth (Jaisson-Hot et al., 2001; Carvalheiro et al., 2005). The toxoplasmic etiology was established in our studies in 14.3% of children with malformations, hydrocephaly, ocular defects, prematurity and low birth weight; the strongest correlation being obtained between ocular defects and positive IgA. We also establish that the best approach to certify diagnosis and identify latent forms of congenital toxoplasmosis, by affordable and available laboratory methods in countries where no screening (prenatal or postnatal) is applied, is by

testing IgG and IgA for newborns which proof more effective than IgG and IgM alone. The benefit of IgA in the screening of neonates was demonstrated because an important percentage of newborns CI=(0.03%-3.2%) in the group of apparently healthy neonates and CI=(3.2%-17.7%) in the group of newborns with malformations, had negative IgM and in the absence of an organized screening program they will be lost if only IgG and IgM are tested, because the eventually positive IgG is considered as placental transmission from mother. In the group of "healthy" newborns cases with latent forms of congenital toxoplasmosis may pass undiagnosed if a screening program on neonates is not considered (Costache, 2008).

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References

Carvalheiro C.G., Mussi-Pinhata M.M., Yamamoto A.Y., De Souza C.B.S., Maciel L.M.Z. 2005. Incidence of congenital toxoplasmosis estimated by neonatal screening: relevance of diagnostic confirmation in asymptomatic newborn infants. Epid. Inf. 133:85-491.

Costache C., Tigan S., Colosi I., Coroiu Z. 2001. Toxoplasmic Infection In Pregnant Women From Cluj County And Neighbouring Area, App. Med.Inform. 23(3-4):31-36.

Costache C.A., Colosi I., Junie M. 2010. Testing for toxoplasmic immunity in women of fertile age - are we prepared? Sci. Parasitol. 11(1):44-46

Dunn D., Wallon M., Peyron F., Petersen E., Peckham C., Gilbert R. 1999. Mother-to-child transmission of toxoplasmosis: risk estimates for clinical counseling. Lancet 353:1829-1833.

Jaisson-Hot I., Wallon M., Al Kurdi M., Thulliez P., Kahi S., Cozon G., Peyron F. 2001. Congenital toxoplasmosis. Transitory negative serology. Presse Med. 1001-1004.

Jamshaid Iqbal J., Nabila K. 2007. Detection of acute *Toxoplasma gondii* infection in early pregnancy by IgG avidity and PCR analysis, J. Med. Microbiol., 56:495-1499.

Montoya J.G., Remington J.S. 2008. Management of *Toxoplasma gondii* Infection during pregnancy. Clin. Infect. Dis. 47(4):554-566.

NCCLS. 2004. Clinical Use and Intrepretation of Serologic Tests for *Toxoplasma gondii*; Approved Guideline. NCCLS document M36-A. NCCLS, 940 West Valley Road, Suite 1400, Wayne, PA, USA, pp.1887-1898.

Sensini A. 2006. *Toxoplasma gondii* infection in pregnancy: opportunities and pitfalls of serological diagnosis. Clin. Microbiol. Infect. 12:504-512.

Wallon M, Mallaret MR, Majon M, Peyron F. 1994. Toxoplasmose congénitale, évaluation de la politique de prévention. Presse Med. 23:1467-70.

THE FUTURE OF THE *TRICHINELLA* SP. CONTROL IN THE EUROPEAN UNION

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Nematodes of the genus *Trichinella* are circulating in most of the 27 member states of the European Union, but these zoonotic parasites have not been documented in Cyprus, Luxembourg, Malta and Great Britain in the last 50 years. The great biomass of these parasites are circulating among wildlife, but when humans fail in the proper management of wildlife and domestic pigs, these parasites can be transmitted into the domestic habitat establishing a domestic cycle. Today, approximately 250 million fattening pigs are slaughtered in the EU per year and all of them are subjected to be individually tested by artificial digestion according to the EU regulation (2075/2005). However, most of these pigs are raised under high containment conditions where *Trichinella* sp. infection has been never documented. Only a small percentage of pigs are backyard or free-ranging and consequently could be a risk to be infected with *Trichinella* sp. parasites, because are not always slaughtered under veterinary control and are more frequently in touch with the sylvatic cycle. By the EU law, all slaughtered pigs and hunted wild animals intended for human consumption should be tested for *Trichinella* with a high economic effort. The recognition of holdings where pigs are under high containment conditions.

TRICHINELLA IN WILD CARNIVORES IN ROMANIA

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Trichinella infection is still a major problem in Romania. The lifecycle of the parasite in nature is achieved through the involvement of wild carnivores: wolf (*Canis lupus*), fox (*Vulpes vulpes*), golden jackal (*Canis aureus*), wild cat (*Felis sylvestris*), lynx (*Lynx lynx*) and mustelids (Mustelidae family). Our aim was to establish the prevalence of *Trichinella* sp. infection in these species.
Between 1999 and 2012 were collected 469 corpses of the mentioned species: 56 wolves, 369 foxes, 3 golden jackal, 32 wild cats and 12 lynx from 19 counties of Romania. The animals came from: wolves – Maramureş (MM), Bistrița-Năsăud (BN), Cluj (CJ), Mureş (MS), Harghita (HG), Neamț (NT), Covasna (CV), Braşov (BV), Argeş (AG), Sibiu (SB), Alba (AB), Hunedoara (HD), Caraş-Severin (CS); foxes – Bihor (BH), Călăraşi (CL), Constanța (CT), Satu-Mare (SM), AB, BV, CJ, CV, HD, HG, MS; golden jackal – Tulcea (TL); wild cats – Sălaj (SJ), AB, CT, NT, TL; lynx – NT.
Between 2009 and 2012, 25 specimens belonging to the family Mustelidae have been examined: *Martes foina* (n=2) – Buzău (BZ), CJ; *M. martes* (n=2) – TL, BV; *Meles meles* (n=6) – Vaslui (VS), AB, CJ, TL; *Mustela erminea* (n=3) – TL; *M. lutreola* (n=3) – TL; *M. nivalis* (n=3) – CJ, HG, SJ; *M. putorius* (n=4) – Suceava (SV), AB, BZ, MS and *Vormela peregusna* (n=2) – CT, TL.
All the corpses were examined by trichinoscopy and positive cases processed with artificial digestion.
The prevalence of *Trichinella* sp. infection in wolves was 33.9% (19 positives from 56 examined). In foxes 63 of the 369 examined resulted positive, the prevalence being 17%. In golden jackal, 2 of 3 examined animals were positive (66.6%). In wild cats, the prevalence was 18.75% (6 of 32) and in lynx 75% (9 of 12). The infection was found in *M. erminea* (3/3), *M. lutreola* (1/3) and *M. foina* (1/2).

STAGE SPECIFIC GENES AND ANTIGENS IN *TRICHINELLA* GENUS

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Trichinella spiralis is a unique intracellular parasitic nematode that is distributed worldwide and can infect almost all mammals, including humans. The life cycle of *T. spiralis* is completed within a single host species and infection starts with the consumption of infective muscle larvae (ML) and digestion of the protective capsule within the host stomach. Larvae undergo four fast molts in intestinal epithelial cells and eventually develop into sexually mature adults (Ad) approximately 2-3 days post infection (pi). Freshly released newborn larvae (NBL) are carried to host tissues by blood flow and invade new host cells. The NBL penetrate striated muscle cells and undergo developmental changes. Larvae that are older than 14 days can be infective to subsequent potential hosts and may remain viable for the entire life span of the host.

To date, little is known about the molecular mechanisms that are involved in parasite development and survival within the cytoplasm of host cell. Identification of stage-specific genes/proteins will be important for elucidation of these mechanisms. ML, Ad and NBL are three major stages in the life cycle of *T. spiralis* that exhibit distinct antigenicity, indicating differential regulation of many parasite proteins (Boireau et al., 1997). Previously, very few developmentally regulated antigens have been characterized, except the ML stage-specific TSL-1 antigens identified by monoclonal antibodies (review Boireau et al., 2004).

In an attempt to identify stage-specific genes of *T. spiralis*, subtracted cDNA libraries of NBL, Ad3 and Ad5 were constructed respectively, using a suppression subtractive hybridization (SSH) technique (Liu et al., 2007). A number of stage-specific cDNAs derived from NBL, Ad3 and Ad5 were identified and analyzed. Six genes were identified as **NBL stage-specific,** including one member of the *T. spiralis* gene family encoding glutamic acid rich proteins, two genes encoding novel serine proteases, two closely related genes encoding proteins that are members of a deoxyribonuclease II (DNase II)-like family and one nucleotidic sequence with no similarity to known genes. The twin genes encoding DNaseII (*DnaseII1*$^{Ts/NBL}$ and *DnaseII2*$^{Ts/NBL}$) (Liu et al., 2008) have a high percentage of identity in their amino acid (aa) sequence (89.6%), and their predicted aa sequences exhibited a N-terminal signal peptide, a potential helix-loop-helix motif and the conserved domains of DNase II. Four stage-specific clones encoding homologues of retinoid X receptor, caveolin, C2H2 type zinc finger protein and a putative protein with no homology to known sequences were obtained from **3-day-old adult worms**. The caveolin-1 gene (*Cav*^{Ts}) was characterized and identified as an adult-specific antigen. *Cav*^{Ts} is gradually accumulated only on the ova surface reaching a maximum at 3 days pi, and decreasing during newborn larva (NBL) development (Hernandez-Bello et al., 2008). Another target (*AdTs1*) (Wu et al., 2005) was analysed in this 3-day-old adult subtractive cDNA library. The selected gene encodes a protein with two putative zinc finger domains. Interestingly, some strong similarities were found between the AdTs1 protein, nuclear hormone receptors of mammals and a molting marker of *C. elegans*. Additional immunodominant stage specific antigens were identified at the very early development of *Trichinella* in the intestinal tract between 14 hours pi and 48 hours p.i. (Zocevic et al., 2011).

Recent sequencing of *T pseudopsiralis* transcriptome was achieved and compared with *T spiralis* one (Liu and Boireau, submitted). We identified a core of shared and constitutively expressed 'housekeeping' genes that evidently are essential to basic *Trichinella* parasite metabolism, as well as elucidated genes and gene families that are differentially expressed in particular parasite species and life history stages.

In brief the *Trichinella* developmentally regulated genes that has been described, can be divided into two main functions: i) the development of the parasite (*Cav*^{Ts} *AdTs1*) ii) the interaction with the host cell (proteases, *DNaseII*) to allow the generation of the nurse cell.

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References

Boireau P., Vallée I., Leguerhier F., Blaga R., Baoquan F., Hernadez-Bello R., Ortega-Pierres G., Mingyuan Liu. 2004. *Trichinella* antigens and immunodominant epitopes. In IX European Multicolloquium of Parasitology-EMOP (Ed. Srj, M.), Valencia, Spain, July 18-23, pp. 181-188.

Boireau P., Vayssier M., Fabien J., Perret C., Calamel M., Soulé C. 1997. Antigenic analysis of L1M of *Trichinella* T1 and T5 using 40 monoclonal antibodies: characterization of eleven antigenic groups and identification of new species markers. Parasitology, 115:641-651.

Hernandez-Bello R., Bermudez-Cruz R.M., Fonseca-Linan R., Garcia-Reyna P., Le Guerhier F., Boireau P., Ortega-Pierres G. 2008. Identification, molecular characterisation and differential expression of caveolin-1 in *Trichinella spiralis* maturing oocytes and embryos. Int. J. Parasitol. 38:191-202.

Liu M., Wang X.L., Fu B., Li C., Xinping W., Le Rhun D., Chen Q.J., Boireau P. 2007. Identification of stage-specifically expressed genes of *Trichinella spiralis* by suppression subtractive hybridization. Parasitology 134:443-1455.

Liu M.F., Wu X.P., Wang X.L., Yu Y.L., Wang W.F., Chen Q.J., Boireau P., Liu M.Y. 2008. The functions of deoxyribonuclease II in immunity and development. DNA and cell biology 27:6.

Wu Z.P., Garcia-Reyna P., Fu B.Q., Yang J., Li C.V., Yang Y.P., Liu M.Y., Ortega-Pierres G., Boireau P. 2005. A stage-specific open reading frame from three-day old adult worms of *Trichinella spiralis* encodes zinc-finger motifs. Parasite 12:151-157.

Zocevic A., Mace P., Vallee I., Blaga R., Liu M., Lacour S.A., Boireau P. 2011. Identification of *Trichinella spiralis* early antigens at the pre-adult and adult stages. Parasitology 138:8.

Trichinellosis in Romania: overview on the past, present situation and future perspectives

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Trichinellosis is a parasitic zoonosis with a high prevalence worldwide and one of the most important zoonotic diseases in Romania. This paper analyzes the main aspects of the disease registered in Romania.

Historically, the first **report of human and pig trichinellosis** in Romania dates back to 1868. Therefore, these two host species have been constituted the main species of the observations along the time, the pig representing the main source for human infection. Thus, 95% of human trichinellosis cases in our country originate from specific cultural food practices involving **pork consumption** (Cristea, 1998; Cironeanu and Ispas, 2002).

The **sylvatic cycle**, such as through the consumption of wild boar and bear meat has been also involved in human cases, but it remains a minor component of the etiology of human trichinellosis in Romania (Cristea, 1998).

Particular epidemiological characteristics of trichinellosis appeared after the first report of human trichinellosis caused by the consumption of infected horse meat, registered in 1975. Thereafter, the domestic horse has appeared as a novel vector of *Trichinella* spp. infection to humans, with outbreaks documented in France, Italy, and Mexico (Boireau et al., 2000; Murrell and Pozio, 2011).

In Romania, the studies for evaluating the prevalence of *Trichinella* spp. infections in horses using both direct and indirect tests were performed, after the year 2000. No *Trichinella* spp. larvae were detected by trichinelloscopy and artificial digestion in horses slaughtered between 2001 and 2004. The false positive results were obtained by serology (Blaga et al., 2009). Furthermore, the lack of detection of *Trichinella* spp. infected horses by artificial digestion, suggests a **very low prevalence of infection in Romanian horses**.

Overall, the **most of human trichinellosis cases** in Romania were reported during the **winter**. This is not surprising considering that, especially during winter period, backyard pigs are slaughtered at home, some of them without veterinary inspection (Barzoi et al., 1999; Radulescu, 2000; Zanc, 2001). These animals are traditionally consumed in a variety of undercooked homemade pork products such as sausages, ham, bacon, blood pudding, and mosaic salami (Barzoi et al., 1999; Neghina et al., 2010). Although summer outbreaks do occur, they are wide-spread over large territories and have low-infective rates because, after slaughter, meat is usually properly cooked and consumed rather than preserved by smoking or salting (Lupascu, 1970).

The epidemiological status of the disease had different **aspects along the time**, registered by diferent authors. The most data are especially after the Romanian authorities declared trichinellosis a notifiable disease, in 1961, and reliable epidemiological information on animal and human infections became available (Lupascu, 1970; Barzoi et al., 1999).

During the 1963-1968 period, 71 human trichinellosis foci were reported, with an incidence of 0.4 cases per 100,000 inhabitants (ranges 0.1-0.7).

Between the 1969 and 1978 years, the limited general data available indicate that 250-400 human cases of trichinellosis were annually diagnosed; the incidence of infection varied between 1.3 and 2 cases per 100,000 inhabitants per year.

From 1979 until 1986, the annual number of human cases was largely constant with an incidence of no more than 2 cases per 100,000 inhabitants per year.

After the political changes of the 1989 year, the annual incidence increased from between 0.1 and 4.1 cases per 100000 inhabitants during the communist period (1963-1989) to 6.2 cases per 100,000 inhabitants, with a range of 2-15.9 per 100,000 between 1990 and 2007. Thus, in only 10 years, the incidence of human trichinellosis increased from 1 per 100,000 inhabitants in 1983 to 15.9 per 100,000 inhabitants in 1993 (Barzoi et al., 1999; Cuperlovic et al., 2005; WHO, 2009).

Many authors mention that in according to the International Commission on Trichinellosis survey in 2004, Romania has the **most cases of trichinellosis in the world**. Thus, the World Health Organization European Region accounted for 87% of cases; 50% of those occurred in Romania, mainly during 1990-1999. Incidence in the region ranged from 1.1 to 8.5 cases per 100000 population (Murrell and Pozio, 2011).

Between the 2002 and 2007 period, as a consequence of the improvement in meat inspection procedures and continuous education of the population regarding sanitary practices, the incidence of human cases was less than 4 per 100,000 inhabitants (WHO, 2009)

Various studies reported the average prevalence of trichinellosis in the main domestic and wild animal populations, as follows: 0.05% in pigs (*Sus scrofa domestica*), 5.7% in dogs (*Canis familiaris*), 9.6% in domestic cats (*Felis catis domestica*), 0.2% in wild boars (*Sus scrofa ferus*), 10.2% in bears (*Ursus arctos*), 22% in foxes (*Vulpes vulpes*), and 38.5% in wolves (*Canis lupus*) (Lupascu, 1970). Between 1980 and 1993 the prevalence of trichinellosis in the major susceptible animal populations was 0.08-0.12% in pigs, 0.1-0.5% in wild boars, and 1.5-25.6% in bears, 5-15% in dogs, and 5-14% in domestic cats (Barzoi et al., 1999). The cumulative prevalence of *Trichinella* infection reported for 1997–2004 was 0.08% in domesticated pigs, 0.9% in wild boars and 13.1% in bears (Blaga et al., 2008).

Regarding the **species of the parasite**, in the past all larvae were assumed to be *Trichinella spiralis*. However, recently, using PCR-based methods, *T. spiralis* and *T. britovi* were found circulating among wild and domestic animals in Romania. *T. spiralis* was the predominant species found in domestic animals (75%), while *T. britovi* was more prevalent in wildlife (86%). No mixed infections were found (Blaga et al., 2008). *T. pseudospiralis* and *T. native* have also been detected (Olteanu, 1996; Cuperlovic et al., 2005), but these two species were not confirmed by molecular biology.

With regards of **the diagnosis** of the disease in Romania, in April 1913, trichinelloscopic examinations were carried out for the first time in the slaughterhouses in Bucharest (Cironeanu and Ispas, 2002). From then on, strict public health measures were implemented in all Romanian slaughterhouses and in the veterinary public system to successfully control trichinellosis using this method. Currently, the trichinelloscopic examination is replaced by the artificial digestion method according to the European Community Regulation (EC) No. 2075/2005.

In conclusion, trichinellosis remains a major public health issue in Romania that requires specific policies to be applied **to advance efficient prevention and control strategies**.

References

Barzoi D., Meica S., Negut M. 1999. Toxiinfectiile alimentare. Bucharest: Editura Diacon Coresi, 605–613

Blaga R., Cretu C.M., Gherman C., Draghici A. 2008. Animal *Trichinella* infection in Romania: geographical heterogeneity for the last 8 years. Vet. Parasitol. 159:290-294

Blaga R., Durand B., Antoniu S., Gherman C. 2007. A dramatic increase in the incidence of human trichinellosis in Romania over the past 25 years: impact of political changes and regional food habits. Am. J. Trop. Med. Hyg. 76:983-986.

Boireau P., Vallée I., Roman T., Perret C., Mingyuan L., Gamble H.R., et al., 2000. *Trichinella* in horses: a low frequency infection with high human risk. Vet. Parasitol. 93:309-20.

Cironeanu I., Ispas A.T. 2002. Totul despre trichineloză. Bucureşti, Romania: Editura MAST, (in Romanian).

Cristea G. 1998. Trichineloza la animale şi riscul îmbolnăvirii omului. Editura Ceres, Bucureşti, România (in Romanian).

Cuperlovic K., Djordjevic M., Pavlovic S. 2005. Re-emergence of trichinellosis in South-Eastern Europe due to political and economic changes. Vet. Parasitol. 132:159-166.

Gottstein B., Pozio E., Nöckler K. 2009. Epidemiology, diagnosis, treatment, and control of trichinellosis. Clin. Microbiol. Rev. 22:127-45.

Liu M., Boireau P. 2002. Trichinellosis in China: epidemiology and control. Trends. Parasitol. 18:553-556.

Lupascu G. 1970. Trichineloza. Editura Academiei Republicii Socialiste Romania, Bucharest, 7-94.

Murrell K.D., Pozio E. 2011. Worldwide occurrence and impact of human trichinellosis, 1986-2009. Emerg. Infect. Dis. 17(12):2194-202.

Neghina R., Neghina A.M., Marincu I., Moldovan R., Iacobiciu I. 2010. Epidemiology and epizootology of trichinellosis in Romania 1868-2007. Vector Borne Zoonotic Dis. 10(4):323-8.

Olteanu G. New study on epidemiology and control of trichinellosis in Romania. 1996. In: Ortega-Pieres, G, Gamble, HR, van Knapen, F, Wakelin D, ed. Trichinellosis ICT 9. Mexico: Centro de Investigation y Estudios Avanzados IPN, 531-538.

Rădulescu S. 2000. Parazitologie medicală. Editura ALL, Bucharest, (in Romanian).

Zanc V. 2001. Parazitologie clinică (Nematodoze. Sindroame de larva migrans). Sincron Publishing House, Cluj-Napoca, pp. 141, (in Romanian).

ONE HEALTH AND GEOSPATIAL TOOLS IN PARASITOLOGY

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The link between animal and human infections and the global environment is unquestionable. This awareness has contributed to an increasing appreciation of the interdependency of human, animal and ecosystem health within the transdisciplinary "One Medicine – One Health" approach to global health. Health is an outcome of multiple determinants. The predisposing factors of health status are often interdependent and interrelated, creating a complex web of causation. Geospatial tools can answer questions about the complex web of causation of many health issues and parasitological infections. For these reasons, the application of geospatial tools, as Geographical Information Systems, Global Positioning System, Satellite based Remote Sensing and Virtual Globes (e.g., Google Earth™) to spatial epidemiology in human and animal parasitology have been firmly established for geo-positioning, collating, exploring, visualizing and analyzing health data in a spatially explicit manner. In the current era of global warming, global movement and globalization, pathogens can move further, faster and in greater numbers than ever before and thus the use of geospatial tools for mapping, forecasting, monitoring, early warning and surveillance is strongly advocated. In addition to climate changes, other important drivers for the (re)-emergence and spread of parasites include habitat changes, alterations in water storage and irrigation habits, pollution, development of resistance to insecticides and drugs, globalization and the increase in international trade, tourism and travel. The last 25 years have witnessed an explosion of environmentally related diseases, with increases in prevalence, incidence and geographical distribution across wide taxonomic ranges, related to climatic and environmental changes and practical changes in land-use. Babesiosis, bluetongue, chikungunya, dengue, encephalitis, erhlichiosis, leishmaniosis, Lyme disease, malaria, plague, trypanosomiasis, West Nile disease, yellow fever, dirofilariosis, are examples of vector-borne diseases that are affected by climate changes and the above-mentioned drivers. The challenge for gaining large-scale control programs for parasitic infections of human and animals in developing and developed countries cannot be addressed without using geospatial tools and considering both abiotic and biotic environmental factors that affect the maintenance and transmission of the parasites.

ECOLOGICAL NICHE MODELS AND THE DISTRIBUTION AND ABUNDANCE OF HOOKWORMS IN BOLIVIA

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The predictive value of Maximum Entropy (Maxent) geostatistical models[1] and empirical models based on the growing degree day-water budget (GDD/WB) concept were compared as methods of mapping the distribution and abundance of hookworm in Bolivia. Maxent is a general purpose ecological niche modeling software that can be used to predict species geographic distribution when only occurrence data are available for analysis (e.g., vector occurrence, case incidence). GDD/WB models are based on known thermal-hydrological preferences and limits of tolerance of a biological system in the environment. A climate grid of Bolivia (18 km^2, monthly long term normal temperature, rainfall, evapotranspiration) was used to calculate the annual number of transmission cycles of the free-living stages (egg-L_3) of *Necator americanus* possible in each grid cell using a base temperature of 15 ^oC (below which no development progresses) conditional on a water budget threshold of > 0.5 soil moisture and reported mean L_3 longevity. A cumulative value was derived of 260 GDD per transmission cycle (annual GDD if > 0.5/260). A risk map based on potential transmission cycles per year revealed an elevation gradient of suitability in Bolivia that ranged from no transmission at high elevation altiplano sites and in arid zones to 13 potential transmission cycles at hot, humid Amazonian sites. Model output was significantly related to 35 municipality level survey prevalence data records (range 0-80%). Maxentgeostatistical model analysis yielded a probability surface map that ranged from a 0.0024 to 0.815 probability of occurrence. Maxent threshold analysis was performed by running separate models based on survey points of < 2% prevalence and > 2% prevalence, revealing a low risk Altiplano zone and a variable predicted probability gradient from the eastern slopes of the Andes to Amazon ecological zones. The potential value and limitations of the two modeling approaches will be discussed.

ZOONOTIC PARASITIC DISEASES IN EUROPE: CLIMATE AND GLOBAL CHANGE EFFECTS

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The capacity of climatic conditions to modulate the extent and intensity of parasitism is well known since long ago. Climate change may be expected to have an important impact on the diseases parasites cause. Protozoans and helminths are organisms with less short generation times and less rapid population growth rates than viruses and bacteria. This explains why the emergence of parasitoses, mainly helminthiases, takes more time to be detected and imply more control difficulties. Climate variables are able to affect prevalences, intensities and geographical distribution of parasites both directly influencing on free-living larval stages, as well as indirectly influencing on mainly invertebrate but also vertebrate hosts. Additionally, monoxenous parasites (one-host life-cycle parasites) are not those presenting bigger health control problems within this context. Zoonotic parasitoses and, among them, those having less definitive host specificity and transmitted by a non-strictly specific vector (or intermediate host) show higher introduction, spreading and emergence capacities.

Climate and global changes represent potential risks for several zoonotic parasitoses in Europe. Several diseases illustrate good examples. In leishmaniasis, intra-European transport of domestic dogs (reservoir) with northern tourists visting southern countries and a global warming inducing a northward spread of phlebotomine vectors imply a possible northward spread from its original south-European distribution. Similar arguments may be highlighted for diroflariasis, which is showing an evident increase of human cases in Europe. Recent human fascioliasis outbreaks in France were related to the unexpected contamination of commercially grown watercress in its turn due to the adaptation of the parasite to the nutria, a sylvatic rodent recently introduced from South America. Cercarial dermatitis and eosinophilic meningoencephalitis are additional examples.

The emergence of Chagas disease in Europe is a completely different situation. Thousands of patients have been diagnosed among Southamerican immigrants, mainly in Spain but also in France, Portugal, Switzerland and Italy. This disease cannot be established in Europe due to the absence of triatomine vectors, but its pathogenic sequelae, mother transmission to newborns, and direct transmission capacity through infected blood donors illustrate large health problematics Europe never expected to face.

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THE USE OF SPATIAL STATISTICS TOOLS AND THE MAXIMUM ENTROPY METHOD FOR STUDY AND FORECASTING OF INFECTIOUS ANIMAL DISEASES SPREAD

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The applied usage of GIS in epidemiology means the mapping of information on the epidemic situation in a particular territory against a set of topical layers (i.e. animal and human population, transportation network, water bodies, vegetation etc.). The availability of geospatial information helps solve one of the principal problems in epidemiology: it allows discovering the causes that contribute to the occurrence and spread of emerging infections in a population, since such causes may be closely connected with both environmental and socio-economic factors of certain localization. Here we consider the application of some spatial analysis functions of ArcMap (Esri, USA) by the example of diseases under study in the Federal Centre of Animal Health.

The basic instruments of geostatistical analysis that are used in the practice of epidemiological analysis are the calculation of *Mean Centre* allowing to estimate the tendency of disease-front advance in time; and *Standard Deviational Ellipse* that allows to define the main direction of the disease spread.

The simplest GIS tool that allows estimating the tendency towards the cases clustering is the *Nearest Neighbor Index*. Other program instruments that are also frequently used are *Moran's I Statistic* and *Getis-Ord Statistic*, based on which it is possible to visualize the existing cases' clusters. *Multi-Distance Cluster Analysis: Ripley K Function* is another useful tool for the estimation of case clustering at different distances from the mean centre.

To define the correlation of disease cases with geographical and socio-economic factors in the study area, a methodology of *Associative Analysis* is applied. This methodology means the application of a regression model that correlates the *Spatial Density* of cases' distribution within the territory with the values of the selected geospatial and socio-economic variables. Based on the obtained regression equation, a risk map can be created that illustrates the probability of emergence of the disease within the study area.

One of the novel methods that are used to detect the habitat of a certain host/causative agent/parasite species is the *Maximum Entropy Modeling*. MaxEnt software (Princeton University, USA) allows to carry out a complex analysis of habitat factors and to create a probability map which illustrates suitable conditions for the habitation of a particular species. We suggest using MaxEnt software for the analysis of possible emergence of disease cases in the territory of study due to an aggregate of geographical and socio-economic factors. This method was used for analyzing of African swine fever cases distribution in the territory of Russian Federation.

VECMAP, A ONE-STOP-SHOP TO VECTOR MAPPING

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The presence and spread of diseases transmissible by vectors (mosquitoes, ticks etc.) depends on diverse and interacting factors such as present distribution, climate, weather and wind patterns, proximity to water bodies, land use and vegetation. Hitchhiking on international trade and travel, foreign species may establish themselves in new environments world-wide if conditions are favorable. Trends such as climate change and land development also contribute to changes in vector distribution. Vector-borne diseases (Chikungunya, Dengue, West Nile, Lyme, Tick-borne encephalitis…) are an increasing public health concern in many European countries. Pilot projects have demonstrated that it is possible to assess abundances of local species and the risk of arrival of foreign species through modeling of the dependencies between habitat conditions, seasonal trends and in-situ sampling results.

VECMAP aims at assessing the viability of a tool and associated service for automated disease vector mapping and forecasting, integrating earth observation (EO) and satellite navigation assets with modeling, mapping and in-situ measurement techniques. To achieve this consortium of three specialists RTD companies have joined efforts with the European Space Agency (ESA) to develop an integrated software package and service. The consortium combines their expertise in spatial modeling, the development of spatial information systems and mobile tools, and of EO time-series production chains.

VECMAP has four main integrated components: (a) a field sampling planner using smartphone and GPS technology linked to a secured centralized database; (b) an area-wide distribution modeling module, fed by the VECMAP field samples and standardized Earth Observation time series; (c) a local landscape scale vector habitat modeling service; and, (d) a Spatial Information System that integrates the VECMAP components and provides access to the required supporting data as well as the means to display and analyze outputs.

After a successful feasibility study VECMAP is currently being tested for mosquito and tick mapping by 12 academic and public health teams in 10 countries. In this presentation we highlight how the concept of a ‘one-stop-shop’ system and service applied to disease vector mapping can contribute to focus resources on improving research outputs based on good quality ground data.

DIAGNOSTICS OF PARASITIC INFECTIONSIN A FLAT WORLD

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Correct diagnosis is the basis for adequate treatment. This has become increasingly evident with large control programs aiming at neglected diseases (Hotez et al., 2006). Emphasis on diagnostics has revealed that the major diagnostic technique to demonstrate infections caused by parasites – microscopy – often is associated with severe limitations. Also epidemiological surveillance reporting, which forms the basis for policy decisions can be affected by uncertainties regarding reliability of primary data. How can novel technologies, "*the Flatteners*", affect these problems?[1]

Poverty and neglected diseases

Chronic parasitic infections are frequently neglected because symptoms are diffuse and the proportion of the population infected so large that the condition is regarded as normal. Increased risk for transmission may result from diverse factors like environmental factors, conflicts, altered food distribution networks.

The inter-relationship between disease – often caused by parasites – and poverty has become increasingly evident and resources have been allocated for the control of infections affecting poor populations e.g., by setting Development Goals up to 2015[2]. Poor populations are extremely vulnerable to medical emergencies. In depth understanding of the mechanisms forcing people into the poverty trap is becoming available. The widespread short-comings of the basic health care system is apparent through careful documentation and we start to understand the impact of disease on economy and the fundamental living conditions of poor people[3]. The disturbing fact is that laboratory medicine in may be a barrier to effective health care (Petti et al., 2006).

Poor diagnostics and the generation of a vicious cycle

Misdiagnosis, like over-diagnosis of malaria is common in endemic areas. If diagnostics is unreliable, distrust rapidly creates a vicious cycle and diagnostics deteriorate.

With decreasing prevalence, malaria is only one of several possible causes of fever and thus the quality of health care declines. The number of individuals carrying chronic worm infections like schistosomiasis is vastly underestimated due to insensitivity of stool examination to detect excreted helminth eggs. These circumstances put a pressure to increase the reliability of diagnostics. At the same time it is evident that high costs are not necessarily reflecting high quality; increasingly we are need to look at performance for the buck!

At the same time changing priorities are putting a pressure on parasitology teaching (Bruschi, 2009) and recruitment of staff with first hand experience of diagnostics in an endemic situation is diminishing in many European regions.

Parasite Diagnostics

Microscopy – the black box

Microscopy is dependent on three major components, the microscope the microscopist and the procedures and reagents associated with sample handling and preparation for examination. Correctly performed microscopy reveals the presence of a vast number of parasites, both protozoa and helminths and the number of parasites counted by anexperienced microscopist is the reference method[4].

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[1]In his book, first released in 2005 , "The World Is Flat: A Brief History of the Twenty-First Century", Thomas Friedman lists the forces flattening the world: Collapse of Berlin Wall, Internet, Communication, Personal digital devices, Outsourcing /Insourcing, Supply-Chaining, Informing.

[2]http://www.guardian.co.uk/global-development/2011/aug/05/millennium-development-goal-one-poverty-hunger

[3]Collins D., Morduch J., Rutherford S., Ruthven O.: Portfolios of the Poor: How the World's Poor Live on $2 a Day.ISBN: 9781400829965

[4]http://www.btinternet.com/~ukneqas.parasitologyscheme/Blood_Scheme/Teaching_Information/Stains_for_malaria_parasites/stains_for_malaria_parasites.html Basic MALARIA microscopy, WHO-OMS 1991 72 pp. [4]In his book, first released in 2005 , "The World Is Flat: A Brief History of the Twenty-First Century", Thomas Friedman lists the forces flattening the world: Collapse of Berlin Wall, Internet, Communication, Personal digital devices, Outsourcing /Insourcing, Supply-Chaining, Informing.

A major problem with microscopy is that it has been very difficult to control all the variables affecting a reliable diagnosis. This is seen in several recent reports of misdiagnosis, both over-diagnosis as in the case of malaria and under-diagnosis as for schistosomiasis. Protozoa in stool samples tend to be overdiagnosed and apathogenic species are often misinterpreted as pathogenic (Leiva, 2006)

The Microscope

The microscope has undergone very little change since it was introduced some 200 years ago. Until recently [5]microscopes were not designed to withstand harsh climate conditions. The quality and performance obviously shows a high degree of variation - largely dependent on maintenance and laboratory standards.

The Microscopist

To identify correctly helminth eggs or protozoan cysts or trophozoites in a biological sample requires skills earned from vast experience and determined time-consuming examination of the sample. It goes without saying that the level of expertise is highly variable – and not so easy to quantify.

Samples/reagents/materials/methods

Even if methods descriptions are available, a high degree of variation occurs and the availability, stability and performance of reagents is a constant problem due to environmental conditions and intermittent electricity supply. Also the sample volume, number of samples, sample treatment, fixation method, concentration technique, staining method etc. cause variation in results and no method exists that is equally suitable for all parasites. Thus a standard method has to be chosen if no specific parasite infection is suspected for which a particular method can be employed. (Utzinger et al., 2010). These problems can be addressed by using standardized kits, but the cost and equipment needed may a problem (Cringoli et al., 2010).

Available established alternatives

Alternative diagnostic methods such as serology, antigen detection and nucleic acid detection by the polymerase chain reaction (PCR) are in use in well-equipped laboratories. Serology is especially useful in identifying infections in travellers and tourists whereas PCR has made possible discrimination and identification of organisms hard or impossible to distinguish based on morphology, such as identification of *Entamoeba histolytica*.

In well-equipped diagnostic laboratories several alternative methods are available e.g., for diagnostics of helminth infections (Bergquist et al., 2010), but essentially they supplement a well functioning microscopy routine. In certain situations as in the case of rapid diagnostic tests (RDTs) for malaria, the test can speed up the differential diagnostics of febrile conditions. The question is, to what extent alternative techniques are useful in situations where microscopy is a problem and if there are tests specifically addressing the situation in poor endemic regions.

Alternative Diagnostic Methods for "the Grass Roots"?

In response to the documented problems to identify malaria in endemic regions by microscopy, rapid diagnostic tests (RDTs) based on the detection of parasite-specific enzymes in the blood of infected individuals with various kits employing specific antibodies. These methods offer a real alternative to poor microscopy, but are far from ideal both with respect to robustness, standardization and availability, sensitivity and costs (Wongsrichanalai, 2007).

Reagent strips similar to the RDTs for malaria are available for the detection of parasite antigens stools. Usually such tests have low sensitivity. However there are exceptions where antigen detection in acute diarrhea has a higher sensitivity than microscopy as shown by ELISAs for *Giardia* and the fact that some reagent strips can detect *Giardia* in acute diarrhea with a sensitivity comparable to microscopy is promising (Oster, 2006).

Diagnostics based on specific properties of the target organism have been developed. This is exemplified by malaria diagnostics based on the demonstration of intraerythrocytic haemozoin paramagnetic nanocrystals, which also are birefringent.

Quality Assurance of Microscopy

Quality assurance of microscopy offers a real possibility to solve the problems associated with standardization of microscopy in endemic situations – but how?

Better microscopes and standardized methods are obvious ways to improve microscopy-based diagnostics.

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[5]eg. Zeiss "Primo Star" iLED, See: http://www.zeiss.de/c12567be0045acf1/Contents- Frame/8a410946841ce684c125746c.

Using tools for standardization, traceability and quality assurance we can get an idea of what is going on in the box. However, this is not easy as shown e.g., an attempt to measure performance using standard specimens containing different stool parasites, distributed to reference laboratories (Utzinger et al., 2010).

Virtual Microscopy for Education and Quality Assurance

Virtual microscopy is gradually expanding as commercial scanning microscopes become available. There are striking advantages, like the possibility by an unlimited number of individuals to examine the same specimen simultaneously. The image files are stored on a network of servers, which guarantees access from various geographical sites (Lundin et al., 2009).

Virtual microscopy was introduced as a tool for education and quality assurance in parasitology (Linder et al., 2008). The merits of the method in higher education – the quality of which is challenged by an insufficient number of teachers and microscopes – was evaluated recently at the National University of Mexico (Alcala, 2012).

Virtual microscopy is based on computer programs for image capture, storage and retrieval essentially developed for Google Earthand when the large image files can be navigated in x, y and z dimensions as in an ordinary microscope (Konsti, 2008; Lundin et al., 2009)[6].

Microscopy-based Diagnostics at a Distance: Image Transfer and Two-way Communication

Communication satellites will soon cover all corners of the globe. Novel communication technologies and the rapid spread of mobile phones open up new possibilities of using mobile phones at the basic levels of health care globally. Recently, software has been developed that enable mobile phones to function as servers, whereby information from a large number of peripheral devices can be analyzed centrally. There is a real possibility to break the isolation of diagnostics performed in remote areas by offering not only education and quality assurance, but also consultation - and even diagnostics-at a distance over the Internet.

Analysis at a Distance by Computer Vision and Crowd Sourcing

The analysis of data can be done either by distant expert(s) in a network (Suhanic et al., 2009) or by computers using specially designed algorithms. Image analysis and computer vision algorithms can be designed to perform the tasks of a microscopist with high accuracy as shown e.g., by the extensive work on malaria diagnostics (Frean, 2008)[7]. Image analysis based on the unique capacity of relatively untrained humans can even be performed by “Crowd Sourcing”[8] (Mavandadi, 2012).

Novel Diagnostic Devices for Imaging and Two-way Communication

The great challenge in parasite diagnostics is to come up with solutions, which work in remote poor endemic areas – the microscope was not – until recently[4] – designed to meet the strains and limitations imposed by conditions in the field.

Miniaturization of the Microscope

In recent years there has been several reports of technological innovations from image transfer from the microscope miniaturized microscopes to a microscope using an attached mobile phone to a mobile phone fitted with a compact portable microscope. Finally the need for a microscope was eliminated completely by developing techniques for lens-less imaging of microscopic objects directly on image sensor chips.

Microfluidics-based diagnostics using Lab-On-a-Chip

We are starting to see the diagnostic technologies based- on miniaturization as envisioned by Richard Feynman[9] “Lab-on-a-chip” (LOC) technologies allow scaling of lab processes down based on mechanical fluid flow control – microfluidics. Such devices integrate multiple laboratory functions on a chip of less than a few square centimeters. For complex analyses, as in PCR fluid reagents are transported simply by capillary force along prefabricated channels (Chin et al., 2011).

Microfluidicsand imaging

High resolution imaging of a sample is achieved by microfluidic flow to scan specimens across submicron apertures directly on a complementary metal-oxide semiconductor (CMOS) image sensor.

[6] http://www.webmicroscope.net
[7] http://www.malariajournal.com/content/8/1/218
[8] Crowd-Sourced Games for Telepathology
[9] “It is a staggeringly small world that is below. In the year 2000, when they look back at this age, they will wonder why it was not until the year 1960 that anybody began seriously to move in this direction.”Feynman's now-famous deliberations on the "plenty of room at the bottom" inspired several generations of scientists to seek to fill this room with nanometer-scale materials and techniques to study them.Feynman, R.P. in Miniaturization (ed., Gilbert H.D.) 282–296 (ReinholPublisCorporation, New York, 1961).

The device called an optofluidic microscope (OFM) (Cui et al., 2008) can detect e.g., malaria-infected red cells (Lee et al., 2011). High resolution of on-chip images can be obtained using a layer of submicron apertures between sensor and sample “holographic pixel super-resolution” (Bisharaet et al., 2010). Finally, techniques for identifying movement similar to those used in simple webcams and smartphones for motion detection and alarms could be used for the detection of parasites in body fluids and excreta[10].

References

Alcala-Canto Y., Ibarra-Velarde F., Vera-Montenegro Y., Alberti-Navarro A. 2012. Virtual microscopy for studying veterinary parasitology. Comp. Parasitol. 79:138-142

Bellina L., Missoni E. 2009. Mobile cell-phones (M-phones) in telemicroscopy: increasing connectivity of isolated laboratories. Diagn. Pathol. 4:19.

Bergquist R., Johansen M.V., Utzinger J. 2009. Diagnostic dilemmas in helminthology: what tools to use and when? Trends. Parasitol. 25:151-156.

Bishara W., Su T., Coskun A., Ozcan A. 2010. Lensfree on-chip microscopy over a wide field-of-view using pixel super-resolution. Optics Express. 18:11181-11191.

Breslauer D., Maamari R., Switz N., Lam W., Fletcher D. 2009. Mobile phone based clinical microscopy for global health applications. PloS One 4:e6320.

Bruschi F. 2009. How parasitology is taught in medical faculties in Europe? Parasitology, lost? Parasitol Res. 105:1759-62.

Chin C.D., Laksanasopin T., Cheung Y.K., Steinmiller D., Linder V., Parsa H., Wang J., Moore H., Rouse R., Umviligihozo G., Karita E., Mwambarangwe L., Braunstein S.L., van de Wijgert J., Sahabo R., Justman J.E., El-Sadr W., Sia S.K. 2011. Microfluidics-based diagnostics of infectious diseases in the developing world. Nat Med. 17:1015-9.

Cringoli G., Rinaldi L., Maurelli M.P., Utzinger J. 2010. FLOTAC: new multivalent techniques for qualitative and quantitative copromicroscopic diagnosis of parasites in animals and humans Nature Protocols 5:503-515.

Cui X., Lee L., Heng X., Zhong W., Sternberg P., Psaltis D., Yang C. 2008. Lensless high-resolution on-chip optofluidic microscopes for *Caenorhabditis elegans* and cell imaging. Proceedings of the National Academy of Sciences 105:10670.

Gascoyne P., Satayavivad J., Ruchirawat M. 2004. Microfluidic approaches to malaria detection. Acta Tropica 89:357-369.

Hotez P.J., Molyneux D.H., Fenwick A., Kumaresan J., Ehrlich Sachs S., Sachs J.D., Savioli L. 2007. Control of neglected tropical diseases. N. Engl. J. Med. 357:1018-1027.

Karl S., Gutiérrez L., House M.J., Davis T.M.E., St. Pierre T.G. 2011. Nuclear Magnetic Resonance: A Tool for Malaria Diagnosis? Am. J. Trop. Med. Hyg. 85:815-817.

Konsti J., Lundin J., Jumppanen M., Lundin M., Viitanen A., Isola J. 2008. A public-domain image processing tool for automated quantification of fluorescence in situ hybridisation signals. J. Clin. Pathol. 61:278-82.

Leiva B., Lebbad M., Winiecka-Krusnell J., Altamirano I., Tellez A., Linder E. 2006. Overdiagnosis of Entamoebahistolytica and Entamoebadispar in Nicaragua: a microscopic, triage parasite panel and PCR study. Arch. Med. Res. 37(4):529-34.

Linder E., Lundin M., Thors C., Lebbad M., Winiecka-Krusnell J., Helin H., Leiva B., Isola J., Lundin J. 2008. Web-based virtual microscopy for parasitology: a novel tool for education and quality assurance. PloS Negl. Trop. Dis. 2:e315.

Lundin M., Szymas J., Linder E., Beck H., de Wilde P., van Krieken H., García Rojo M., Moreno I., Ariza A., Tuzlali S., Dervişoğlu S., Helin H., Lehto V.P., Lundin J. 2009. A European network for virtual microscopy-design, implementation and evaluation of performance. Virchows Arch. 454:421-9.

[10] Linder E et al., Excreted live *Schistosome* eggs detectable by motion recognition using a modified web camera. In manuscript 2012.

Mavandadi S., Dimitrov S., Feng S., Yu F., Sikora U., Yaglidere O., Padmanabhan S., Nielsen K., Ozcan A. 2012. Distributed medical image analysis and diagnosis through crowd-sourced games: a malaria case study. PloS One 7(5):e37245.

Oster N., Gehrig-Feistel H., Jung H., Kammer J., McLean J.E., Lanzer M. 2006. Evaluation of the immunochromatographicCORIS Giardia-Strip test for rapid diagnosis of *Giardia lamblia*. Eur. J. Clin. Microbiol. Infect. Dis. 25(2):112-5.

Petti C.A., Polage C.R., Quinn T.C., Ronald A.R., Sande M.A. 2006. Laboratory medicine in Africa: a barrier to effective health care. Clin. Infect. Dis. 42:377-382.

Suhanic W., Crandall I. Pennefather P. 2009. An informatics model for guiding assembly of telemicrobiology workstations for malaria collaborative diagnostics using commodity products and open-source software. Malar. J. 8:16

Tek F.B., Dempster A.G., Kale I. 2009. Computer vision for microscopy diagnosis of malaria. Malar J. 8:153.

Utzinger J., Botero-Kleiven S., Castelli F., Chiodini P.L., Edwards H., Köhle N., Gulletta M., Lebbad M., Manser M., Matthys B., N'Goran E.K., Tannich E., Vounatsou P., Marti H. 2010. Microscopic diagnosis of sodium acetate-acetic acid-formalin-fixed stool samples for helminths and intestinal protozoa: a comparison among European reference laboratories Clin. Microbiol. Infect. 16:267-273

Weigl B., Domingo G., Labarre P., Gerlach J. 2008. Towards non- and minimally instrumented, microfluidics-based diagnostic devices. Lab. Chip. Be. 8:1999-2014.

Wongsrichanalai C., Barcus M.J., Muth S., Sutamihardja A., Wernsdorfer W.H. 2007. A review of malaria diagnostic tools: microscopy and rapid diagnostic test (RDT). Am. J. Trop. Med. Hyg. 77:119.

Zheng G., Lee S.A., Yang S., Yang C. 2010. Sub-pixel resolving optofluidic microscope for on-chip cell imaging. Lab Chip. 10:3125-3129.

HUMAN GIARDIOSIS REPORT IN ROMANIA: THE PRINCIPLE OF SNOWBALL!

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The starting point for this conference was the information about giardiasis being "a big problem in Romania", information based on an ECDC report showing that almost 9 out of 10 giardiasis cases are reported from Romania. In this report appears that in 2006-2008 in Romania there were an average of 150000 cases of giardiasis and, suddenly, in 2009 report there were 76671 cases reported number that was modified, in the 2011 report for 2009, to 16574. The epidemiological concepts state that there is no possibility to decrease 10 times the prevalence of any disease in a particular population in one year, whatever the prevention/treatment methods would be applied! The conclusion is that there is obvious a mistake either in the diagnosis (false positive diagnosis) or in the reporting procedure that lead to this data. The motivation offered by Romanian authorities was that non-confirmed cases and cases from screening programs were included. The mistake made by one of the reporters drug other mistakes from the rest of them, on the principle of the snowball. Based on our research and experience this is a correct motivation responsible for 90% of the exaggerated numbers particularly because in our country the reporting is made from hospitals and not from laboratories, as in most other European countries. On the other hand there is a false positive report, particularly before 2008 when the majority of laboratories did not have an external quality control on parasitology, which was compulsory with that year on in all standardized laboratories. Explanation about the false positive diagnosis in Romanian parasitology laboratories were given in a paper published in 2007 (Costache et al., 2007). However there are still authorized but not standardized laboratories which may offer false positive results to the general practitioner which will report a false positive case.
We perform an analytical study in children communities and general infantile population of Cluj county and neighbouring area between 2008-2011, and the overall incidence of giardiasis on 18486 children was 0.41%, with a higher incidence in disadvantaged children communities (social assistance centres) of 9.27%. The overall incidence of intestinal parasitic infestation in general infantile population was 1.62% when symptomatic cases with opportunistic protozoa were considered. We also perform an analytical study in adult general population (17645 adults) in the same period as for the infantile population (2009-2011) and we found an overall incidence of 0.8% with a higher incidence in the active group of population (19-55 years of age). The incidence of giardiasis was also analyzed for a larger period of time in hospitalized patients from Cluj county hospital (4713 cases) and it was 0.36%. These data are much closer to the data reported in 2011. The problem which had still to be solved is the small difference from the reality due to the false positive diagnosis. The aspect will be solved by the implementation of external quality control in all laboratories and maybe with the help of virtual microscopy.

References

Costache C., Junie M., Colosi I., Minica F. 2007. Intestinal Parasitosis in Adults from Infectious Disease and County Clincal Hospital Cluj. Sci. Parasitol. 8(1):39-44.

PARASITIC INFECTIONS OF AFRICAN GREAT APES: HOW MUCH DO WE SHARE WITH OUR NEAREST EXTANT RELATIVES?

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Diversity and host specificity of parasites of great apes

The interaction with infectious agents is one of the major forces generating and maintaining the biodiversity. However, infectious diseases also pose serious threat to populations of endangered animals and biosphere alterations apparently increase the disease risk. Pathogens have been implicated in the extinction or decline of species as ecologically and taxonomically diverse as rain frogs, island land birds, marine tortoises, carnivores etc. and there is growing concern that emerging infectious diseases (EID) may be impeding efforts to conserve threatened and endangered species (Daszak et al., 2000). African great apes are of particular interest and importance because (i) they are essential components of the tropical forest systems and (ii) they are fundamental for understanding of human evolution and human diseases.

Since Darwin's time, there is no doubt about the common ancestry of man and great apes. However, African apes and hominids probably evolved without significant contacts and it is possible that the communities of parasites of individual metapopulations of these hosts were shaped independently, without much of cross-transmissions. Nowadays, both humans and primates are forced to live in close proximity and risk of direct disease transmission in the wild has increased (Leendertz et al., 2006). Several ape populations have been habituated for research and ecotourism purposes, enabling very close contact of humans with immunologically naïve ape populations. The same way as in other mammals, EIDs can have a devastating impact on wild ape populations. The most prominent case is Ebola virus, respiratory viruses and anthrax; however, several other outbreaks (e.g., poliomyelitis, scabies, and measles) were recorded in habituated populations of chimpanzees and gorillas. Besides bacterial and viral diseases, parasitic infections are often listed among the serious threats for wild great apes. Among them the helminths (*Trichuris, Ascaris, Oesophagostomum, Strongyloides, Enterobius*) and protozoans (*Cryptosporidium, Giardia*) are among the most frequently mentioned.

Great apes are large, herbivorous, long-aged social animals. As such, they host diverse communities of parasites, commensal and mutualists. The diversity of helminth fauna is considerably well studied in gorillas and chimpanzees and in lesser extent also in bonobos. Parasite communities then include the species with high host specificity (i.e., *Probstmayiria gombensis*, *Enterobius anthropopitheci, Anoplocephala gorillae*), as well as parasites infecting broad range of hosts (e.g., *Strongyloides* spp., *Protospirura muris*, *Capillaria hepatica*, etc.). However, exact assessment of host spectrum is complicated by fact that the material available (mostly eggs or larvae passed in feces) does not allow for exact determination. Logically, close evolutionary relationship of African great apes and humans results in partial overlap of parasite fauna. However, the direct proofs of transmission are rare and several pathogens are tentatively assumed as zoonotic just based on the fact, that they are morphologically indistinguishable. Especially in protozoans, such approach can be greatly misleading and lead to overestimation of zoonotic transmissions (in both directions). Despite many speculations, proofs of direct parasite transmission between humans and primates, in particular wild great apes, are restricted to few studies. It is probable, that real diversity of protistan and metazoan parasites in great apes is larger than expected, as documented in case of malaria just recently (i.a., Duval et al., 2010, Rayner et al., 2011).

Anthropogenic impact and parasite communities

Increased anthropogenic impact on populations of great apes and their environment can results both in (i) general changes in communities of parasites, but also into (ii) direct increase of exchange of parasites between individual parasite xenocommunities (humans versus apes=zoonotic transmissions). Anthropogenic disturbance can result in a suite of alternations in host ecology and its environment, which can induce changes of infection risk and host-parasite dynamics. Anthropogenic pressure in shrinking wildlife habitat can cause increased wildlife population densities and promote

emergence of diseases. Compared to helminths, gastrointestinal protistan parasites represent neglected part of parasites' communities in apes. Protists are more diversified than helminths, despite the fact, that this diversity is not always reflected in their taxonomy. The communities of protists of gastrointestinal tract in great apes consist of wide range of organisms with various phylogenetic affinities, including ciliates (*Balantidium*), apicomplexans (*Cryptosporidium*), range of amoebae (e.g., *Entamoeba*), flagellates (esp. trichomonads and *Giardia*), *Blastocystis* spp. and microsporidia (esp. *Enterocytozoon* and *Encephalitozoon*). Nowadays, the available diagnostic tools are powerful enough not only to detect and determine these parasites in any material, but also to analyze the epidemiological consequences of these findings. Direct mode of transmission of all these organisms greatly facilitates their exchange between individuals and populations of various hosts and the absence of intermediate hosts makes these infections to be less dependent on particular ecological conditions.

In past seven years, we have had a chance to survey several wild populations of great apes throughout the Africa and we also examined hundreds of fecal samples of great apes originating from European zoological gardens and African sanctuaries. Using molecular tools we confirmed remarkable diversity of studied parasites and tour efforts led to description of new species of ciliates and flagellates (e.g., Tokiwa et al., 2010). Data resulting from examination of such extensive material, the team work and collaborations that we have established enable us to elucidate at least some aspects of diversity and transmission patterns of protistan and metazoan parasites of African great apes. Presented lecture summarizes these results, based mostly on the examples given below.

Selected examples

Blastocystis

Blastocystis were historically classified as yeasts, fungi or sporozoans, but currently referred to as members of the Stramenopiles. Although often considered harmless commensals, it has been shown that *Blastocystis* may be involved in human irritable bowel syndrome. As species/strains of *Blastocystis* colonizing particular mammalian hosts are morphologically indistinguishable, differentiating among particular lineages is based on molecular genotyping (e.g., Stensvold et al., 2009). Since 2006, we have been monitoring these parasites in populations of four primate species (including humans, reintroduced chimpanzees and guerezas and indigenous vervet monkeys) on Rubondo Island, Tanzania (Petrášová et al., 2010). Besides important lessons for future reintroduction projects, Rubondo offers a unique place to study the patterns of transmission of primate parasites and their host specificity. *Blastocystis* was detected using standard microscopy, together with PCR-based determination and the prevalence and subtype identification of *Blastocystis* (Petrášová et al., 2011). Subtype (ST) 1 was detected in all three Rubondo primate populations, ST2 and ST3 were found in colobus and vervet monkeys and ST5 was found only in colobus monkeys. All chimpanzee-originating isolates of *Blastocystis* belonged exclusively to ST1, which formed a discrete group, suggesting that Rubondo chimpanzee are colonized by a single, host specific *Blastocystis* strain. Genotyping of the obtained isolates showed limited or even no exchange of individual genotypes between syntopic primate species living on the island.

From a few studies focusing on primates addressing the cross-transmissions of *Blastocystis* to man (Parkar et al., 2010; Stensvold et al., 2009), none worked with chimpanzees. Any experimental work on primates is almost impossible. Thus, in our study, we carefully selected model situations that partly compensate the lack of experimental opportunities and proved the high host specificity of *Blastocystis* in two model situations: (i) in captive primates in zoos and (ii) in free ranging chimpanzees. Captive chimpanzee groups were colonized by genotypes of *Blastocystis* differing from other primates in same facilities (with one exception) and also the sequences from primate keepers differed from those obtained from primates. The second model situation involved the chimpanzees sampled in the wild at two research sites in East Africa, together with researchers that were in contact with these animals. Interestingly, all SSrDNA sequences of *Blastocystis* obtained from two geographically separated chimpanzee populations formed a discrete strain, being further subdivided according to their locality. The close relationship between *Blastocystis* strains from two geographically distant populations of free-ranging chimpanzees suggests the existence of a host-specific lineage within these hosts, indicating the co-evolution, which might be a driving force in the evolution of the diversity of *Blastocystis*.

Balantidium coli

Balantidium coli (Vestibuliferida: Balantidiidae) is a cosmopolitan ciliate colonizing the intestine of many mammalian hosts. Pigs are considered as major reservoir. Our previous research on the occurrence of *B. coli* in great apes revealed a common presence of trophozoites and/or cysts in captive individuals in European facilities and African sanctuaries, contrary to its absence in wild apes' populations (Pomajbíková et al., 2010). To elucidate the diversity and transmission pattern of balantidia in primates, we performed an extensive evaluation of genetic diversity of *B. coli* in non-human primates based on SSrDNA and ITS1-5.8SrDNA-ITS2 markers. Results showed two subtypes of *B. coli* differing in host specificity: the B genotype that includes predominantly the pig isolates, and the A group comprising isolates from great apes and other hosts. There is no evidence that the contact with humans plays role in the occurrence of balantidia in captive African great apes. Interestingly, several isolates from both captive and wild other primates clustered outside the *B. coli* clade, suggesting possibly new ciliate taxa close to this genus.

Trichuris

The whipworms, *Trichuris* spp., are parasitic nematodes of variety of mammals, the human whipworm, known as *Trichuris trichiura,* was named already by Linné in 1771. *T. trichiura*, *T. lemuris* and *T. cynocephalus* are the only *Trichuris* taxa described from primates. Such a low diversity of *Trichuris* in primates strongly contrasts with high number of species described in other mammals. During past decades, nuclear and mitochondrial DNA genes have been used for species identification and phylogenetic analyses of *Trichuris* from rodents, pigs and cattle (Cutillas et al., 2009; Callejón et al., 2010). We addressed the diversity of *Trichuris* in primates (including humans) based on nuclear (18S, ITS2) and mitochondrial (*cox*1) genes. Phylogenetic analyses using individual markers gave different results; however, all of them revealed that primate whipworms divide into two evolutionary clades: (i) the *Trichuris trichiura* clade with majority of whipworm sequences from man and other primates and, (ii) clade containing primarily *Trichuris suis* where some isolates from primates formed a separate sub-clade suggesting the existence of more *Trichuris* taxa. Obvious existence of (at least) two different species in man (and their possible hybridization), broad distribution of trichuriasis in human population together with unresolved zoonotic aspects call for further research addressing the diversity of whipworms in primates. Findings of eggs in feces of non-human primates are commonly referred to as *Trichuris trichiura* (e.g., Ooi et al., 1993). Such tentative taxonomic treatment implies zoonotic cross-transmissions which can be misleading in most of the cases. On the other hand, limited historical experimental data published (i.a. Imada et al., 1980) demonstrated zoonotic transmissions of *T. trichiura* between non-human primates and man, which is somehow supported by close phylogenetic proximity of some of the *Trichuris* isolates in presented trees. Most of the so far sequenced *Trichuris* are from captive hosts and individual taxa might differ in their host specificity/preferences in free ranging populations. In the wild, various species of primates often overlap in their home ranges and share habitats and/or foraging sites. Similarly to our previous work on *Blastocystis*, careful selection of model field sites and survey of the genetic diversity of *Trichuris* in such natural multi-host primate communities can answer persisting questions about the host specificity of whipworms.

Conclusions? Coming soon...

Broad applications of molecular tools (e.g., Stensvold et al., 2009, Rayner et al., 2011, Sak et al., 2011, Jirku et al., 2012) in parasitological research on free ranging African primates partly uncovered remarkable diversity of both protistan and metazoan parasites/commensals. It seems, that close relationship of man and other primates had greatly deluded almost one generation of anthropologists into swampy area full of zoonotic hobgoblins. Undoubtedly, several infectious diseases possess serious threat for endangered primates. However, the simple fact that parasite fauna of primates and man is superficially similar does necessarily imply neither the extensive exchange of protistan and metazoan pathogens nor the disease risk. Diversity of parasites is tightly connected with phenomenon of host specificity and with epidemiology of parasitic diseases. Firstly we have to properly characterize the communities of symbionts (in its broadest meaning) of individual primate species under natural conditions, and then we can address their overlaps and pathogen transmissions.

This work is an outcome of the HPI-lab, Laboratory for Infectious Diseases Common to Humans and (non-Human) Primates. As such, it present results of joint effort of all the team members and dozens of collaborators from various research institutions, captive facilities (Zoo, sanctuaries) and field sites across Africa.

References

Callejón R., de Rojas M., Nieberding C., Foronda P., Feliú C., Guevara D., Cutillas C. 2010. Molecular evolution of *Trichuris muris* isolated from different Muridae hosts in Europe. Parasitol. Res. 107:631-641.

Cutillas C., Callejón R., de Rojas M., Tewes B, Ubeda J.M., Ariza C., Guevara D.C. 2009. *Trichuris suis* and *Trichuris trichiura* are different nematode species. Acta Trop. 111:299-307.

Daszak P., Cunningham A.A., Hyatt A.D. 2000. Wildlife ecology – Emerging infectious diseases of wildlife – Threats to biodiversity and human health. Science 287:443-449.

Duval L., Fourment M., Nerrienet E., Rousset D., Sadeuh S.A. et al. 2010. African apes as reservoirs of *Plasmodium falciparum* and the origin and diversification of the *Laverania* subgenus. Proc. Natl. Acad. Sci. USA 107:10561-10566.

Imada I., Horii Y., Usui M. 1980. The experiment of artificial infection to men and monkeys by eggs of *Trichuris trichiura* from Japanese monkeys. Jap. J. Parasitol. 29:30.

Jirků M., Pomajbíková K., Petrželková K.J., Hůzová Z., Modrý D., Lukeš J. 2012. Detection of *Plasmodium* spp. in human feces. Emerg. Inf. Dis. 18:634-636.

Leendertz F.H., Pauli G., Maetz-Rensing K., Boardman W., Nunn C., Ellerbrok H., Jensen S.A., Junglen S., Boesch C. 2006. Pathogens as drivers of population declines: The importance of systematic monitoring in great apes and other threatened mammals. Biol. Conserv. 131:325-337.

Ooi H.K., Tenora F., Itoh K., Kamiya M. 1993. Comparative study of *Trichuris trichiura* from non-human primates and from man, and their difference with *T. suis*. J. Vet. Med. Sci. 55:363-366.

Parkar U., Traub R.J., Vitali S., Elliot A., Levecke B., Robertson I., Geurden T., Steele J., Drake B., Thomson R.C. 2010. Molecular characterization of *Blastocystis* isolates from zoo animals and their animal-keepers. Vet. Parasitol. 169:8-17.

Petrášová J., Modrý D., Huffman M.A., Mazoch V., Bobáková L., Mapua M.I., Kaur T., Singh J., Petrželková K.J. 2010. Gastrointestinal parasites of indigenous and introduced primate species of Rubondo Island National Park, Tanzania. Int. J. Primatol. 31:920-936.

Petrášová J., Uzlíková M., Kostka M., Petrželková K.J., Huffman M.A., Modrý D. 2011. Diversity and host specificity of *Blastocystis* in syntopic primates on Rubondo Island, Tanzania. Int. J. Parasitol. 41:1113-1120.

Pomajbíková K., Petrželková K.J., Profousová I., Petrášová J., Modrý D. 2010. Discrepancies in the occurrence of *Balantidium coli* between wild and captive African great apes. J. Parasitol. 96:1139-1144.

Rayner J.C., Liu W., Peeters M., Sharp P.M., Hahn B.H. 2011. A plethora of Plasmodium species in wild apes: a source of human infection? Trends Parasitol. 27:222-229.

Sak B., Kváč M., Petrželková K.J., Květoňová D., Pomajbíková K., Mulama M., Kiyang J., Modrý D. 2011. Diversity of microsporidia (Fungi: Microsporidia) among captive great apes in European zoos and African sanctuaries: evidence for zoonotic transmission? Folia Parasitol. 58:81-86.

Stensvold C.R., Nielsen H.V., Molbak K., Smith H. 2009. Pursuing the clinical 457 significance of *Blastocystis* - diagnostic limitations. Trends Parasitol. 25:23-29.

Tokiwa T., Modrý D., Ito A., Pomajbíková K., Petrželková K.J., Imai S. 2010. A new entodiniomorphid ciliate, *Troglocorys cava* n. g. n. sp., from the intestine of the wild eastern chimpanzee (*Pan troglodytes schweinfurthii*) from Uganda. J. Euk. Microbiol. 57:115-120.

STUDYING PARASITES IN MARINE MAMMALS (SEA LIONS AND NORTHERN FUR SEALS): ROMANIAN EXPERIENCE

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The conservation of marine mammals is a matter of public concern, since many populations are currently threatened or endangered, and the status of many others is little known. Therefore, the design and implementation of adequate conservation plans of marine mammals has become a pressing issue (Raga et al., 1997).
Marine mammals (*Pinnipedia*), includes three families: Otariidae (eared seals), Odobenidae (walruses) and Phocidae (true seals).
Toxins, neoplasia, and viral, bacterial, and parasitic diseases have all recently been identified as causing, or being associated with significant morbidity or mortality in pinnipeds, especially in free-ranging populations (Miller et al., 2001).
Parasites have been known to cause major health problems in marine mammals since research scientists and veterinarians first began study of these animals (Daily, 2001). The pathologic impact of parasites on pinnipeds can be overt where they cause obvious chronic problems, debilitation, and even death of the host. They may be also present without any indication of affecting the pinnipeds. In the latter situation, it is difficult to discern whether the parasites cause insidious or actually no negative effects to the host.
The effect of parasitism in marine mammals is still poorly known, mostly due to methodological and logistical difficulties. However, the available evidence strongly suggests that parasites can play a role in the population dynamics of many species either by acting as a density-dependent manner or as a form of stochastic event (die-offs).
In pinnipeds, perhaps the best documented case illustrating the regulatory potential of parasites is that of the hookworm *Uncinaria lucasi* in populations of northern fur seals (*Callorhinus ursinus*) in the Pribilof Islands, Alaska. As early as 1899, this nematode was reported as the most important cause of mortality to the pups, producing severe intestinal hemorrhages and is occasionally responsible for large scale die-offs (Olsen and Lyons, 1965). The population size of pups born in St. Paul Island peaked around 1940 and declined more or less steadily until the present. Fowler (1990) presented data of hookworm mortality from previous surveys showing a decrease with pup numbers, which means that recent mortality is less than past. This data suggested a density-dependent relationship, and therefore, a regulatory effect of *U. lucasi* on the northern fur seal population (Raga et al., 1997).
In this paper, emphasis is placed on the hookworm parasitism in northern fur seals and sea lions in the Pribilof Islands, AK, USA and San Miguel Island, CA, USA, as result of participation in scientific expeditions in these areas, in 2007 and 2008, respectively.
Hookworms are typically present in the Otariidae (fur seals and sea lions) but uncommon in Phocidae (George-Nascimento et al., 1992). They are intestinal parasites that suck blood, and can cause anemia, debilitation and death of pups. So far, two species, *Uncinaria lucasi* and *Uncinaria hamiltoni*, have been described. However, recently, specimens with molecular and morphologic differences have also been reported (Dailey and Hill, 1970; Nadler et al., 2000; Castinel et al., 2006; Nadler et al., 2009) suggesting the existence of more than two species of hookworms infecting otariids worldwide.
U. lucasi is the only pinniped hookworm for which detailed life-cycle information is known (Olsen and Lyons 1965; Lyons and Keyes, 1978; 1984). Unlike hookworms from most terrestrial hosts, infection of adult *Uncinaria* occur only from parasitic L_3 larvae passed to nursing pups in their mother's first milk. Adult hookworms are eliminated spontaneously from pups at a few months of age, and further mature hookworm infections are not found in older fur seals.
The first data on the prevalence of hookworms in the northern fur seals were reported in 1899 by Lucas. He stated that *Uncinaria* spp. infestations were the most important factor in pup mortality on Saint Paul Island (SPI; Pribilof Islands, Alaska), and the prevalence of hookworms was 61%.
From the time of their description until the early 1980s, hookworms were highly prevalent in fur seal pups and were responsible for a substantial amount of pup mortality in the Pribilof Islands. Lyons

(1963) likewise reported a high prevalence of hookworms (between 56 and 85%) in northern fur seals from SPI, for the period 1960 to 1962.
More prevalence surveys for hookworms were done in northern fur seal and California sea lion pups on San Miguel Island, CA, in 1996 (Lyons et al., 1997), 2000, and 2002 (Lyons et al., 2001; 2003), confirming a high prevalence of adult *Uncinaria* spp. (95% in fur seal pups, 100% in sea lion pups, respectively). More than that, in the last study (Lyons et al., 2005) unexpected findings were reported: adult hookworms completely within the peritoneal cavity and an adult hookworm penetrating through the intestinal wall of the host. These findings indicate the potential life-threatening pathogenicity to California sea lion pups, predisposing to bacteremia ans peritonitis.
More recently, comparative prevalence surveys (Ioniță et al., 2008; Lyons et al., 2011) for hookworm (*Uncinaria* spp.) infections in northern fur seals (*Callorhinus ursinus*) and California sea lion (*Zalophus californianus*) pups were performed.
The purpose of these investigations was to obtain current data on prevalence and intensity of *Uncinaria* spp. in California sea lion (*Zalophus californianus*) and northern fur seals (*Callorhinus ursinus*) pups on San Miguel Island (SMI), California, and in northern fur seals pups on Saint Paul Island (SPI), Alaska. The studies were carried out on Saint Paul Island (SPI), Alaska (57° 09' N, 170° 13' W) (SUA) in 2007 (July-August) and San Miguel Island (SMI) (34°2'N, 120°26'W) in the California Channel Islands (CA, USA), in 2008 (August). The findings verify the low current prevalence (6.25%) of *Uncinaria* spp. in fur seals on SPI, and a high prevalence rate (up to 100%) of *Uncinaria* spp. in both species of pinnipeds from SMI.
Therefore, the current data indicate that hookworm infections are still a health problem in pinnipeds on San Miguel Island, where in 2008 pup mortality rates to 4 months of age were 15% for California sea lions and 32% for northern fur seals.
Contrary, studies in later years on fur seals on St. Paul Island showed the tremendous decline in the prevalence of hookworms. This may be related to the huge decrease in the number of fur seals resulting in perturbation of the hookworm transmission (Lyons et al., 2011). In addition to density-dependent factors, another hypothesis is that the major histocompatibility complex (MHC) genes present in the population may be more effective in creating immune responses that resist hookworm infections (DeLong, 2007).
In conclusion, is important to continue monitoring endoparasite infections such as in pinnipeds because these organisms can reflect the seemingly ever-changing status of these hosts.
Moreover, conservation biologists are increasingly aware of the potential role of both micro- and macroparasites in the dynamics of animal populations, as evidence by both theoretical and empirical studies. In the case of marine mammals, recent epizootic events resulting in mass mortality have raised concern about the potential of transmission to endangered populations (Harwood et al., 1989).

References

Castinel A. 2006. Causes of neonatal mortality in the New Zealand sea lion (*Phocarctos hookeri*). A thesis in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Veterinary Pathology at Massey University. Palmerston North, New Zealand, pp. 293.

Dailey M.D. 2001. Parasitic diseases. In: Dierauf LA, Gulland FMD (Ed.) CRC handbook of marine mammal medicine, 2nd ed. CRC Press, NY, pp. 357-379.

Dailey M.D., Hill B.L. 1970. A survey of metazoan parasites infecting the California (*Zalophus californianus*) and Steller (*Eumetopias jubatus*) sea lion. Bull. Soc. Cal. Acad. Sci. 69:126-132.

DeLong R.L. 2007. The dynamics of hookworm disease in northern fur seals. Alaska Fisheries Science Center, quarterly research reports April-June. AFSC, Washington, pp. 5.

George-Nascimento M., Lima M., Ortiz E. 1992. A case of parasite mediated competition? Phenotypic differentiation among hookworms *Uncinaria* sp. (Nematoda: Ancylostomatidae) in sympatric and allopatric populations of South American sea lions *Otaria byronia*, and fur seals *Arctocephalus australis* (Carnivora: Otariidae). Mar. Biol. 112:527-533.

Harwood J., Carter S.D., Hughes D.E., Bell S.C., Baker J.R., Cornwell H.J. 1989. Seal disease predictions. Nature 339(6227):670.

Ioniță M., Varela M.G., Lyons E.T., Spraker T.R., Tolliver S.C. 2008. Hookworms (*Uncinaria lucasi*) and acanthocephalans (*Corynosoma* spp. and *Bolbosoma* spp.) found in dead northern fur seals (*Callorhinus ursinus*) on St. Paul Island, Alaska in 2007. Parasitol. Res. 103:1025-1029.

Lyons E.T. 1963. Biology of the hookworm, *Uncinaria lucasi Stiles*, 1901, in the northern fur seal *Callorhinus ursinus Linn*. on the Pribilof Islands, Alaska. PhD Dissertation, Colorado State University.

Lyons E.T., Keyes M.C. 1978. Observations on the infectivity of parasitic third- stage larvae of *Uncinaria lucasi Stiles*, 1901 (Nematoda: Ancylostomatidae) of northern fur seals (*Callorhinus ursinus Linn*.), on St. Paul Island, Alaska. J. Parasitol. 64:454-458

Lyons E.T., Keyes M.C. 1984. Further indication of viability of the hookworm (*Uncinaria lucasi*) for several years in tissues of northern fur seals (*Callorhinus ursinus*). J. Parasitol. 70:459-460

Lyons E.T., DeLong R.L., Melin S.R., Tolliver S.C. 1997. Uncinariasis in northern fur seal and California sea lion pups from California. J. Wildl. Dis. 33:848-852.

Lyons E.T., Melin S.R., DeLong .R.L., Orr AJ., Gulland F.M., Tolliver S.C. 2001. Current prevalence of adult *Uncinaria* spp. in northern fur seal (*Callorhinus ursinus*) and California sea lion (*Zalophus californianus*) pups on San Miguel Island, California, with notes on the biology of these hookworms. Vet. Parasitol. 97:309.318.

Lyons E.T., DeLong R.L., Spraker T.R., Melin S.R., Tolliver S.C. 2003. Observations in 2001 on hookworms (*Uncinaria* spp.) in otariid pinnipeds. Parasitol. Res. 89:503-505.

Lyons E.T., Spraker T.R., De Long R.L., Ionita M., Melin S.R., Nadler S.A., Tolliver S.C. 2011. Review of research on hookworms (*Uncinaria lucasi Stiles*, 1901) in northern fur seals (*Callorhinus ursinus Linnaeus*, 1758). Parasitol. Res. 109(2):257-65.

Miller M.A., Sverlow K., Crosbie P.R., Barr B.C., Lowenstine L.J., Gulland F.M., Packham A., Conrad P.A. 2001. Isolation and characterization of two parasitic protozoa from a Pacific harbor seal (*Phoca vitulina richardsi*) with meningoencephalomyelitis. J. Parasitol. 87(4):816-22.

Nadler S.A., Adams B.J., Lyons E.T., DeLong R.L., Melin S.R. 2000. Molecular and morphometric evidence for separate species of *Uncinaria* (Nematoda: Ancylostomatidae) in California sea lions and northern fur seals: hypothesis testing supplants verification. J Parasitol 86:1099-1106.

Nadler S.A., Beckman K., Bell C., Berón-Vera B., Castinel A., Burek Huntington K., Lyons E.T., Morgades D., Norman R., Pagan C., Spraker T.R. 2009. Delimiting hookworm species parasitizing pinniped hosts using gene trees: phylogenetic evidence for hostsharing and switching. Abstract Paper No. 93, Am Soc of Parasitol 84th Annual Meeting, Knoxville, 14-17 August.

Olsen O.W., Lyons E.T. 1965. Life cycle of *Uncinaria lucasi Stiles*, 1901 (Nematoda: Ancylostomatidae) of fur seals, *Callorhinus ursinus Linn*., on the Pribilof Islands, Alaska. J. Parasitol. 51:689-700.

Raga J.A., Balbuena J.A., Aznar J., Fernández M. 1997. The impact of parasites on marine mammals: a review. Parassitologia 39(4):293-6.

REINDEER PARASITOLOGY

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The reindeer is a northern circumpolar cervid ruminant species with a number of subspecies classified into three major ecological groups: the woodland form, continental tundra form, and high Arctic island form (e.g., Flagstad and Røed, 2003). The Nearctic reindeer are called caribou, and one of the subspecies, the Eurasian tundra reindeer (*Rangifer tarandus tarandus*) has been semi-domesticated to become the major production animal of indigenous peoples in the Arctic and sub-Arctic of Russia and Fennoscandia. Reindeer husbandry is an essential part of the Saami culture in Norway, Sweden, and Finland. The reindeer is the only cervid species with both sexes carrying antlers. Reindeer are adapted to the barren environment eating very protein and mineral poor lichen during the winter. To facilitate this, reindeer have a system recirculating urea in saliva into the rumen where microbes utilize it in protein synthesis. Most reindeer populations are migratory, which helps them utilize pastures when they are at best. Migration may also help them to avoid parasites.

There are reasons making parasitism of this one host species especially interesting. Herders of the semi-domesticated production animals have during centuries developed many control measures to protect their stock. Also, the origin of reindeer parasites is exciting as some of them have their closest current relatives really far away. So, reindeer parasites can elucidate the structure of prehistoric ecosystems. The coexistence of wild and semi-domesticated populations makes it possible to study the effects of domestication in the parasite fauna. Thus, it is no wonder that reindeer parasites have been of interest to naturalists and veterinarians in the North, both in Eurasia and in America.

The most prominent reindeer parasites are the warble (*Hypoderma tarandi)* and throat bot (*Cephenemyia trompe)* fly larvae, which have been seen to cause obvious discomfort and production losses to the developing reindeer husbandry, even exceeding 25% of reindeer production (Saval'ev, 1968). That is why many control measures were developed against these oestrid dipterans. The most natural control measure was based on the annual rhythm of the life cycle causing the larvae to drop in May, which gives herders a couple of months to drive the herd at least 50 to 60 km away before the emergence of adult flies, making it difficult for the flies to find the reindeer hosts (Saval'ev, 1968). Smokes and shelters were used to protect reindeer from insect harassment (Hadwen, 1926), which consists of both oestrid flies and blood-sucking insects. Because of their specific attraction to light coloured reindeer, warble fly females were lured to lay eggs on white reindeer pelts where they were easily killed by young herders with twigs. Children could with their small hands also pick out throat bots from adult reindeer throats. When warbles developed under the back of reindeer, they could be manually compressed out and to death in late winter (Bergman, 1917). Later on, experiments were done covering the reindeer back with tar to suffocate warbles, and even later reindeer in the Soviet Union were sprayed with DDT during the summer to kill egg-laying and larvae-squirting females of the two fly species, in addition to diverse blood-sucking insects. Also the specific mating hilltops of throat bot flies were sprayed with hexachlorane. Then, during the 1950s and 1960s, organophosphates were experimented and taken into use by injecting overwintering reindeer at the autumn/winter roundups (Saval'ev, 1968). When macrocyclic lactones were invented, organophosphates were rejected and any routine antiparasitic treatments of reindeer have been done with them (mostly ivermectin) almost for three decades (Laaksonen et al., 2008). Even though ivermectin is extremely efficacious against warbles and throat bots, no really significant reduction has been seen in their distribution, even in areas where treatment of all reindeer is targeted for years, even decades (Åsbakk et al., 2012), most probably because no round-ups ever are perfectly inclusive, leading to a small percentage of animals always escaping the treatment. Ivermectin is also efficacious against deer keds (*Lipoptena cervi*) (Paakkonen et al., 2011), an in Finland important ectoparasite of the moose, or European elk, spreading to the north and causing serious itch to reindeer, and also against various nematode parasites, including abomasal nematodes and lungworms, as well as the abdominal filarioid nematode *Setaria tundra* (Laaksonen et al., 2008).

Effect of domestication

Domestication of reindeer means limiting herd movements, thus often preventing migration useful also for parasite avoidance. Stocking rate is increased, especially when animals are corralled for winter feeding, which is a common practice in Finland now because of lack of winter pastures. Corralling also helps against losses caused by predators and traffic. Increase in animal density increases parasite transmission, also of some parasites to which the reindeer is an intermediate host, such as the coccidian *Toxoplasma gondii*, which is found to be the more common the more reindeer are corralled, as this will expose them to closer contact with cats, and cat faeces (Oksanen et al., 1997). Also the herd structure will be changed, as in a semi-domesticated reindeer herd; the sex ratio is more female dominated than in wild herds. Lactating females are more susceptible to nematode infections, while males are more susceptible to warbles. While many factors in domestication favour parasitism, on the other hand, herders also control parasites, now mostly with macrocyclic lactone antiparasitics. Treatment of hinds in autumn/winter has been shown to increase birth weight of reindeer calves.

Where did they come from?

Some reindeer parasites have close relatives in other boreal wild ruminants, but some do not, e.g., the throat bot fly has a relative species (*Cephenemyia ulrichii*) in the moose, but the reindeer warble fly is probably the only species of its genus in major parts of the reindeer husbandry area. The phylogenetics of the reindeer pentastomid *Linguatula arctica* and the apicomplexan coccidian parasite *Besnoitia tarandi* are especially intriguing (Haugerud and Nilssen, 1990; Madubata et al., 2012). The probably closest relatives of *L. arctica* are parasites of hyenas and lions (Haugerud, 2011 pers. comm.) with herbivores as intermediate hosts, while *L. arctica* parasitizes only reindeer with no intermediate hosts. *Besnoitia tarandi* has reindeer as an intermediate host and the definitive host is unknown, but perhaps not much involved in the life cycle currently. Obviously the closest relative to *B. tarandi* is the bovine species *Besnoitia besnoiti* presently spreading to north in Western Europe from the Mediterranean countries. The origin of these two reindeer parasite species may well be in host switch during the Upper Pleistocene (approximately 10000 to 120000 years ago) when e.g., in the current Czech Republic area, reindeer, bovines, hyenas and lions coexisted among other mammal species. As it appears improbable that European wild reindeer would have migrated to Nearctic Canada since the Upper Pleistocene (Flagstad and Røed, 2003), the presumably young age of *B. tarandi* presents a question: if *B. tarandi* was born in Europe, how was it introduced to the caribou? A plausible answer might be: with semi-domesticated reindeer introduced into Alaska during the 1890s. At least in 1922, *B. tarandi* was already known to cause “cornmeal disease” in Alaska (Hadwen, 1922), which would require a rather fast spread of the parasite, however, not necessarily much faster than that of the reindeer host, the population of which increased from 1280 introduced to about 400000 by the late 1920s. After introduction, it would have needed to spread to the wild caribou herds, where is at least now widely distributed (Ducrocq et al., 2008; Madubata et al., 2012).

Effect of climate change

The filarioid nematode parasite *Setaria tundra* was first described in the Arkhangelsk area in Russia during the 1920s (Rajewski, 1928), but has since been found in roe deer widely in Europe (e.g., Favia et al., 2003). The roe deer spread to the Fennoscandian reindeer husbandry area during the 1960s and 1970s, and *S. tundra* caused an outbreak of peritonitis in reindeer in northern Fennoscandia in 1973, and again in Finland in 2003-2006. It appears that the roe deer is the principal and usually asymptomatic host of the parasite, which can become widespread in the reindeer population and cause disease outbreaks following two consecutive warm summers, meaning that the three summer month (June-August) mean temperature exceeds 14 ºC. Global warming will probably increase the frequency of peritonitis outbreaks (Laaksonen et al., 2010).

Even though reindeer parasitology research has been active for more than a century, it is a fascinating field of study with many still open questions, and new ones emerging almost continually. The same is probably true with many other host species.

References

Åsbakk K., Kumpula J., Hautaniemi M., Oksanen A., Kurkilahti M., Laaksonen S. 2012. Reindeer warble fly (*Hypoderma tarandi*) in Finland and factors affecting infestation rate. Manuscript in prep. Bergman A.M. 1917. Om renens oestrider. Entomologisk Tidskrift 38:1-34 (in Swedish).

Ducrocq J., Lair S., Kutz S. 2008. Prevalence and intensity of *Besnoitia tarandi* in caribou herds: preliminary results. Poster, Carma 2008 Conference (http://www.carmanetwork.com/display/public/Vancouver+2008+Conference, accessed June 15th, 2012).

Favia G., Cancrini G., Ferroglio E., Casiraghi M., Ricci I., Rossi L. 2003. Molecular assay for the identification of *Setaria tundra*. Vet. Par. 117:139-45.

Flagstad Ø., Røed K. H. 2003. Refugial origins of reindeer (*Rangifer tarandus L.*) inferred from mitochondrial DNA sequences. Evolution 57:658-70.

Hadwen S. 1922. Cyst-forming protozoa in reindeer and caribou, and a sarcosporidian parasite of the seal (*Phoca richardi*). J. Am. Vet. Med. Ass. 61:374-82.

Hadwen S. 1926. Notes on the life history of *Oedemagena tarandi L*. and *Cephenomyia trompe* Modeer. J. Parasitol. 13:56-65.

Haugerud R. E., Nilssen A. C. 1990. Life history of the reindeer sinus worm, *Linguatula arctica* (Pentastomida), a prevalent parasite in reindeer calves. Rangifer. Spec. Iss. 3:333-334

Laaksonen S., Oksanen A., Orro T., Norberg H., Nieminen M., Sukura A. 2008. Efficacy of different treatment regimes against setariosis (*Setaria tundra*), Nematoda: Filarioidea) and associated peritonitis in reindeer Acta Vet. Scand. 50:49:1-9.

Laaksonen S., Pusenius J., Kumpula J., Venäläinen A., Kortet R., Oksanen A., Hoberg E. 2010. Climate change promotes the emergence of serious disease outbreaks for filarioid nematodes. Eco. Health 7:7-13.

Madubata C. M., Dunams-Morel D. B., Elkin B., Oksanen A., Rosenthal B. M. 2012. Evidence for a recent population bottleneck in an Apicomplexan parasite of caribou and reindeer, *Besnoitia tarandi*. Infection, Gen. Evol. in press.

Oksanen A., Åsbakk K., Nieminen M., Norberg H., Näreaho A. 1997. Antibodies against *Toxoplasma gondii* in Fennoscandian reindeer – Association with the degree of domestication. Parasit. Int. 46:255-261.

Paakkonen T., Mustonen A.M., Käkelä R., Kiljander T., Kynkäänniemi S. M., Laaksonen S., Solismaa M., Aho J., Kortet R., Puukka K., Saarela S., Härkönen L., Kaitala A., Ylönen H., Nieminen P. 2011. Experimental infection of the deer ked (*Lipoptena cervi*) has no negative effects on the physiology of the captive reindeer (*Rangifer tarandus tarandus*). Vet. Parasitol. 179:180-8.

Rajewsky S.A. 1928. Zwei bisher unbekannten Nematoden (Setarien) von *Rangifer tarandus* und von *Cervus canadensis asiaticus*. Z. Infekt. Krankh. Hyg. Haustiere 35:40-52. (In German).

Saval'ev D.V. 1968. Control of warble flies and bloodsucking diptera. – In: P.S. Zhigunov (Ed.). Reindeer Husbandry, pp. 294-311. Translated from Russian. Israel Program for Scientific Translations. Jerusalem.

Stich R.W., Rikihisa Y., Ewing S.A., Needham G.R., Grover D..L, Jittapalapong S. 2002. Detection of *Ehrlichia canis* in canine carrier blood and in individual experimentally infected ticks with a p30-based PCR assay. J. Clin. Microbiol. 40:540-546.

ARTHROPOD PARASITES OF ENDANGERED VERTEBRATES: THREATENED OR THREATENING?

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Most of the species on Earth are invertebrates and about half of them are parasitic in at least one stage of their development. Yet, the general human perception of parasites is usually negative and several dictionaries derogatorily associate this concept with exploitation (Mihalca et al., 2011). From evolutionary point of view, parasites and hosts are two separate units of selection and compete for the same resources. The concept of '*coextinction*' was intuited by Darwin in 1862 but introduced in the scientific literature rather recently (Stork and Lyal, 1993). As a logical consequence, the term '*coendangered*' came few years later (Koh et al., 2004). Generally, extinction has four main causes: habitat loss, species invasion, overkill and cascades of extinctions (Diamond, 1989). Cascades of extinctions (also known as coextinctions) are in the majority of situations related to habitat loss in species for which the habitat is another organism, like the case of all symbionts (mutualists, commensals and parasites). In most symbiotic interactions the extinction of the host could result in the extinction of several associated species (Dunn et al., 2009). In the case of parasites, the coendangered status applies with predilection to species with high host-specificity (Mihalca et al., 2011).

For ectoparasites, not only the endangered status of the host makes them endangered. As part of conservation efforts of threatened vertebrates, actions often involve artificial breeding, re-introduction or relocations. During these processes, a common practice is the removal of external parasites, with devastating impact on their population (Durden and Keirans, 1996; Mihalca et al., 2011). Several cases are well documented. One relevant example is the case of lice species *Colpocephalum californici* (now extinct) which were intentionally removed from the endangered California condor, *Gymnogyps californianus* during the captive breeding project at Los Angeles Zoo (Dunn, 2009). Another example is the extinction of *Ixodes nitens* described from *Rattus macleari* on Christmas Island. The last report of the host species was in 1903 (Lamoreux, 2009). Moreover, the other endemic rat species *Rattus nativitatis* (sympatric with *R. macleari*) does not harbor *I. nitens*, so the possibility that *I. nitens* might have re-adapted as a parasite of the introduced black rats, *Rattus rattus* can be excluded. Additionally, Mihalca et al. (2011) proposed 63 hard-tick species to be considered coendangered.

On the other side of the coin, the most important issue regarding arthropod-host associations is vectorial transmission of microbial pathogens (i.e. viruses, bacteria, protozoans). Vector-borne diseases of threatened vertebrates are sometimes fatal to their hosts. Mortality associated with vector-borne pathogens has been documented in several cases, mostly after translocations. Are ectoparasitic arthropods a real threat to their coendangered host and should they be eliminated?

References

Diamond J.M. 1989. Overview of recent extinctions. In Conservation for the twenty-first century. Edited by Western D., Peral M., Oxford: University Press; pp. 37–41.

Dunn R.R., Harris N.C., Colwell R.K., Koh L.P., Sodhi N.S. 2009. The sixth mass coextinction: are most endangered species parasites and mutualists? Proc. R. Soc. B Biol. Sci. 276:3037-3045.

Dunn R.R. 2009. Coextinction: anecdotes, models, and speculation. In Holocene Extinctions. Edited by Turvey ST. Oxford: University Press. 167-180.

Durden L.A., Keirans J.E. 1996. Host-parasite co-extinction and the plight of tick conservation. Am. Entomol. 42:87-91.

Koh L.P., Dunn R.R., Sodhi N.S., Colwell R.K., Proctor H.C., Smith V.S. 2004. Species coextinctions and the biodiversity crisis. Science. 305:1632-1634.

Lamoreux J. 2012. *Rattus macleari*. http://www.iucnredlist.org/apps/redlist/details/19344/0.

Mihalca A.D., Gherman C.M., Cozma V. 2011. Coendangered hard-ticks: threatened or threatening? Parasit. Vectors 4:71.

Stork N., Lyal C.H. 1993. Extinction or 'co-extinction' rates? Nature 366:307.

BIRDS AND FEATHER LICE: CO-SPECIATION OR EVOLUTIONARY ARMS-RACE

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Adopting an ectoparasitic lifestyle provides a reliable source of food and habitat but requires the evolution of adaptations for remaining attached to the host's integument. Some 18% of all animal species use a parasitic lifestyle and live on the body of another animal species.
Birds have a number of ectoparasites, like flies, fleas, bugs, feather lice, ticks and other acarians, feather lice being the most common among them. Lice are ectoparasites of birds and mammals, however most lice species (88.7%) being parasites of only birds. They complete their entire life cycle on the body of one host individual, feeding on feathers, skin or blood, and two thirds of all species are confined to a single host species. Ectoparasites may severely reduce the fitness of avian hosts when they occur in large numbers by reducing feather quality and in consequence imperilling flight or insulation capacities. However, in most cases, ectoparasites occur in small numbers with little or no effect on hosts. These small populations may be the result of host-parasite coevolution.
If a parasite exerts a selection pressure on its host and the host exerts a reciprocal selection on the parasite, repeated adaptive evolutionary changes can lead to a process called co-adaptation of host defence and parasite resistance. Continuous host defensive actions may prevent the build-up of high parasite loads, but these may require high resource utilization or extreme adaptations, thus prohibiting the development of ideal parasite defence mechanisms. On the other side, parasites do coevolve to counteract the defensive measures of hosts, thus leading to parasite resistance. Persistence of small loads of ectoparasites on avian hosts may therefore reflect a balance in this coevolutionary arms race.
Birds tend to avoid parasite rich environments. This can be manifested at small scale, like nest or roost sites, or at larger scale, when birds select habitats which are unfavourable for ectoparasite development. There are a number of species which reuse nest-holes for successive breeding events. These holes may harbour parasites, thus for reducing the chances of being parasited birds use a number of techniques. It was shown that a number of species look for signs of parasite presence (Great Tits *Parus major* avoid nest holes with spots of parasite faeces), or actively remove old nest lining, or do not reuse the same nest in successive nesting attempts in the same season. Another measure is to use green material like leaves or stems of herbaceous species with high content of volatile chemicals which have antiparasitic or immune-system enhancer properties. Another defence against parasites is to occupy habitats that are relatively free from parasites. In a study comparing feather lice on birds in a wide range of habitats it was found that a positive correlation exists between loose prevalence and ambient humidity, further it was showed that lice cannot survive on birds kept at low relative humidity.
Moreover, birds manifest parasite avoidance behaviour in avoiding parasited partners, and actively selecting mates with low parasite burdens when mating. Most lice species are transferred from one host to another by direct contact. Thus for birds is crucial to avoid parasited congeners. As body contact is most common in the mating period, several bird species developed mate selection techniques which account for the parasitic load of the eventual future mate. Birds when assessing a possible partner may look for ques of parasite burdens, thus resulting in the evolution of handicap signalling. Barn Swallow (*Hirundo rustica*) females actively select males with lesser whole numbers in white tail streamers, an indicator of low feather lice number of the host (parasite mediated sexual selection).
While chances of host-hopping for lice are scarce and may be reduced to contacts between mating birds, or parent-offspring, a number of lice species evolved techniques which favour fast direct or indirect transfer to another host. Most important adaptations for these are the distribution of lice on host body areas which are in close contact with partners through mating, like the congregation of lice in the anal region in periods of mating (*Rallicola ortygometrae* lice gather in high numbers on outer shafts of feathers surrounding the anus of male corncrakes *Crex crex* only in mating period, other ways they are distributed on the wing). Lice use transport for passing barriers by hitchhiking hippoboscid flies, thus overcoming a major obstacle (phoresis).
One of birds' major defences against feather lice is grooming, which consists of preening and

scratching. During preening, birds move their bills throughout their plumage and by pressing feather barbules between the bills, they extract lice. The importance of preening for controlling feather lice has been shown by experiments that impair preening. Birds that cannot preen effectively usually experience large increases in their louse populations. Preening is of two types: self-preening and allo-preening, this is later case is when one individual preens another. There is a large bulk of scientific studies and empirical observations regarding self-preening and its importance in defence against ectoparasites. Bill morphology is the most important component, while birds with deformed bills usually have high loads of ectoparasites. Allo-preening is important for species with long-lasting pair bonds, as shown in case of several penguin species.

As most feather lice are host specific, the major host defence against them is preening, a presumably visual process. Therefore, crypsis might be selectively advantageous for feather lice to escape from host defence. The data show that the colour of congeneric lice is correlated with host colours, while colours of head lice, which a bird cannot preen, are not. This suggests that feather lice are cryptically coloured. Studies regarding correlation between louse colour and host colour across a broad array of avian taxa showed that colour of congeneric lice are correlated with host colour while the colour of congeneric head lice is not. This suggests that feather lice might have evolved cryptic coloration and avian vision is important in the evolution of louse colour. At a much finer taxonomic scale it was found that the colour of host-specific subspecies of the louse *Quadraceps punctatus* is correlated with the colour of the different host species. These results suggest that feather lice are cryptically coloured. However, experimental tests with Rock Pigeon lice failed to prove this.

Moreover, host species which have well adapted bills for preening have higher number of wing lice which evolved a dorsoventrally flattened body, thus they can slide between the barbs of flight feathers and escape a preening bill, while lice from the same family parasiting species of wading birds (long bills, thus reduced preening capability) do not show this specialisation. Scratching with the feet controls ectoparasites in regions where preening is not possible, like on the head. Birds with deformed or missing leg usually have high ectoparasite loads concentrated on the head and neck. The efficiency of scratching by legs may be enhanced by the presence of a pectinate claw on the middle toe. There are higher numbers of bird species with pectinate claw present among species with long bills, thus compensating for the handicap presented by the long bill in preening efficiency.

Lice evolved a number of morphological adaptations against host grooming. Most important are related to size and shape, tegument structure and strong mouthparts and grasping claws (for tight attachment). The size of lice is strongly correlated with host body size, thus with bill size, as well. The microhabitat distribution of lice also appears to reflect grooming imposed selection, with slim, elongated body species occurring mostly on flight feathers, while larger, round-bodied, sluggish species occurring on head and neck, where they are more protected from preening.

Though plumage may deter feeding ectoparasites, in analogous way to structurally resistant foliage (high cellulose content/low palatability) it deters feeding by herbivores. Some recent work suggests that feather toughness may be an important defence against ectoparasites. Melanin content of feathers is positively correlated with feather toughness, and darker feathers are known to be more resistant to mechanical abrasion, than feathers without this pigment. There are several studies which suggest that melanin may also limit damage by feather-feeding lice. For example, feather lice (*Hirundoecus malleus*) of swallows use to chow more holes in white regions of feathers in tail-streamers of barn swallows. However, there are no experimental tests yet performed, while direct choice tests with pigeon lice does not confirmed this hypothesis. Toxic feathers or skin is known to protect animals from parasites. Feather and skin of several members of the *Pitohui* genus contain a toxic chemicalia caller homobatrachotoxin which affects a wide range of invertebrates, thus may deter ectoparasites, too. Homobatrachotoxin is a neurotoxin, analogous to the skin-toxin of poison dart frogs (*Dendrobatidae*) that use this toxin against predators. Although there are hypotheses that toxic plumage of Pitohui may be an antipredatory mechanism, its antiparasitic functions were already tested in experimental settings. In a choice trial feather lice were offered Pitohiu and non-toxic feathers. Lice avoided feeding or resting on Pitohui feathers, furthermore they showed higher mortality if chosen.

There are a number of hypotheses describing major governing rules of bird and lice coevolutionary processes, however most of them lack experimental evidences or failed to be proven (like the morphological specialisation reinforcement hypothesis, which suggested that morphological specialisation leads to host specificity, proven for certain taxa, but the opposite proven for a number of other taxa). In conclusion, bird-parasite coevolution is a continuous arms-race, which provided more questions and study topics than answers yet, while the large amount of information gathered (especially in taxonomy and distribution studies) still lacks proper analysis.

CHEMOTHERAPY OF LEISHMANIASIS: PRESENT AND FUTURE CHALLENGES

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Introduction

There have been significant differences in progress and approaches to drug development for visceral leishmaniasis (VL) but far fewer for cutaneous leishmaniasis (CL). As progress and the approaches taken are different for these two manifestations, they will be presented separately. However, there are aspects of the biology of *Leishmania* parasites that affect approaches to drug development, including (i) the intracellular location of the target form of the pathogen, the amastigote, in the low pH phagolysosomal compartment of different macrophage populations, (ii) the varying sensitivities of strains and species, and (iii) the inter-relationship with the host immune response. Differences in drug development of compounds for VL and CL relate to the different pharmacokinetics properties, that is the different needs for drugs that distribute to the viscera (liver, spleen, bone marrow in VL) compared to the skin (in CL), and to the pharmaceutical formulation of drugs that aid these different distributions.

Visceral leishmaniasis

Visceral leishmaniasis, caused by *L. donovani* (in Asia and Africa) and *L. infantum* (in Southern Europe and South America) is potentially fatal and has received the most focus for drug R & D. The need for new drugs is urgent as pentavalent antimonials (sodium stibogluconate, meglumine antimoniate), the standards drugs for 60 years (Alvar et al., 2006), are now almost obsolete in the key endemic area in Bihar state, India due to parasite resistance (Croft et al., 2006), though still useful in the rest of the world. Amphotericin B, previously a second line drug, is now a first line in Bihar. A number of amphotericin B lipid formulations, developed for treatment of systemic mycoses in immunocompromised patients, have proved effective in the treatment VL, although only the liposomal formulation AmBisome®, has become a standard treatment, it's use prescribed by a WHO working group (Bern et al., 2006). Recently, a single course therapy of 10 mg/kg has been shown to cure 95% patients in India (Sundar et al., 2010). One of the limitations of AmBisome, cost, has been partially met by a significant reduction in price negotiated by WHO with the producers (Gilead), currently $18/50 mg ampoule), and more recently by a donation from Gilead. A parenteral formulation of the aminoglycoside paromomycin (aminosidine, monomycin), first shown to have a curative effect in VL in the 1980s, showed 94% efficacy (at 15mg/kg for 21 days, intramuscularly) in phase III clinical trials in India (Sundar et al., 2007) and was registered in that country for VL in 2006. The anti-leishmanial activity of the phospholipid derivative, miltefosine, first identified in the 1980s (Croft et al., 1987), also showed 94% efficacy in adults and children (Sundar et al., 2002) and was registered as the first oral treatment for treatment of VL in India in 2002. It was the first anti-leishmanial to undergo phase IV studies (Bhattacharya et al., 2007), and has been incorporated into the VL elimination programme for the sub-continent. Miltefosine is not perfect as there is a potential for teratogenicity, requiring women of child bearing age to take contraception, and the 28-day oral treatment can lead to poor compliance. Drug combinations have proved to be a successful strategy to shorten course of therapy, reduce toxicities through lower dosage and reduce the selection of resistant mutations for malaria and tuberculosis. Clinical studies of co-administration (either concomitant or sequential) of available anti-leishmanial drugs, based on experimental studies (Seifert and Croft, 2006), led to a Phase 3 study in India involving three co-administration regimens. All regimes, namely, (a) single dose intravenous (iv) AmBisome® + sequential 7 days oral miltefosine, (b) single dose iv AmBisome® + sequential 10 days intramuscular (im) paromomycin and (c) concomitant 10 days oral miltefosine + 10 days im paromomycin achieved a 98% cure rate (Sundar et al., 2011). The significantly reduced treatment time, from 30 days to potential 8 days, is important for both patient treatment and VL control. Another co-administration, sodium stibogluconate plus paromomycin, has also led to a reduction in treatment course to 17 days in East Africa.

The major challenges for improvement of VL treatment are: (a) the regional differences in response rates, for example, paromomycin, in East Africa using the same the regimen that was successful in India, showed lower efficacy, particularly in Sudan where the cure rate was < 50% and even the increased dose of 20 mg/kg for 21 days gave only a 85% cure rate, insufficient for consideration as a

monotherapy (Hailu et al., 2010), (b) the dermal manifestation, post kala-azar dermal leishmaniasis (PKDL), which appears months to years after the end of treatment of visceral disease, for which there is no recommended treatment for PKDL, and (c) co-infections with HIV , with increasing numbers of cases in East Africa, for example, 23% of all VL cases in NE Ethiopia, which have not respond to a range of treatment regimens with all standard drugs (Alvar et al., 2008).

Cutaneous leishmaniasis (CL)

There are few treatment options for CL. Pentavalent antimonials have proved inconsistent in their effectiveness across the different *Leishmania* species, and pentamidine and amphotericin B are limited to specific types of CL (see Alvar et al., 2006). Paromomycin has been tried in different topical formulations (El-On et al., 1992) with variable clinical results (see Garnier and Croft, 2002). A recent formulation of 12% paromomycin, containing also gentamicin and surfactants, showed efficacy in *L. major* CL in Tunisia (Ben-Salah et al., 2009), and is currently in phase III. Oral miltefosine also has some variable, species dependent effectiveness against CL. Two recent Cochrane analyses of clinical trials of CL emphasized that most clinical studies could not be analyzed as they did not meet standards of randomized placebo controlled trials. For *L. major* and *L. tropica* infections in the Old World, there was some evidence of the activity of antifungal azoles, fluconazole and itraconazole (Gonzalez et al., 2008), whilst in the New World, in addition to antimonials, the anti-inflammatory drug pentoxyphylline as adjunct therapy, miltefosine and ketoconazole, and oral allopurinol were active in a limited number of trials (Gonzalez et al., 2009). As an approach to CL is to accelerate self-cure (Garnier and Croft, 2002), immunomodulators as adjunct therapies have been tested. In studies on CL patients in Peru the anti-viral TLR7 agonist, imiquimod, gave a 75% cure when used with antimonials compared to 58% cure rate for antimonials alone (Miranda-Verastegui et al., 2009).

Challenges to advance chemotherapy of CL include: (a) the establishment of a standardized protocol (Gonzalez et al., 2010), (b) treatments for the complex manifestations of CL like mucocutaneous and diffuse forms of the disease, probably 5% of all cases, where there are metastatic sites of infection, remain a major challenge, and (c) improved topical formulations including rational pharmaceutical design (Garnier and Croft, 2002). Attempts are being made to increase the visibility of CL on the research agenda and to define objectives, for example target product profiles for key forms of CL caused by *L. tropica* and *L. braziliensis* (Modabber et al., 2007).

Conclusion

There have been significant advances in the treatment of VL but there have been none for CL, simple or complex forms, or for HIV co-infected patients. Although we now have single-dose AmBisome® and short course co-administrations for VL, the goal still remains a safe cheap oral drug requiring a 10-14 day course of treatment. This goal appears to be distant for both VL and CL. However, there is potential for further development of topical formulations for simple CL. We are some way from having the ideal treatments for VL and CL and drug R&D for these diseases needs to be kept high on the agenda.

References

Alvar J., Aparicio P., Aseffa A., Den Boer M., Cañavate C., Dedet J.P., Gradoni L., Ter Horst R., López-Vélez R., Moreno J. 2008. The relationship between leishmaniasis and aids: The second 10 years. Clin. Microbiol. Rev. 21:334-359

Alvar J., Croft S., Olliaro P. 2006. Chemotherapy in the treatment and control of leishmaniasis. Adv. Parasitol. 61:223-274.

Bhattacharya S.K., Sinha P.K., Sundar S., Thakur C.P., Jha T.K., Pandey K., Das V.R., Kumar N, Lal C., Verma N., Singh V.P., Ranjan A., Verma R.B., Anders G., Sindermann H., Ganguly N.K. 2007. Phase 4 trial of miltefosine for the treatment of indian visceral leishmaniasis. J. Infect. Dis. 196:591-598.

Ben Salah A., Buffet P.A., Morizot G., Ben Massoud N., Zâatour A., Ben Alaya N., Haj Hamida N.B., El Ahmadi Z., Downs M.T., Smith P.L., Dellagi K., Grögl M. 2009. WR279,396, a third generation aminoglycoside ointment for the treatment of leishmania major cutaneous leishmaniasis: A phase 2, randomized, double blind, placebo controlled study. PloS Negl. Trop. Dis. 3:e432.

Bern C., Adler-Moore J., Berenguer J., Boelaert M., den Boer M., Davidson R.N., Figueras C, Gradoni L., Kafetzis D.A., Ritmeijer K., Rosenthal E., Royce C., Russo R., Sundar S., Alvar J. 2006. Liposomal amphotericin b for the treatment of visceral leishmaniasis. Clin. Infect. Dis. 43:917-924

Croft S.L., Neal R.A., Pendergast W, Chan J.H. 1987. The activity of alkyl phosphorylcholines and related derivatives against leishmania donovani. Biochem. Pharmacol. 36:2633-2636.

Croft S.L., Sundar S., Fairlamb A.H. 2006. Drug resistance in leishmaniasis. Clin. Microbiol. Rev. 19:111-126.

El-On J., Halevy S., Grunwald M.H., Weinrauch L. 1992. Topical treatment of old world cutaneous leishmaniasis caused by leishmania major: A double-blind control study. J. Am. Acad. Dermatol. 27:227-231.

Garnier T., Croft S.L. 2002. Topical treatment for cutaneous leishmaniasis. Curr. Opin. Investig. Drugs. 3:538-544.

González U., Pinart M., Rengifo-Pardo M., Macaya A., Alvar J., Tweed J.A. 2009. Interventions for american cutaneous and mucocutaneous leishmaniasis. Cochrane Database Syst. Rev. CD004834.

González U., Pinart M., Reveiz L., Alvar J. 2008. Interventions for old world cutaneous leishmaniasis. Cochrane Database Syst. Rev. CD005067.

González U., Pinart M., Reveiz L., Rengifo-Pardo M., Tweed J., Macaya A., Alvar J. 2010. Designing and reporting clinical trials on treatments for cutaneous leishmaniasis. Clin. Infect. Dis. 51:409-419.

Hailu A., Musa A., Wasunna M., Balasegaram M., Yifru S., Mengistu G., Hurissa Z., Hailu W., Weldegebreal T., Tesfaye S., Makonnen E., Khalil E., Ahmed O., Fadlalla A., El-Hassan A., Raheem M., Mueller M., Koummuki Y., Rashid J., Mbui J., Mucee G., Njoroge S., Manduku V., Musibi A., Mutuma G., Kirui F., Lodenyo H., Mutea D., Kirigi G., Edwards T., Smith P., Muthami L., Royce C., Ellis S., Alobo M., Omollo R., Kesusu J., Owiti R., Kinuthia J. Leishmaniasis East Africa Platform (LEAP) group. 2010. Geographical variation in the response of visceral leishmaniasis to paromomycin in east africa: A multicentre, open-label, randomized trial. PloS Negl. Trop. Dis. 4:e709.

Miranda-Verastegui C., Tulliano G., Gyorkos T.W., Calderon W., Rahme E., Ward B., Cruz M., Llanos-Cuentas A., Matlashewski G. 2009. First-line therapy for human cutaneous leishmaniasis in Peru using the tlr7 agonist imiquimod in combination with pentavalent antimony. PloS Negl. Trop. Dis. 3:e491.

Modabber F., Buffet P.A., Torreele E., Milon G., Croft S.L. 2007. Consultative meeting to develop a strategy for treatment of cutaneous leishmaniasis. Institute Pasteur, Paris. 13-15 June, 2006. Kinetoplastid Biol. Dis. 6:3.

Seifert K., Croft S.L. 2006. *In vitro* and *in vivo* interactions between miltefosine and other antileishmanial drugs. Antimicrob. Agents Chemother. 50:73-79.

Sundar S., Chakravarty J., Agarwal D., Rai M., Murray H.W. 2010. Single-dose liposomal amphotericin b for visceral leishmaniasis in india. N. Engl. J. Med. 362:504-512.

Sundar S., Jha T.K., Thakur CP, Sinha PK, Bhattacharya SK. 2007. Injectable paromomycin for visceral leishmaniasis in india. N. Engl. J. Med. 356:2571-2581.

Sundar S., Jha T.K., Thakur C.P., Engel J., Sindermann H., Fischer C., Junge K., Bryceson A., Berman J. 2002. Oral miltefosine for indian visceral leishmaniasis. N. Engl. J. Med. 347:1739-1746.

Sundar S., Sinha P.K., Rai M., Verma D.K., Nawin K., Alam S., Chakravarty J., Vaillant M., Verma N., Pandey K., Kumari P., Lal C.S., Arora R., Sharma B., Ellis S., Strub-Wourgaft N, Balasegaram M., Olliaro P., Das P., Modabber F. 2011. Comparison of short-course multidrug treatment with standard therapy for visceral leishmaniasis in india: An open-label, non-inferiority, randomised controlled trial. Lancet 377:477-86.

PHLEBOTOMINE SPECIES CONCEPT: A LARGE INTRASPECIFIC MOLECULAR VARIABILITY OR DIFFERENT CRYPTIC SPECIES INVOLVED

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The epidemiology of leishmaniases, arboviroses and Carrion's disease is directly linked to the taxonomy of Phlebotomine sand flies (Diptera, Nematocera, Psychodidae, Phlebotominae).
We are unable to really define what is a species (Lherminier and Solignac, 2005) but three broad conventional categories of species concept are usually recognized: the taxonomic, the evolutionary and the biological one. Ready (2011), reviewed these concepts: the medical entomologists should not expect all taxonomic species to be biological species and should realize that it is only the latter that usually provide barriers to gene flow and the sharing of biomedically important phenotypes or traits. Taxonomic species can be valid names, if described correctly using a designated type specimen and following other rules of zoological nomenclature, and yet they may not be good biological species (especially because the colonization of Phlebotomine sandflies is difficult) or even phylogenetic species. Often, however, taxonomic species are also good phylogenetic species, because an experienced taxonomist tends to search intuitively for synapomorphic morphological characters, rather than a small combination of ancestral characters that might be diagnostic only for local populations (Ready, 2011).
The taxonomic problems in many groups of insect vectors have been detailed in general by Lane and Crosskey (1995) who distinguish three categories: i) arthropods that require careful preparation before identification is possible. It applies to Phlebotomine sand flies, ii) species that can be easily distinguished but only during a single life stage. It applies to Phlebotomine sand flies not about the life stage (sensu larvae versus imago) but according to the sex taking into account that for many groups (*Adlerius, Sergentomyia*,…), males or females can be indistinguishable and iii) closely related but reproductively isolated species that are morphologically identical, corresponding to the cryptic species.
Black and Munstermann (2004) defined as follow the species complexes: "When two populations of a species become reproductively isolated either through geographic separation or as a consequence of the evolution (…) isolation mechanisms, they gradually accumulate genetic differences. Given sufficient time, these genetic differences may eventually become manifest as subtle morphological differences.
Over an extended time, these may become manifest as distinct morphological differences. However, character differences arising from recent or incipient (ongoing) reproductive isolation are frequently difficult to use in routine identification. Closely related but reproductively isolated species that appear morphologically identical are often referred to as cryptic or sibling species, and a group of cryptic species is referred to as a species complex." In fact, the sand flies recognized species complex listed in Black and Munstermann (2004) for American taxa or *P. perniciosus* complex (Pesson et al., 2004) includes in fact populations morphologically closely related but identifiable, which is not in agreement with this definition. Cryptic species can be detected by sequencing of molecular markers. To detect introgression, a common phenomenon in Phlebotomine sandflies, it is important to combine at least one mitochondrial marker with a non mitochondrial one. We discuss this point in this communication. Curiously, the status of subspecies, very commonly used in the past (Abonnenc, 1972) has been completely forgotten since 1980, the taxa described being ranked to the specific level. This taxonomic rank, and its use, is also discussed in the present communication.

Key words: Phlebotomine sandflies, Systematics, molecular biology, mtDNA, species concepts, evolution.

References

Abonnenc E. 1972. Les Phlébotomes de la Région Éthiopienne (Diptera, Psychodidae). Mémoires ORSTOM, pp. 55:239.

Black W.C., Munstermann L.E. 2004. Molecular taxonomy and systematic of arthropods vectors. In biology of disease vectors, Ed. William C. Marquardt, 2004. Amsterdam: Elsevier Academic Press, pp. 505-528.

Lane R.P., Crosskey R.W. 1995. Medical Insects. Chapman and Hall, London.

Lherminier P. Solignac M. 2005. De l'espèce, Ellipses.

Pesson B., Ready J.S., Benabdennbi I., Martín-Sánchez J., Esseghir S., Cadi-Soussi M., Morillas-Marquez F., Ready P.D., 2004. Evidence of mitochondrial introgression and cryptic speciation involving the sandflies *Phlebotomus perniciosus* and *P. longicuspis* from the Moroccan Rif. Med. Vet. Entomol. 18: 25-37.

Ready P.D. 2011. Should sand fly taxonomy predict vectorial and ecological traits? J. Vector Ecol. 36(suppl. 1): S17-S22.

STRATEGIES FOR CONTROL OF *TAENIA SOLIUM* IN MEXICO

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Between the 1940's and 1970's several reports of human necropsies performed in Mexico revealed the presence of cysticerci in the brain, documenting an average of 2%. Hospital-based reports provided data on the magnitude of cysticercosis in patients being 4% among neurological patients. Immunodiagnostic assays facilitated the shift to epidemiological information performed in open populations of rural and urban settings. Reports of predictive values indicate that for screening purposes western blot is the test of choice, and for diagnosis, the addition of an imaging test yields the best results, although with clinical data suggestive of NCC even ELISA is useful to support diagnosis.

A number of seroepidemiological studies focusing on cysticercosis/taeniosis have been conducted in Mexico using different immunodiagnostic techniques that have contributed to the growing body of evidence on endemicity, on risk factors for transmission and on the success of interventions for prevention and control. In a study undertaken in El Sotano, a small community in the state of Hidalgo, 6% of the 124 inhabitants had antibodies detected by ELISA, 25% of their pigs had cysticerci that were palpated in their tongues and 3% of the people had *Taenia* eggs. *Ascaris, Trichuris* and *Toxocara* eggs were found in soil samples but no *Taenia* eggs were identified. The clustered distribution of infected pigs, tapeworm carriers and people with serologic or clinical evidence of cysticercosis suggested intra-household transmission. Furthermore, although the correlation of seropositivity and clinical history suggestive of NCC in individual residents was poor, there was an apparent spatial association between tapeworm carriers and persons with serologic or clinical histories suggestive of NCC. This information was very interesting because it identified, for the first time, the main risk factor for NCC: the presence of a person infected with an adult tapeworm at home.

In order to determine markers of *T. solium* transmission and risk factors in an urban community, 1000 soldiers from a military camp in Mexico City and their families were studied. Serum samples were used to detect antigens and antibodies and faecal specimens were examined for *Taenia* CpAg and eggs. Antibodies were detected in 12% of soldiers and 6% and 10% of relatives of positive and negative soldiers, respectively. Antigens in serum were detected in 3% of the soldiers and in 4% of the relatives of antibody-positive soldiers. *Taenia* CpAg were found in 0.5% and *Taenia* eggs in 0.1% of soldiers but were not found in their families. Interestingly, 12% of the family members of positive soldiers had had a history of proglottid release, compared to only 4% of the family members of negative soldiers. Lastly, 86% of the family members of positive soldiers had eaten in street food stores, compared to only 62% of those of negative soldiers. Both risk factors identified were statistically significant, indicating again, that the main risk factor was association with the presence of a tapeworm carrier at home. Therefore, a clinical history of taeniosis in a family member, defined as elimination of proglottids in faeces, should be taken into account by health personnel to prevent other members of the family from becoming infected. The results obtained by the simultaneous search for antigens and antibodies suggest that the prevalence of human cysticercosis in Mexico is, as indicated by previous autopsy findings, around 2%. Therefore, the higher prevalence of antibodies suggests exposure to the parasite but not current infection.

On one hand it has been considered for a long time in Mexico that fruits, such as strawberries, and vegetables, such as lettuce, that are eaten unpeeled and uncooked and that grow at ground level, are the main sources of *Taenia* eggs that cause human NCC. But in a study carried out in the counties of Irapuato in the state of Guanajuato and Zamora, Michoacan, which are important locations for the production of strawberries, no *Taenia* eggs were identified in large amounts of homogenized strawberries collected throughout one year (low numbers of protozoan cysts and one *Ascaris* egg were found). This indicated that, although there was contamination with human faeces, strawberries did not carry tapeworm eggs. Also, the absence of *Taenia* eggs in domestic flies was demonstrated in the community of Tianquizolco, where over one thousand flies were caught in homes and assessed for their role in the transmission of *Taenia solium*.

On the other hand, multiple studies have demonstrated that the prevalence of tapeworm carriers is higher among household members of NCC patients than in the rest of the population. A clear

association exists between the presence of taeniosis and the severity of NCC, since most massive cerebral infections probably result from a maintained infective source in patients harbouring the adult tapeworm in the intestine. The perception that *T. solium* tapeworms are silent guests causing no harm to humans is erroneous, and tapeworm carriers should be regarded as potential sources of contagion to both themselves and to those living in their close environment. Just how important is the presence of a tapeworm carrier is demonstrated by the case of 4 orthodox Jewish families from New York, in which 4 neurological cases and 7 seropositive people were detected. Although these families did not ingest pork meat, the maid who cooked for these families was from Mexico and she had an intestinal *T. solium.* A study of 31 families of NCC patients showed that 2% were CpAg positive, 14% had a family history of taeniosis and 10% had self-reported being a tapeworm carrier.

The information presented above clearly demonstrates a clustered distribution of persons with serological or clinical evidence of cysticercosis, infected pigs and tapeworm carriers, thus allowing to evaluate intervention measures: health education, self-detection of tapeworm carriers and mass treatment against human taeniosis. Regarding health education as a community-based intervention measure a comprehensive study was undertaken in Chalcatzingo, Morelos with 2000 inhabitants. An educational program was developed to study the local knowledge of both diseases (taeniosis and cysticercosis) and of both parasites (the tapeworm and the cysticercus), to promote recognition and knowledge of the transmission of the parasites and to improve hygienic behaviour and sanitary conditions that foster transmission. This was performed by in-depth questionnaires developed by anthropologists. Based on the information obtained, an educational intervention was developed which included explanation of the life cycle, diseases, risk factors and control measures. For this purpose the anthropologists trained local leaders, selected among students, housewives, and teachers as well as the priest, to be in charge of promoting and providing health education, so that education remained in the community after the project ended. The effects of this educational intervention were evaluated by measuring changes in knowledge, attitudes and practices and prevalences of human taeniosis and swine cysticercosis before and after the campaign. The prevalence in pigs at the start of the education intervention was 2.6% and 5.2% by tongue examination and WB for antibody detection, respectively. Approximately one year after the intervention they were 0% and 1.2%, and remained so for almost 4 years. These changes were accompanied by a significant reduction in reported access of pigs to sources of infection and freedom to roam, since people knew that infected pigs had to be sold at lower prices. In Coapeche, Veracruz, where swine cysticercosis was ascertained by western blot, none of the 53 pigs studied had antibodies or cysticerci. Latrines were present in 91% of houses and pigs were kept in restrained areas, demonstrating that adequate basic sanitary conditions and pig breeding practices are effective and practical to control *T. solium* in rural communities. High standards of meat inspection and proper disposal of infected pig carcasses will also aid in preventing infected pigs from entering the food chain.

Self-identification of tapeworm carriers as a community based intervention alternative to health education was evaluated in the municipality of Irapuato, Guanajuato. Clinical and animal health care practitioners and schoolteachers were trained in the life cycle, risk factors and control measures related to infection with *Taenia solium.* Over 120 small glass bottles, each containing a few tapeworm segments fixed in formaldehyde and an instructional guide were distributed among all clinical practitioners (physicians and nurses) working in health centres. The guide contained 10 key points on how to ask questions about tapeworm infections. The small bottles were shown to people during questionnaire administration to determine if they had seen such parasites in their faeces. Information on taeniosis and cysticercosis was also provided to the general population via different media. Seven tapeworm carriers were recorded in the official epidemiology surveillance system the year previous to the study, compared to the year after the study, when 41 tapeworm carriers (37 *T. saginata*; 4 *T. solium*) were recorded. Thus six times more tapeworm carriers were notified after the study. All four persons with *T. solium* were treated, thereby eliminating the parasite and subsequently preventing new cases of human and swine cysticercosis that might have arisen from them. This study demonstrates that self-detection is a feasible tool for control of *T. solium.*

The use of mass treatment with praziquantel to eliminate tapeworms from human carriers as a community-based intervention measure was evaluated in two studies. In a small community (559 inhabitants) in La Curva, Sinaloa, over 70% of the population over 5 years of age was treated with a 10 mg/kg dose. One year later, no infections with *Taenia* sp. eggs were found and no pigs with cysticercosis were detected. Seropositivity using ELISA was 11% before treatment and 7% afterwards, in the 30-39 year age group, antibody detection decreased from 30% to 7% suggesting that elimination

of tapeworms reduces the possibility of contact with infective eggs. Interestingly, in the geographic section of the community where 3 of the 4 tapeworm carriers were found and treated, seropositivity was reduced from 19% to 2%, indicating that serum antibodies to *Taenia* antigens are short-lived and diminish, as contact with the parasite is lost. In the second study performed in Atotonilco (3007 inhabitants), 87% of the community received a single dose of 5 mg/kg following a recommendation of Pawlowski from WHO. The prevalence of taeniosis was reduced by 53% after 6 months and by 56% after 42 months, as measured by CpAg ELISA or egg detection; late onset general seizures decreased by 70%. Anti-cysticercus antibodies in the human population were reduced by 75% after 42 months and antibodies in pigs also showed a significant reduction (55%) after 6 months. In conclusion, the impact of mass chemotherapy against taeniosis to control cysticercosis in the short and long term was successfully demonstrated. Experience with praziquantel however suggested that it should not be given at doses lower than 10mg/kg. Furthermore, a history of late onset convulsions and specific antibodies are good indicators of NCC and exposure to the parasite, respectively. This population-based cestocidal treatment eliminated tape worm carriers level but generated symptomatology in a previously asymptomatic neurological case. This observation highlights the importance of weighing a mass chemotherapy approach against selective treatment for at-risk individuals.
The efforts and advancements described above, and others, drove Mexico onto a new stage regarding NCC: it is not anymore a public health problem in Mexico. This proposal is based on the dramatic decrease in the frequency of human NCC and human taeniosis obtained from the Information System for Epidemiological Surveillance of the Ministry of Health in Mexico. The reasons that may have caused this change are: 1) the results published by the Mexican scientific and medical communities working on cysticercosis; 2) the establishment of a National Program for the Control of *Taenia solium* since 1994; 3) Ana Flisser and Dolores Correa, authors of the paper **“**Neurocysticercosis may no longer be a public health problem in Mexico. PLoS NTD 2010” show data obtained from national and international agencies that are of major importance, since they demonstrate that living conditions in Mexico have had great improvements in social, economy and health sectors.

If readers are interested in original data, most can be found in articles by Flisser Ana.

MOLECULAR IDENTIFICATION OF HUMAN *TAENIA* TAPEWORMS

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Taenia solium, *T. saginata*, and *T. asiatica* are cestodes that cause taeniases in humans and cause cysticercosis in intermediate host animals (cows and pigs). *T. asiatica* is the most recently described *Taenia* species, among them, based on its morphological characteristics and life cycle (Eom and Rim, 1993). The larval tapeworm of *T. asiatica* (*Cysticercus viscerotropica*) also differs morphologically from that of *T. saginata*. The phylogenetic relationships analyses of morphological characters and molecular data (Horberg et al., 2000), ITS2 (Eom et al., 2002), the complete mtDNA (Jeon et al., 2005; 2006) indicated *T. asiatica* is a distinct sister species to *T. saginata*.
Epidemiologically, *T. asiatica* has been found in China, Indonesia, Korea, the Philippines, Taiwan, Thailand, and Vietnam and Japan (Eom et al., 2009). *T. asiatica* and *T. saginata* exhibit morphological similarities, particularly in the eggs and proglottids of adult worms, which are often impediments to correct identification in many Asian countries where the distributions of these two species overlap. To this end, it is necessary to distinguish these two *Taenia* species precisely and rapidly.
Molecular approaches to the differential diagnosis of these 2 morphologically similar species, *T. saginata* and *T. asiatica,* have been developed, including the use of sequence-specific DNA probes (Flisser et al., 1988; Rishi et al., 1988; Harrison et al., 1990; Chapman et al., 1995), PCR-RFLP (restriction fragment length polymorphisms: Bowles and McManus, 1994; Mayta et al., 2000; Rodrignez-Hidalgo et al., 2002; Yamasaki et al., 2002; McManus, 2006), multiplex PCR (Gonzales et al., 2004; Yamasaki et al., 2004; Jeon et al., 2009), nucleotide sequencing based on 10% formalin-fixed paraffin-embedded specimens (Yamasaki et al., 2007; Jeon et al., 2011a), and DNA genotype (Eom et al., 2011; Jeon et al., 2011b).
The mitochondrial genome is one of the useful molecular marker resources for not only studying evolutionary relationships among distantly related taxa, but also for investigating the phylo-biogeography of closely related species, including species level taxonomy. The mitochondrial genome each was 13703 bp (*T. asiatica*), 13670 bp (*T. saginata*) and 13709 bp (*T. soilum*) long containing 12 protein-coding genes, two ribosomal RNAs (rRNAs, a small and a large subunit), and 22 transfer RNAs (tRNAs). They do not encode the *atp8* gene. Overlapping regions were found in between *nad4L* and *nad4*, *nad1* and *trnN*, and *cox1* and *trnT*. The ATG initiation codon was used for 10 protein-coding genes, and the GTG initiation codon was used for the remaining 2 genes (*nad4* and *atp6*). The size of the protein-coding genes of the 3 human *Taenia* tapeworms did not vary, except for *Taenia solium nad1* (891 aa) and *nad4* (1212 aa) and *Taenia asiatica cox2* (576 aa). The tRNA genes were 57-75 bp long, and the predicted secondary structures of 18 of these genes had typical clover-leaf shapes with paired dihydrouridine (DHU) arms. The genes in all human *Taenia* tapeworms for the 2 mitochondrial rRNA subunits *rrnL* and *rrnS* were separated by *trnC*. The putative *T. saginata rrnL* ranged from 972 to 980 bp long and *rrnS* are ranged from 705 to 732 bp long, respectively. The non-coding regions of the mt genomes of human *Taenia* tapeworms consisted of 2 regions: a short non-coding region (SNR, 66-71 nucleotides) and a long non-coding region (LNR, 159-192 nucleotides). The overall sequence difference in the full mitochondrial genome between *T. saginata* and *T. asiatica* was 4.6%, while *T. solium* differed by 11%.
The multiplex PCR assay with the Ta4978F, Ts5058F, Tso7421F, and Rev7915 primers using the full sequence information were used for the differential diagnosis, molecular characterization, and epidemiological surveys of *T. asiatica*, *T. saginata*, and *T. solium*. Three species-specific forward primers were prepared based on the nucleotide sequences of valine transfer RNA (tRNA) and NADH dehydrogenase subunit 2 from the human taeniid cestodes. The 3 forward primers were designed to amplify products of different size as fellow: (1) Ta4978F, specific for *T. asiatica* (5'-GGG TTT AAG TTA TAA ATG TGA TGT-3'; nucleotides 4978 to 5001 from GenBank accession number AF445798); (2) Ts5058F, specific for *T. saginata* (5'-ACT ACA TTT GGT TTG TTT TTG TAG-3'; nucleotides 5058 to 5081 from AY684274), and (3) Tso7421F, specific for *T. solium* (5'-CTA GGC CAC TTA GTA GTT TAG TTA-3'; nucleotides 7421 to 7444 from AB086256). The reverse primer, Rev7915 (5'-CAT AAA

ACA CTC AAA CCT TAT AGA-3'; nucleotides 5659 to 5685 from AF445798, nucleotides 5657 to 5683 from AY684274, and nucleotides 7870 to 7895 from AB086256), was from a highly conserved sequences common to all of 3 human *Taenia* tapeworms.

The improvement of genomic DNA extraction methods from various tissue sources is prerequisite for applying molecular genetics techniques to the relevant study fields. The majority of *Taenia* tapeworm specimens in the museum collections are fixed in formalin fixative for permanent preservation and morphological examination purposes. Molecular diagnosis of specimens preserved for a long time in formalin can be useful in identifying the species even though the DNA molecules were degraded or very small in volume. High-resolution multiplex PCR assay and DNA sequencing based on formalin-fixed specimens or Copro-DNA that could be used for the differential diagnosis of human-infecting *Taenia* tapeworms.

References

Bowles J., MacManus D.P. 1994. Genetic characterization of the Asian *Taenia*, a newly described taeniid cestodes of human. Am. J. Trop. Med. Hyg. 5:33-44.

Chapman A., Vallejo V., Mossie K.G., Oritiz D., Agabian N., Flisser A. 1995. Isolation and characterization of species-specific DNA probes from *Taenia solium* and *Taenia saginata* and their use in an egg detection assay. J. Clin. Microbiol. 33:1283-1288.

Eom K.S., Rim H.J. 1993. Morphologic descriptions of *Taenia asiatica* sp. n. Korean J. Parasitol. 31:1-6.

Eom K.S., Jeon H.K., Kong Y., Hwang U.K., Yang Y., Li X., Xu L., Feng Z., Rim H.J. 2002. Identification of *Taenia asiatica* in China: molecular, morphological and epidemiological analysis of a Luzhai isolate. J. Parasitol. 88:758-764.

Eom K.S., Jeon H.K., Rim H.J. 2009. Geographical distribution of *Taenia asiatica* and related species. Korean J. Parasitol. 47(Suppl.):115-124.

Eom K.S., Chai J.Y., Yong T.S., Min D.Y., Rim H.J., Kihamia C., Jeon H.K. 2011. Morphologic and genetic identification of *Taenia* tapeworms in Tanzania and DNA genotyping of *Taenia solium*. Korean J. Parasitol. 49(4):399-403.

Flisser A., Reid A., Garcia-Zepeda E., McManus D.P. 1988. Specific detection of *Taenia saginata* eggs by DNA hybridization. Lancet. ii:1429-1430.

Gonzales L.M., Montero E., Morakote N., Pueute S., Diaz De Tuesta J.L., Serra T., Lopez-Velez R., McManus D.P., Harrison L.J.S., Parkhouse R.M.E., Garate T. 2004. Differential diagnosis of *Taenia saginata* and *Taenia saginata asiatica* taeniasis through PCR. Diagn. Microbiol. Infect. Dis. 49:183-188.

Harrison L.J.S., Delgado J., Parkhouse R.M.E. 1990. Differential diagnosis of *Taenia sagianta* and *Taenia solium* with DNA probes. Parasitology 100:459-461.

Hoberg E.P., Jones A., Rausch R.L., Eom K.S., Gardne, S.L. 2000. A phylogenetic hyphothesis for species of the genus *Taenia* (Eucestoda: Taeniidae). J.Parasitol. 86:89-98.

Jeon H.K., Lee K.H., Kim K.H., Hwang W.U., Eom K.S. 2005. Complete sequence and structure of the mitochondrial genome of the human tapeworm, *Taenia asiatica* (Platyhelminthes; Cestoda). Parasitology 130:717-726.

Jeon, H.K., Eom, K.S. 2006. *Taenia asiatica* and *Taenia saginata*: Genetic divergence estimated from their mitochondrial genomes. Exp. Parasitol. 113: 58-61.

Jeon H.K., Chai J.Y., Kong Y., Waikagul J., Insisienhmay B., Rim H.J., Eom K.S. 2009. Differential diagnosis of *Taenia asiatica* using multiplex PCR. Exp. Parasitol. 121:151-156.

Jeon H.K., Yong T.S., Sohn W.M., Chai J.Y., Hong S.J., Han E.T., Jeong H.G., Chhakda T., Sinuon M., Socheat D., Eom K.S. 2011a. Molecular identification of *Taenia* tapeworms by cox1 gene by Koh Kong, Cambodia. Korean J. Parasitol. 49(2):195-197.

Jeon H.K., Kim K.H., Eom K.S. 2011b. Molecular identification of *Taenia* specimens after long-term preservation in formalin. Parasitol. Int. 60:203-205.

Mayata H., Tally A., Gilman R.H., Jimenez J., Verastegui M., Ruiz M., Garcia H.H., Gonzalez A.E. 2000. Differentiating *Taenia solium* and *Taenia saginata* infections by simple hematoxylin-eosin staining and PCR-restriction enzyme analysis. J. Clin. Microbiol. 38:133-137.

McManus D.P. 2006. Molecular discrimination of taeniid cestodes. Parasitology Int. 55(suppl.):S31-37.

Rishi A.K., McManus D.P. 1988. Molecular cloning of *Taenia solium* genomic DNA and characterization of taeniid cestodes by DNA analysis. Parasitology 97:161-176

Rishi A.K., McManus D.P. 1988. DNA probes which unambiguously distinguish *Taenia solium* from *T. saginata*. Lancet. i:1275-1276.

Rodrignez-Hidalgo R., Geysen D., Benitez-Ortiz W., Geerts S., Brandt J. 2002. Comparison conventional techniques to differentiate between *Taenia solium* and *Taenia saginata* and an improved polymerase chain reaction-restriction fragment length polymorphism assay using a mitochondrial 12S rDNA fragment. J. Parasitol. 88:1007-1011.

Yamasaki H., Nakao M., Sako Y., Nakaya K., Sato M.O., Mamuti W., Okamoto M., Ito A. 2002. DNA differential diagnosis of human taeniid cestodes by base excision sequence scanning thymine-base reader analysis with mitochondrial genes. J. Clin. Microbiol. 40:3818–3821.

Yamasaki H., Allan J.C., Sato M.O., Nakao M., Sako Y., Nakaya K., Qiu D., Mamuti W., Craig P.S., Ito A. 2004. DNA differential diagnosis of taeniasis and cysticercosis by multiflex PCR. J. Clin. Microbiol. 42:548-553.

Yamasaki H., Nakaya K., Nakao M., Sako Y., Ito A. 2007. Significance of molecular diagnosis using histophatological specimens in cestode zoonoses. Trop. Med. Health 35:307-3.

TSOL18 VACCINE AGAINST SWINE CYSTICERCOSIS

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Taenia solium is an important human pathogen causing neurological disease, particularly in people living in poor, developing countries. The parasite is transmitted between humans acting as the definitive host for the tapeworm parasite, and pigs which harbour the larval stage (cysticercus) in their muscles and other organs. The tapeworm eggs contain an infective stage known as an oncosphere; these are not only infective for pigs but can also infect humans with cysticerci should the eggs be ingested. In humans the parasite has a propensity to encyst in neural tissue, causing neurocysticercosis.

Past attempts to control neurocysticercosis have been reviewed by Lightowlers (2010a). Control efforts have generally relied on the treatment of humans with drugs to kill tapeworms, education about the importance of sanitation for prevention of this and other diseases, and confinement of pigs so as to reduce their access to items contaminated with human faeces. Generally these efforts have had limited success. The effectiveness of mass treatment of the human population or treatment of identified tapeworm carriers as control measures is undermined by the presence of infected pigs which serve as a reservoir for re-infection of the population with the tapeworm.

The potential for vaccination to play a role in controlling neurocysticercosis has been recognised for more than 20 years (Molinari et al., 1993). In 1989 the creation of the 45W vaccine against *Taenia ovis* (Johnson et al., 1989) was a pivotal moment in the quest to develop a practical vaccine against neurocysticercosis. The *T. ovis* vaccine was the first recombinant vaccine against any parasitic infection and it proved that a high level of protection could be achieved against a complex metazoan parasite using a single recombinant antigen. It also provided the blueprint for development of vaccines against other taeniid cestode infections, including *T. solium*. Although there may be potential for application of metacestode antigens as vaccines (Sciutto et al., 2008; Lightowlers, 2010b), antigens derived from the oncosphere life cycle stage have provided the most consistent and highest level of protection (Lightowlers, 2006).

The *tsol*18 gene was cloned by Gauci et al. (2003) and the protein expression product of the gene, TSOL18, was shown by Flisser et al. (2004) to be capable of inducing a very high level of protection in vaccinated pigs against an experimental challenge infection. In five separate experiments undertaken in Mexico (2 trials), Peru, Honduras and Cameroon (Flisser et al., 2004; Gonzalez et al., 2005; Lightowlers, 2006), immunisation with TSOL18 induced > 99% protection against the development of cysticerci. The vaccine comprises 200µg of the TSOL18 protein expressed as a glutathione *S*-transferase fusion protein in *Escherichia coli,* plus Quil A as adjuvant, and is injected intramuscularly.

The first field trial of the TSOL18 vaccine was described by Assana et al. (2010). The trial was undertaken in the Mayo-Danay administrative department of the Far North region of Cameroon and involved two hundred and forty, 3-month-old piglets. The animals were distributed to 114 different households and the farmers paid to keep the animals for the duration of the trial, using identical husbandry procedures to those used with their other pigs. In this region, pigs are commonly free roaming during the dry season and the area is highly endemic for *T. solium* (Assana et al., 2010). The pigs were distributed to households as pairs, with one animal to be vaccinated and one animal acting as a non-vaccinated control. The TSOL18 vaccine targets an antigen that is present only in the oncosphere and very early developing parasite, hence is not expected to be effective against established cysticercus infections. The animals that were used in the field trial were derived from a region known to be endemic for cysticercosis and they were placed with the farmers in circumstances such that it was possible that the animals could have been exposed to *T. solium* prior to the first vaccination. This situation is similar to that which would pertain to young animals being born and raised as part of normal farm practice where cysticercosis transmission was occurring. In this situation, any of the experimental animals in the vaccination group that had been infected prior to being vaccinated, would potentially have appeared as vaccine failures when infection was assessed at the end of the trial. For this reason, the pigs were given a single treatment with oxfendazole at the time they were given their second immunisation. This treatment kills all viable cysticerci in muscle

tissues (Gonzales et al., 1996; Sikasunge et al., 2008). The treatment was given at the time of the second immunisation because TSOL18-induced immunity would be effective shortly after the second injection hence any pre-infection as cured by oxfendazone and any subsequent infection prevented by vaccination. Animals that may have been infected prior to the beginning of the trial would not only have their infections cured but also lesions in the muscles caused by the death of cysticerci had sufficient time to resolve completely (Gonzales et al., 1996; Sikasunge et al., 2008) by the time the trial was concluded, when the animals were approximately 12 months of age. Control pigs were given the same oxfendazole treatment so that any difference between the controls and the vaccination group was clearly related to vaccination. When the field trial animals were necropsied and the number of cysticerci counted, 97 vaccinated animals and 102 controls were available for assessment (Table 1). Twenty controls were infected with viable *T. solium* cysticerci in their muscles whereas none of the vaccinated animals was detected with any evidence of *T. solium* infection, either viable or not viable. This difference was highly statistically significant ($P < 0.001$).

Table 1. Numbers of pigs assessed for infection with *T. solium* at the culmination of a field trial of the TSOL18 vaccine undertaken in Cameroon, the number of infected animals detected in controls and vaccinates and the numbers of cysticerci detected (Assana et al., 2010)

Group	Number of animals at necropsy	Number infected with *T. solium* cysticerci	Cyst numbers in muscles (range)
Controls	102	20	3 – 37080
TSOL18 vaccinated	97	0	-

In the Cameroonian field trial, vaccination with TSOL18 completely eliminated the transmission of *T. solium* by the animals involved in the trial. The combination of both vaccination and a single treatment with oxfendazole removed any potential for infections acquired prior to vaccination from being present when pigs are of an age suitable for slaughter and consumption. Future research will seek to confirm the efficacy of the vaccine in other field-based experiments and to scale-up and register TSOL18 as a vaccine against porcine cysticercosis. It is likely that the TSOL18 vaccine would also be effective if it were used directly in humans, however the parasite's life cycle is restricted almost entirely to poor people living in poor countries and for this reason it is unlikely that the substantial resources required to register and use an new human vaccine would be forthcoming for cysticercosis. For this reason, implementation of vaccination for prevention of neurocysticercosis seems likely to concentrate on vaccination of pigs because this is a substantially less expensive option than vaccinating humans. A control program based on pig vaccination alone would not immediately lead to a reduction in transmission of cysticercosis to humans because of the persistence of cases of taeniasis. A combined program involving the vaccination of pigs together with anthelmintic treatment of humans to treat taeniasis would be more immediately affect transmission to humans (Lightowlers 2010a). The available evidence indicates that *T. solium* tapeworms have a lifespan of 2-3 years (Lightowlers 2010a) so that even a control program directed entirely towards pigs would be expected to impact transmission of cysticercosis to humans within about 2-3 years.

There are many challenges that remain before the TSOL18 vaccine is ready to be implemented as widely available tool for control of *T. solium* transmission, however the extraordinary effectiveness of the vaccine in both experimental and field studies bodes well for the vaccine contributing to a reduction in the global burden of neurocysticercosis.

References

Assana E., Kyngdon C.T., Gauci C.G., Geerts S., Dorny P., De Deken R., Anderson G.A., Zoli A.P., Lightowlers M.W. 2010. Elimination of *Taenia solium* transmission to pigs in a field trial of the TSOL18 vaccine in Cameroon. Int. J. Parasitol. 40:515-519.

Flisser A., Gauci C.G., Zoli A., Martinez-Ocana J., Garza-Rodriguez A., Dominguez-Alpizar J.L., Maravilla P., Rodriguez-Canul R., Avila G, Aguilar-Vega L., Kyngdon C., Geerts S., Lightowlers

M.W. 2004. Induction of protection against porcine cysticercosis by vaccination with recombinant oncosphere antigens. Infect. Immun. 72:5292-5297.

Gauci C., Lightowlers M.W. 2003. Molecular cloning of genes encoding oncosphere proteins reveals conservation of modular protein structure in cestode antigens. Mol. Biochem. Parasitol. 127:193-198.

Gonzales A.E., Garcia H.H., Gilman R.H., Gavidia C.M., Tsang V.C., Bernal T., Falcon N., Romero M., Lopez-Urbina M.T. 1996. Effective, single-dose treatment or porcine cysticercosis with oxfendazole. Am. J. Trop. Med. Hyg. 54:391-394.

Gonzalez A.E., Gauci C.G., Barber D., Gilman R.H., Tsang V.C., Garcia H.H., Verastegui M., Lightowlers M.W. 2005. Vaccination of pigs to control human neurocysticercosis. Am. J. trop. Med. Hyg. 72:837-839.

Johnson K.S., Harrison G.B., Lightowlers M.W., O'Hoy K.L., Cougle W.G., Dempster R.P., Lawrence S.B., Vinton J.G., Heath D.D., Rickard M.D. 1989. Vaccination against ovine cysticercosis using a defined recombinant antigen. Nature 338:585-587

Lightowlers M.W. 2006. Cestode vaccines: origins, current status and future prospects. Parasitology 133:S27-42.

Lightowlers M.W. 2010a. Eradication of *Taenia solium* cysticercosis: a role for vaccination of pigs. Int. J. Parasitol. 40:1183-1192.

Lightowlers M.W. 2010b. Fact or hypothesis: *Taenia crassiceps* as a model for *Taenia solium*, and the S3Pvac vaccine. Parasite Immunol. 32:701-709.

Molinari J.L., Soto R., Tato P., Rodriguez D., Retana A., Sepulveda J., Palet A. 1993. Immunization against porcine cysticercosis in an endemic area in Mexico: a field and laboratory study. Am. J. Trop. Med. Hyg. 49:502-512.

Sciutto E., Fragoso G., Aluja ASd., Hernandez M., Rosas G., Larralde C. 2008. Vaccines against cysticercosis. Curr. Top. Med. Chem. 8:415-423.

Sikasunge C.S., Johansen M.V., Willingham A.L. 3rd., Leifsson P.S., Phiri I.K. 2008. *Taenia solium* porcine cysticercosis: viability of cysticerci and persistency of antibodies and cysticercal antigens after treatment with oxfendazole. Vet. Parasitol. 158:57-66.

HUMAN FASCIOLIASIS: CONTROL STRATEGIES IN THE PRESENT WORLDWIDE EMERGENCE SITUATION

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Human helminthiases are of considerable public health importance in sub-Saharan Africa, Asia, and Latin America. However, fascioliasis, caused by the two liver fluke species *Fasciola hepatica* and *F. gigantica* (*Trematoda*), is an important parasitic disease which shows a worldwide distribution, with *F. hepatica* presenting a cosmopolitan distribution, mainly in temperate zones, while *F. gigantica* is found in tropical regions of Africa and Asia giving rise to an overlap of the two fasciolid species in large regions (Mas-Coma et al., 2005; 2009a). Fascioliasis in livestock has always been recognized as a great problem worldwide (Spithill et al., 1999; Torgerson and Claxton, 1999), but in humans the disease was only considered of secondary importance until the end of the 1980 decade owing to the relative short number of human reports. Only about 2500 human cases were reported in the 25 years previous to 1990 (Chen and Mott, 1990). This scenario changed dramatically after the results obtained in different countries within the initiative launched by the World Health Organization at the beginning of the 90s. At present, estimations in all continents reach several millions and this may even be underestimating the real situation if the total lack of knowledge about numerous Asian and African countries is taken into account (Mas-Coma et al., 2005; 2009a).

In human helminthiases, updated knowledge of relevant and fundamental parasite biology is crucial. Successful and sustainable intervention depends on optimal utilisation of available control measures and development of new tools and strategies, as well as an understanding of the evolutionary implications of prolonged intervention on parasite populations and those of their hosts and vectors (Lustigman et al., 2012). With regard to fascioliasis, several geographical areas have already been described as endemic for the disease in humans (Mas-Coma et al., 2005), including hypoendemic, mesoendemic and hyperendemic situations, with prevalences and intensities ranging from low to very high (Mas-Coma et al., 2009a). Moreover, the disease in humans appears to be emerging in different areas of developing countries of Latin America, Europe, Africa and Asia, in a phenomenon which has been in part related to climate change in certain endemic areas (Mas-Coma et al., 2009b). In these human endemic areas, children and females are the most affected by both prevalences and intensities (Mas-Coma et al., 2005). They mostly show to be in the chronic phase, contrarily to the situation of patients in developed countries who are diagnosed in hospitals or other health centres usually at the acute phase or only beginning of the chronic phase. Infected subjects detected in surveys in human endemic areas are mainly in the advanced stage of chronicity, when not already reinfected due to the high contamination risk, as the liver fluke is able to survive up to more than 10 years in human beings (Mas-Coma et al., 2005). The public health impact of such scenario is becoming of an importance never expected before, owing to (i) the great pathogenicity of liver fluke infection at long term proven experimentally (Valero et al., 2003, 2006, 2008) and (ii) the immune modulation effect of fasciolids in the acute phase (Brady et al., 1999) and their immune suppression effect in the acute and advanced chronic phases (Girones et al., 2007) which is in the background of coinfections with other parasitic and infectious diseases (Mas-Coma et al., 2005).

In front of this emerging scenario and the capacity of liver flukes to underdevelop the rural human communities affected, the World Health Organization (WHO) decided in October 2006 to launch a worldwide initiative against this disease (WHO, 2007) based on the success of obtaining a donation of Egaten®, the only highly efficient drug presently available for human treatments (WHO, 2008). After an initial pilot phase in Vietnam, Egypt, Peru and Bolivia, actually this initiative is being progressively implemented in many additional countries of different continents.

Many new concepts have been reached on human fascioliasis from the 90s and have furnished a new baseline for the human disease which is very different from a simple extrapolation from fascioliasis in livestock. The number of human infection reports and the description of human endemic areas, in which children are mainly affected, have been increasing in the last two decades. The great pathogenicity on individuals and underdeveloping impact on human communities fascioliasis requests fast actions to help people affected.

The acknowledgement of the disease burden due to helminth infections, the availability of donated or affordable drugs that are mostly safe and moderately efficacious and the implementation of viable mass drug administration interventions have prompted the establishment of various large-scale control and elimination programmes. These programmes have benefited from improved epidemiological mapping of the infections, better understanding of the scope and limitations of currently available diagnostics and of the relationship between infection and morbidity, feasibility of community-directed or school-based interventions, and advances in the design of monitoring and evaluation protocols (Boatin et al., 2012).

In human fascioliasis, unfortunately, the pronouncedly different situations do not offer an easy frame to deal with. Studies have shown that human fascioliasis presents a marked heterogeneity including different epidemiological situations and transmission patterns in the different endemic areas (Valero et al., 2012). The establishing of general control measures becomes a problem due to this heterogeneity, which added to the present emergence/re-emergence of the disease both in humans and animals in many regions conforms a worrying, global scenario. Different situations appear related to, for instance:

-presence of only one (*F. hepatica* or *F. gigantica*), the two species (endemic area presenting total, partial or no overlapping of two fasciolid species), or the two species plus hybrids;

-presence of only one lymnaeid species transmitting one fasciolid species, several lymnaeid species transmitting one fasciolid species, two lymnaeid species transmitting the two fasciolids or several transmitting each one of the two fasciolids in an overlapping area;

-one or more domestic animal species playing a role of reservoir;

-wild mammals species playing a role of reservoirs;

-seasonal or permanent transmission;

-a stable endemic situation or an emergent one;

-physiographically uniform or heterogeneous endemic area;

-one or more human infection sources;

-more or less problems with diagnostic methods (impossibility to get blood samples in the endemic area not allowing for serological tests; peculiar fluke characteristics related to absence of egg shedding or shedding of only a very reduced number of eggs; so far impossibility to differentiate fluke hybrids in coprological and serological diagnostic techniques);

-rare or usual massive infections in humans;

-children or adults the subjects mainly infected.

The heterogeneity is of such a level, that thinking at convenient, simplified and uniform control schemes does not appear feasible. For many areas or countries, concrete control measures should be recommended which may include several measures peculiar for only that given place. The World Health Organization (WHO) has already recognized such heterogeneity and has accordingly implemented different pilot strategies for each one of the initially selected countries of Peru, Bolivia, Egypt and Vietnam.

The question immediately arises about how to define different control measures for each. If several years will be needed for the appropriate multidisciplinary assessment each time an area with human fascioliasis is detected, the global process will take too much time. Moreover, noting many different control measure packages related to the different transmission patterns and epidemiological situations known would give rise to a complicate list which could easily lead to confusion of the national health responsible in charge for the implementation of the control measures in each country. Pragmatism indicates the convenience of looking for markers which could easily and quickly distinguish each type of transmission pattern and epidemiological situation.

If appropriate markers able to distinguish the different types of situations could be found, there would be a way to both accelerate the global process and facilitate the general protocol for human fascioliasis control. Owing to the fact that all aforementioned differential characteristics may be related to different combinations of different species and strains of both liver flukes and lymnaeid vectors, genetic markers appear to be the targets in the front line (Mas-Coma et al., 2009a), and climatic-physiographic markers those in the second line. Indeed, the latter need a previous field and experimental characterization of flukes and snails. In human fascioliasis, accurate mathematical modelling (Fuentes et al., 1999) and remote sensing and GIS (Fuentes et al., 2005; Mas-Coma et al., 2009b), only genetic markers considered useful for molecular epidemiology of fascioliasis are analysed in the following.

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References

Boatin B.A., Basañez M.G., Prichard R.K., Awadzi K., Barakat R.M., Garcia H.H., Gazzinelli A., Grant W.N., McCarthy J.S., N'Goran E.K., Osei-Atweneboana M.Y., Sripa B., Yang G.J., Lustigman S. 2012. A research agenda for helminth diseases of humans: Towards control and elimination. PLoS Negl. Trop. Dis. 6(4):e1547.

Brady M.T., O'Neil S.M., Dalton J.P., Mills K.H. 1999. *Fasciola hepatica* supresses a protective Th1 response against *Bordetella pertussis*. Infect. Immun. 67:5372-5378.

Chen M.G., Mott K.E. 1990. Progress in assessment of morbidity due to *Fasciola hepatica* infection: a review of recent literature. Trop. Dis. Bull. 87(4):1-38.

Fuentes M.V., Valero M.A., Bargues M.D., Esteban J.G., Angles R., Mas-Coma S. 1999. Analysis of climatic data and forecast indices for human fascioliasis at very high altitude. Ann. Trop. Med. Parasitol. 93(8):835-850.

Fuentes M.V., Sainz-Elipe S., Nieto P., Malone J.P., Mas-Coma S. 2005. Geographical Information Systems risk assessment models for zoonotic fasciolosis in the South American Andes region. Parassitologia (special issue) 47:151-156.

Girones N., Valero M.A., Garcia-Bodelon M.A., Chico-Calero M.I., Punzon C., Fresno M., Mas-Coma S. 2007. Immune supression in advanced chronic fascioliasis: an experimental study in a rat model. J. Inf. Dis. 195(10):1504-1512.

Lustigman S., Geldhof P., Grant W.N., Osei-Atweneboana M.Y., Sripa B., Basañez M.G. 2012. A Research agenda for helminth diseases of humans: Basic research and enabling technologies to support control and elimination of helminthiases. PloS Negl. Trop. Dis. 6(4):e1445.

Mas-Coma S., Bargues M.D., Valero M.A. 2005. Fascioliasis and other plant-borne trematode zoonoses. Int. J. Parasitol. 35:1255-1278.

Mas-Coma S., Valero M.A., Bargues M.D. 2009a. *Fasciola*, lymnaeids and human fascioliasis, with a global overview on disease transmission, epidemiology, evolutionary genetics, molecular epidemiology and control. Adv. Parasitol. 69:41-146.

Mas-Coma S., Valero M.A., Bargues M.D. 2009b. Climate change effects on trematodiases, with emphasis on zoonotic fascioliasis and schistosomiasis. Vet. Parasitol. 163(4):264-280.

Spithill T.W., Smooker P.M., Copeman D.B. 1999. *Fasciola gigantica*: epidemiology, control, immunology and molecular biology. In: Dalton J.P. (Ed.), Fasciolosis, CAB International Publishing, Wallingford, Oxon, pp. 377-410.

Torgerson P., Claxton J. 1999. Epidemiology and Control. In: Dalton J.P. (Ed.), Fasciolosis, CAB International Publishing, Wallingford, Oxon, pp. 113-149.

Valero M.A., Santana M., Morales M., Hernandez J.L., Mas-Coma S. 2003. Risk of gallstone disease in advanced chronic phase of fascioliasis: an experimental study in a rat model. J. Inf. Dis. 188:787-793.

Valero M.A., Navarro M., Garcia-Bodelon M.A., Marcilla A., Morales M., Garcia J.E., Hernandez J.L., Mas-Coma S. 2006. High risk of bacterobilia in advanced experimental chronic fasciolosis. Acta Trop. 100(1-2):17-23.

Valero M.A., Girones N., Garcia-Bodelon M.A., Periago M.V., Chico-Calero I., Khoubbane M., Fresno M., Mas-Coma S. 2008. Anaemia in advanced chronic fasciolosis. Acta Trop. 108:35-43.

Valero M.A., Perez-Crespo I., Khoubbane M., Artigas P., Panova M., Ortiz P., Maco V., Espinoza J.R., Mas-Coma S. 2012. *Fasciola hepatica* phenotypic characterisation in Andean human endemic areas: valley versus altiplanic patterns analysed in liver flukes from sheep from Cajamarca and Mantaro, Peru. Inf. Genet. Evol. 12:403-410.

World Health Organization 2007. Report of the WHO Informal Meeting on use of triclabendazole in fascioliasis control. World Health Organization, Headquarters Geneva, 17-18 October 2006: WHO/CDS/NTD/PCT/2007.1.

World Health Organization 2008. Fact sheet on fascioliasis. In: Action Against Worms, World Health Organization, Headquarters Geneva (December 2007), Newsletter 10:1-8.

PHENOTYPING OF PURE AND HYBRID FASCIOLIDS IN AFRICA AND ASIA

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Fascioliasis is a serious human disease caused by *Fasciola hepatica* and *F. gigantica*, transmitted by freshwater lymnaeid snails and with many livestock species acting as reservoirs. The geographical distribution of both causal agents overlaps in many African and Asian countries (Mas-Coma et al., 2009). Despite the importance to differentiate between the infection by either fasciolid species, due to their distinct epidemiological, pathological and control characteristics, there is, unfortunately, neither a direct coprological nor an indirect immunological test available for their diagnosis. The specific differentiation can only be made by either a morphological study of adult flukes (Periago et al., 2006) or by molecular tools (Mas-Coma et al., 2009). Hence, subjects diagnosed are currently referred to as infected by *Fasciola* sp. The overlapping distribution of *F. hepatica* and *F. gigantica* has also led to a long ranging controversy on the taxonomic identity of the *Fasciola* species found in Far East countries, in which some resemble *F. hepatica*, whereas others resemble *F. gigantica*, with intermediate forms also being present and involving phenomena such as abnormal gametogenesis, diploidy, triploidy and mixoploidy, parthenogenesis, and hybridization events between different genotypes. The existence of hybrid forms was confirmed when it was shown that Japanese fasciolids from animals presented ribosomal DNA sequences almost identical to those of *F. hepatica* and mitochondrial DNA sequences almost identical to those of *F. gigantica* (Itagaki and Tsutsumi, 1998). Initially, such peculiar phenomena were only described in animal endemic areas of South-East Asia. Human Fascioliasis hot spots have been described in some areas of Africa and Asia. WHO decided in October 2006 to launch a worldwide initiative against this disease (WHO, 2007) based on the success of obtaining a donation of Egaten®, the only highly efficient drug presently available for human treatments. After an initial pilot scheme in Vietnam, Egypt, Peru and Bolivia, this initiative is currently being extended to many other countries. In these endemic areas of human fascioliasis, environmental characteristics favour liver fluke transmission as well as lymnaeid presence, and both *F. hepatica* and *F. gigantica* are present simultaneously in individual definitive host species. The co-existence of the two fasciolids in these areas suggests a complicated picture of possible ways of circulation of the causal agents, and additional studies about the identification of the species implicated necessary for epidemiological and transmission studies are needed to obtain the baseline on which to establish appropriate control measures of the disease to humans.

In this study the morphometric characteristics of fasciolid adults/eggs infecting the main livestock species and eggs from humans from Africa and Asia were analysed. The areas chosen were severely affected human fascioliasis endemic zones, where our team had the opportunity to carry out fieldwork within the WHO initiative. In Africa, the study was performed in the Nile Delta region of Egypt, where both *F. hepatica* and *F. gigantica* are encountered, and endemic areas present high prevalences and intensities in humans. The Kutaisi region in Georgia, on the border between Europe and Asia, was chosen as *F. hepatica* is widely distributed there, but *F. gigantica* was also sporadically detected several decades ago and human fascioliasis is currently emerging. In the Middle East, Iran presents a large number of human cases in the Northern provinces situated along the shore of the Caspian Sea, especially in Gilan province. In southwest Asia, the analysis was carried out in Vietnam, where this disease has emerged in humans throughout the whole country in recent years. The aforementioned populations were compared to standard natural populations, i.e. from geographical areas where both species do not co-exist of European, Latin American (*F. hepatica*) (Valero et al., 2012) and African (*F. gigantica*) origin. The adult populations analysed were: (i) *F. hepatica* (European Mediterranean area) and *F. gigantica* (Burkina Faso) standard populations, and (ii) *F. hepatica* and *F. gigantica* populations from Iran (Gilan area) and Egypt (the Nile Delta). Only adult flukes found in livers of naturally infected bovines (cattle and buffaloes) were used, since previous studies revealed that the definitive host species decisively influences the size of fasciolid adults and eggs (Valero et al., 2001). Fasciolid adult specimens included in the study were gravid adult flukes. The samples analysed included the largest possible worm variability (different stages of maturity, body size and gravid uteri).

The eggs analysed were from: (i) the northern Bolivian Altiplano and the Cajamarca valley in Peru); (ii) the Kutaisi region of Georgia, the Nile Delta in Egypt, and the Quy Nhon province in Vietnam. Egg

material used remained without fixation, suspended in water, preserved in darkness at 4 ºC until required and were measured in the shortest time possible. A computer image analysis system (CIAS) was applied (Valero et al., 2005) on the basis of standardized measurements known to be useful for the differentiation of both fasciolid species (Periago et al., 2006). The study of phenotypic variability was carried out by a principal component analysis (PC).

According to the criteria proposed by Periago et al. (2006) for the differentiation of liver fluke adults of both fasciolid species, the results of this study indicate the presence of *F. hepatica, F. gigantica* and intermediate forms (*Fasciola* sp.) in Egypt and Iran (Ashrafi et al., 2006; Periago et al., 2008). The changes in size in the populations analysed are reflected in the PC analyses. In populations from Egypt and Iran, intermediate forms are located between *F. hepatica* and *F. gigantica* standard populations (Spain, France and Burkina Faso). Furthermore, the intermediate forms detected in populations from Iran have a different size than those from Egypt. Body roundness, body length over body width, and distance between the ventral sucker and the posterior end of the body provide useful tools for studying inter- and intraspecific morphological diversity in *Fasciola* adults and the application of these markers to specimens from Egypt and Iran, suggest a strong population-level variation in *Fasciola* adult morphology.

Classically, it is considered that at the abopercular end of the shell of *Fasciola* eggs there is often a roughened or irregular area that is not seen in *Fasciolopsis* eggs (Ash and Orihel, 1997). Nevertheless, our observations show that the frequency of the presence of this feature in *F. hepatica* is population-dependent, and therefore is not a pathognomonic criterion in diagnosis. Whether the peculiar abopercular and/or lateral irregularities in the eggshell surface observed in this study are related to the geographical origin or due to egg production abnormalities caused by human treatment (as, for instance, the usual albendazole treatments in the two Andean countries) could neither be analysed nor established.

The study of the influence of the host species on morphological traits reveals that eggs shed by humans are different from eggs shed by animals. In humans, *F. hepatica* eggs are bigger and *F. gigantica* eggs are smaller than reported to date from livestock, and their measurements overlap when compared.

The PC analysis of fasciolid egg variables from human samples from Bolivia, Peru, Georgia, Egypt and Vietnam clearly illustrate global size differences in the populations analysed. Two zones can be distinguished: one zone is made up of Bolivia, Peru, Georgia and Egypt, while the other zone consists only of Vietnam and overlaps with the Bolivian and Peruvian samples. The material analysed in this study shows that the size of eggs shed by humans from Georgia and Egypt corresponds to the *F. hepatica* morph, while the size of eggs shed by humans from Vietnam corresponds to the *F. gigantica* morph.

Fascioliasis is traditionally considered a worldwide veterinary problem, and consequently the egg size range found in the literature applied to human diagnosis was in fact obtained through the analysis of samples from domestic animal hosts. Therefore, the borderlines allowing differentiation between the two species were traditionally considered to be 150 µm in length and 90 µm in width (Ash and Orihel, 1997), lower values representing *F. hepatica* and higher values *F. gigantica*. Our study reveals that humans, in comparison to other host species, have a decisive influence on the size of *F. hepatica* and *F. gigantica* eggs, showing a greater variation than the above-mentioned classic size range, i.e. in humans *F. hepatica* eggs are bigger and *F. gigantica* eggs are smaller than the classic sizes reported. Measurements of *F. hepatica* and *F. gigantica* eggs originating from humans and animals from sympatric areas overlap, and, therefore, they do not allow differential diagnosis when within this overlapping range. In this sense, the new results should aid clinicians since the application of the classic egg size range in human samples may lead to erroneous conclusions (Valero et al., 2009).

The adult and egg stages of *F. hepatica* have been morphologically studied by numerous parasitologists throughout many decades, although neglecting the influence of the host species or geographical origin on morphology. The application of the morphological markers used in this study to specimens from geographical areas where *F. hepatica* and *F. gigantica* co-exist, such as Egypt and Iran, have allowed phenotyping these populations and the results suggest a strong population-level variation. In this sense, our team is presently carrying out the phenotypic characterisation of *Fasciola* eggs and adults from many Asian and African human endemic areas applying these tools.

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References

Ash L., Orihel T. 1997. Atlas of Human Parasitology, 4th edition. American Society of Clinical Pathologists, Chicago.

Ashrafi K., Valero M.A., Panova M., Periago M.V., Massoud J., Mas-Coma S. 2006. Phenotypic analysis of adults of *Fasciola hepatica*, *Fasciola gigantica* and intermediate forms from the endemic region of Gilan, Iran. Parasitol. Int. 55:249-260.

Itagaki T., Tsutsumi K., 1998. Triploid form of *Fasciola* in Japan: genetic relationships between *Fasciola hepatica* and *Fasciola gigantica* determined by ITS-2 sequence of nuclear rDNA. Int. J. Parasitol. 28:777-781.

Mas-Coma S., Valero M.A., Bargues M.D. 2009. *Fasciola*, lymnaeids and human fascioliasis, with a global overview on disease transmission, epidemiology, evolutionary genetics, molecular epidemiology and control. Adv. Parasitol. 69:41-146.

Periago M.V., Valero M.A., Panova M., Mas-Coma S. 2006. Phenotypic comparison of allopatric populations of *Fasciola hepatica* and *Fasciola gigantica* from European and African bovines using a computer image analysis system (CIAS). Parasitol. Res. 99:368-378.

Periago M.V., Valero M.A., El Sabed M., Ashrafi K., El Wakeel A., Mohamed M.Y., Desquesnes M., Curtale F., Mas-Coma S. 2008. First phenotypic description of *Fasciola hepatica/Fasciola gigantica* intermediate forms from the human endemic area of the Nile Delta, Egypt. Infect. Genet. Evol .8:51-58.

Valero M.A., Darce N.A., Panova M., Mas-Coma S. 2001. Relationships between host species and morphometric patterns in *Fasciola hepatica* adults and eggs from the Northern Bolivian Altiplano hyperendemic region. Vet. Parasitol. 102:85-100.

Valero M.A., Panova M., Mas-Coma S. 2005. Phenotypic analysis of adults and eggs of *Fasciola hepatica* by computer image analysis system. J. Helminthol. 79:217-225.

Valero M.A., Perez-Crespo I., Periago M.V., Khoubbane M., Mas-Coma S. 2009. Fluke egg characteristics for the diagnosis of human and animal fascioliasis by *Fasciola hepatica* and *F. gigantica.* Acta Trop. 111:150-159.

Valero M.A., Perez-Crespo I., Khoubbane M., Artigas P., Panova M., Ortiz P., Maco V., Espinoza J.R., Mas-Coma S. 2012. *Fasciola hepatica* phenotypic characterization in Andean human endemic areas: valley versus altiplanic patterns analysed in liver flukes from sheep from Cajamarca and Mantaro, Peru. Infect. Genet. Evol. 12:403-410.

WHO, 2007. Report of the WHO Informal Meeting on Use of Triclabendazole in Fascioliasis Control. World Health Organization, Headquarters Geneva, 17-18 October 2006 (WHO/CDS/NTD/PCT/2007.1).

DNA ASSESSMENT OF FOSSARINE VECTORS OF FASCIOLIASIS: DIFFERENCES BETWEEN THE AMERICAS AND EUROPE, WITH EMPHASIS ON *LYMNAEA SCHIRAZENSIS*

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In the Americas and in Europe, fascioliasis is caused by *Fasciola hepatica* and gives rise to a serious public health problem. Human endemic areas throughout the world present different epidemiological and transmission patterns among which, the freshwater lymnaeid species involved in the transmission prove to be a crucial factor (Mas-Coma et al., 2005; 2009). Hence the importance of the correct classification of the lymnaeid species in each endemic area. Pronounced susceptibility differences to absolute resistance have been described among lymnaeid populations (Bargues et al., 2011a). When assessing disease characteristics in different endemic areas, unexpected results were obtained in studies on lymnaeid susceptibility to *Fasciola.*

Disease prevalences and intensities also depend on the ecological characteristics (population dynamics, anthropophylic characteristics, type of water bodies, etc.) of the different lymnaeid vector species. That is why different lymnaeid species appear linked to the different transmission patterns and epidemiological scenarios of this very heterogeneous disease in humans (Mas-Coma 2005; Mas-Coma et al., 2009). The continental differences in lymnaeid faunas also explain that in the Americas fascioliasis is only caused by *F. hepatica*, owing to the absence of lymnaeids of the genus *Radix* which act as transmitters of *F. gigantica* (Bargues et al., 2001).

Despite the applied interest of lymnaeid snails, the present knowledge on the genetics of this gastropod group as well as on their parasite-host interrelationships is far from being sufficient. A good example of this situation is the systematic-taxonomic confusion in which this molluscan family has been immersed (Bargues et al., 2001). At lymnaeid species level, the problems are found mainly due to the interspecific morphological and anatomic uniformity numerous species present, usually resulting in serious difficulties in specimen classification, sometimes even impeding it (Bargues et al., 2003, 2007a; Bargues and Mas-Coma, 2005). Moreover, intraspecific variation of shell shape is particularly well marked within lymnaeids depending on environmental conditions (Burch, 1968; Burch and Lindsay, 1973) although a genetic component in shell shape has been shown at least in some lymnaeid populations (Samadi et al., 2000). Thus, there are many specimen classification problems, mainly related to: (i) species of the “stagnicoline” group in Europe and North America (Glöer and Meier-Brook, 1998; Bargues et al., 2001); (ii) the “radix” group in Europe and Asia (Glöer and Meier-Brook, 1998); (iii) the “fossarine” or “*Galba*/*Fossaria*“ group in the Americas (Bargues et al., 2007) (*Fossaria* is a synonym of *Galba* (ICZN, 1998); terms “fossarine” or “*Galba*/*Fossaria*“ group here used only in the meaning frequently found in American malacological literature of the last century).

Evidence suggests that *F. hepatica* is linked to the species of the originally Holarctic and Neotropical *Galba*/ *Fossaria* group, with *G. truncatula* as the most probable original intermediate host species. Species of the *Galba/Fossaria* group, have been frequently involved in the transmission in Central and South America, in spite of the serious difficulties or even impossibility of lymnaeid species classification within this morphologically and anatomically confusing group (Mas-Coma et al., 2001; Bargues et al., 2007b, 2011b; Mera y Sierra et al., 2009; Artigas et al., 2011; Bargues, 2011, etc.). By the contrary, in Europe, only one lymneid species of this group has been reported has *F. hepatica* transmitter, *Galba truncatula*.

However, the aforementioned different susceptibility phenomena in lymnaeids have to be considered with great caution. When comparing different lymnaeid DNA sequences, several populations originally classified as belonging to different species showed identical DNA marker sequences, and other populations originally classified as pertaining to the same species presented different DNA marker sequences. Sometimes sequence differences were very few, suggesting intraspecific variability (different haplotypes). However, occasionally differences detected among populations classified as pertaining to the same species were numerous, sufficient as to consider different species involved. Moreover, the number of sequence differences between species sometimes appeared lower than that between populations of the same species (Bargues et al., 2001, 2003, 2007). This clearly underlines both the classification problems and the systematic-taxonomic confusion present in *Lymnaeidae*.

Consequently, several susceptibility differences described could in fact be related to different lymnaeid species instead of different populations of the same lymnaeid species.
A ten-year study in different countries of the Americas and Europe demonstrated that this disease transmission heterogeneity is not due to susceptibility differences, but to a hitherto overlooked cryptic species, *Lymnaea schirazensis*, confused with the main vector *Galba truncatula* and/or other *Galba*/*Fossaria* vectors. Nuclear rDNA and mtDNA sequences and phylogenetic reconstruction highlighted an old evolutionary divergence from other *Galba*/*Fossaria* species, and a low intraspecific variability suggesting a recent spread from one geographical source.
These results demonstrate that, *Lymnaea* (*s. l.*) *schirazensis*, genetically distant but phenotypically very close, has always been confused with *G. truncatula* and/or other similar lymnaeid vector species in all these continents (Bargues et al., 2011a). Studies showed that *L. schirazensis* is not a vector species. The implications for fascioliasis are evident, as this hitherto overlooked species has been distorting results of fasciolid specificity/susceptibility analyses as well as the geographical distribution of the disease. The existence of this *G. truncatula*-like lymnaeid species frequently present in animal fascioliasis endemic areas and usual in human fascioliasis endemic areas ought be henceforth considered to avoid misunderstandings concerning transmission.

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References

Artigas P., Bargues M.D., Mera y Sierra R., Agramunt V.H., Mas-Coma S. 2011. Characterisation of fascioliasis lymnaeid intermediate hosts from Chile by DNA sequencing, with emphasis on *Lymnaea viator* and *Galba truncatula*. Acta Trop. 120:245-257.

Bargues M.D. 2011. Haplotipificación combinada de limneidos vectores de fascioliasis: evaluación y aplicabilidad de marcadores ribosómicos y mitocondriales. Biomédica 31:157-159.

Bargues M.D., Artigas P., Khoubbane M., Flores R. Glöer P., Rojas-Garcia R., Ashrafi K., Falkner G., Mas-Coma S. 2011a. *Lymnaea schirazensis*, an overlooked snail distorting fascioliasis data: genotype, phenotype, ecology, worldwide spread, susceptibility, applicability. PloS One 6:e24567.

Bargues M.D., Artigas P., Khoubbane M., Mas-Coma S. 2011b. DNA sequence characterisation and phylogeography of *Lymnaea cousini* and related species, vectors of fascioliasis in northern Andean countries, with description of *L. meridensis* n. sp. (Gastropoda: Lymnaeidae). Parasit. Vectors 4:132.

Bargues M.D., Artigas P., Mera y Sierra R., Pointier J.P., Mas-Coma S. 2007a. Characterisation of *Lymnaea cubensis*, *L. viatrix* and *L. neotropica* n. sp., the main vectors of *Fasciola hepatica* in Latin America, by analysis of their ribosomal and mitochondrial DNA. Ann. Trop. Med. Parasitol. 101:621-641.

Bargues M.D., Horak P., Patzner R.A., Pointier J.P., Jackiewicz M., et al. 2003- Insights into the relationships of Palaearctic and Nearctic lymnaeids (Mollusca: Gastropoda) by rDNA ITS-2 sequencing and phylogeny of stagnicoline intermediate host species of *Fasciola hepatica*. Parasite 10:243-255.

Bargues M.D., Mas-Coma S. 2005. Reviewing lymnaeid vectors of fascioliasis by ribosomal DNA sequence analyses. J. Helminthol. 79:257-267.

Bargues M.D., Mera y Sierra R., Gomez H., Artigas P., Mas-Coma S. 2007b. Molecular characterisation of *Galba truncatula*, main vector of fascioliasis, in Argentina. Implications for public health. Enf. Emerg. 9:77-82.

Bargues M.D., Vigo M., Horak P., Dvorak J., Patzner R.A., et al. 2001. European Lymnaeidae (Mollusca: Gastropoda), intermediate hosts of trematodiases, based on nuclear ribosomal DNA ITS-2 sequences. Infect. Genet. Evol. 1:85-107.

Burch Burch J.B., Lindsay G.K. 1973. Apparent hybrids from a laboratory culture of *Lymnaea stagnalis* and *Bulimnea megasoma*. Malacol. Rev. 6:61-62.

Burch J.B. 1968. *Erinna newcombi* of Hawaii and *Limnaea onychia* of Japan. Malacol. Rev. 1:15-30.

Glöer P., Meier-Brook C. 1998. Süsswassermollusken. Ein Bestimmungsschlüssel für die Bundesrepublik Deutschland (12th Edition). Hamburg: Deutscher Jugendbund für Naturbeobachtung, pp. 136.

International Commission on Zoological Nomenclature (ICZN) (1998) Opinion 1896. *Galba* Schrank, 1803 (Mollusca, Gastropoda): *Buccinum truncatulum* Müller, 1774 designated as the type species. Bull. Zool. Nomencl. 55:123.

Mas-Coma S. 2005. Epidemiology of fascioliasis in human endemic areas. J. Helminthol. 79:207-216. Mas-Coma S., Bargues M.D., Valero M.A., 2005. Fascioliasis and other plant-borne trematode zoonoses. Int. J. Parasitol. 35:1255-1278.

Mas-Coma S., Funatsu I.R., Bargues M.D. 2001. *Fasciola hepatica* and lymnaeid snails occurring at very high altitude in South America. Parasitology 123:S115-S127.

Mas-Coma S., Valero M.A., Bargues M.D. 2009. *Fasciola*, lymnaeids and human fascioliasis, with a global overview on disease transmission, epidemiology, evolutionary genetics, molecular epidemiology and control. Adv. Parasitol. 69:41-146.

Mera y Sierra R., Artigas P., Cuervo P., Deis E., Sidoti L. 2009. Fascioliasis transmission by *Lymnaea neotropica* confirmed by nuclear rDNA and mtDNA sequencing in Argentina. Vet. Parasitol. 166:73-79.

Samadi S., Roumegoux A., Bargues M.D., Mas-Coma S., Yong M., et al. 2000. Morphological studies of lymnaeid snails from the human fascioliasis endemic zone of Bolivia. J. Moll. Stud. 66:31-44.

EMERGING OPISTHORCHIASIS IN THE EUROPEAN UNION

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The liver fluke *Opisthorchis felineus* is one of the zoonotic trematodes that circulates in the European Union (EU). It is transmitted from freshwater snails to fish and then to fish-eating mammals, including humans, in which it causes opisthorchiasis. Humans acquire the infection by consuming raw freshwater fish of the family Cyprinidae, which harbor the larval stage, metacercariae, in their muscles. In the XX century, infections in humans have been reported in Byelorussia, Lithuania, Poland, Romania, Russia, Spain and Ukraine, and in Siberia. In EU in the last fifty years, the parasite has been detected in humans of Germany, Greece and Italy, and in red foxes, cats, fish and mollusks of Germany, Italy, Poland, Portugal and Spain. In Italy from 2003 to 2011, four individual cases and eight outbreaks of opisthorchiasis were reported. All of the infected persons had consumed raw fillet of tench (*Tinca tinca*) fished from three lakes in central Italy, but a percentage of infected people were tourists who developed the disease when they returned home in other Italian regions or in other European countries. The symptomatology of opisthorchiasis due to *O. felineus* in humans can range from asymptomatic to severe. In endemic regions where people frequently consume raw fish, the number of worms in the bile ducts can be very high, inducing chronic infection, which is characterized by anorexia, dyspepsia, dryness and bitter taste in the mouth, fatigue, nausea, and pain in the right hypochondrium. In EU, where the chance to get infected is low, only a percentage of infected people show a symptomatology characterized by fever, abdominal pain, headache, asthenia, arthralgia, diarrhea and nausea. All infected patients show eosinophilia and increased liver enzymes. In most of these patients, the symptomatology disappears within 2-3 months after the infection, whereas the laboratory features decrease slowly independently from the presence of adult worms shedding eggs. The clinical diagnosis of *O. felineus* should be confirmed by the detection of eggs in stool. However in non-endemic areas such as the EU, the infection is generally due to a low number of worms, there can be very few eggs in faecal samples and they can be detected only after faecal concentration and examination of at least three samples collected on different days. To identify cryptic infections, a very sensitive and specific method is the detection of parasite DNA in faecal samples by PCR and sequencing. Alternatively, serology (e.g., ELISA) using excretory/secretory (ES) antigens produced by adult worms in vitro can be used, although the possibility of cross-reaction with other helminths (both trematodes and nematodes) cannot be ruled out. *Opisthorchis felineus* infection in humans has been treated with praziquantel or albendazole. The treatment with praziquantel is as a role resolutive, whereas, the treatment with albendazole can fail and the individual should be treated again with the other drug. *Opisthorchis felineus* infection has been documented in humans and/or animals in 13 EU countries; however, in EU countries, with the exception of Germany and Italy, the available information on the circulation of this pathogen in the past 30-40 years is very limited or non-existent. For the most part, this can probably be explained by underdiagnosis; however, the lack of cases in the past 50 years could also be related to changes in eating behaviour. In Italy, the recent occurrence of many human cases has revealed an unexpected epidemiological situation, in that it demonstrates how a parasite can circulate with a high prevalence in animal hosts without interacting with humans until eating behaviour changes. Humans probably play another important role in the epidemiology of this parasite; in particular, fishermen frequently throw tenches away on the shores of lakes because of their low economic worth or if they are too small. Furthermore, many restaurants in lake areas improperly dispose of leftovers. In both scenarios, the fish are eaten by stray cats and dogs. In humans, a consistent number of *O. felineus* infections are asymptomatic; furthermore, in symptomatic persons, the clinical symptoms disappear after a short period of time. For this reason, there could be a number of persons with cryptic and chronic infection in the EU. Moreover, even if the pathogenesis (e.g., cholangiocarcinoma) associated with *O. felineus* infection is poorly documented, there exits clinical and epidemiological data suggesting its involvement in severe clinical forms. This stresses the urgent need to deeply investigate the circulation of this zoonotic trematode in the EU and to develop new diagnostic tools for the diagnosis in humans and animals

HUMAN TREMATODIASES IN THE FAR EAST OF ASIA

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Abstract
Human trematodiases had long been neglected but now are acknowledged as an important group of parasitic diseases in public health points of view. These diseases are prevaling particularly in South East Asia, including the Far East (Korea, Japan, China, Taiwan, Russia, and Philippines). The diseases prevailing in the Far East include clonorchiasis, paragonimiasis, fascioliasis, echinostomiasis, heterophyidiasis (that include metagonimiasis, heterophyiasis, haplorchiasis, and various others), plagiorchiasis, neodiplostomiasis, and gymnophalloidiasis. Clinical manifestations due to *Clonorchis sinensis* infection include cholangitis, jaundice, liver cirrhosis, and cholangiocarcinoma. Infection with *Paragonimus* can cause chest pain and hemoptysis, with nodule formation, in pulmonary infections, and can also cause variable other manifestations, including neurologic symptoms, in cerebral infections. *Fasciola hepatica* or *F. gigantica* can cause bile duct infection but can also cause ectopic fascioliasis in the abdominal cavity or other areas. In intestinal trematode infections, including *Metagonimus, Heterophyes, Haplorchis, Echinostoma, Neodiplostomum,* and *Gymnophalloides* infection, abdominal pain, diarrhea, weight loss, and mucosal bleeding are the major clinical manifestations. A wide variety of human food materials, i.e. aquatic vegetations, snails, clams, crustaceans, fish (freshwater or brackish-water), amphibians, and reptiles are the sources of these trematode infections. For prevention of these diseases, it is strongly recommended to adequately cook the food before consumption. Proper disposal of human and animal excreta containing eggs is also required for their control.

Introduction

Many parasitic helminths, including trematodes, have a complex life cycle, in which specific intermediate hosts are involved. These kinds of intermediate hosts may become a source of human or animal infections. When an intermediate host, i.e. a food material, is involved in the transmission of trematodes, the disease is called as a food-borne trematodiasis. Food-borne trematodiases are nowadays recognized as a group of highly important parasitic diseases in public health points of view. In the present paper, trematode species transmitted via consumption of food and the diseases caused by them are briefly summarized.

Clonorchiasis

Clonorchis sinensis infects the bile duct of humans and animals, and is capable of causing significant morbidity and mortality of infected hosts. This disease is distributed in China, Taiwan, Korea, and Vietnam (Chai et al., 2005). The infection is caused by ingestion of raw or improperly cooked flesh of various species of freshwater fish, including *Gnathopogon* sp., *Zacco platypus, Hypomesus olidus,* and *Ctenopharyngodon idellus*. The infected people may develop cholangitis, gall stone, liver cirrhosis, and cholangiocarcinoma.

Paragonimiasis

At least seven species, including *Paragonimus westermani. P. skrjabini, P. heterotremus, P. africanus, P. uterobilateralis, P. mexicanus*, and *P. kellicotti*, are known to cause human infections around the world (Narain et al., 2010). This infection is contracted to humans by eating improperly cooked freshwater crayfish and crabs. The parasite invades the lung parenchyma normally, but in not a few instances, it can cause extrapulmonary infections that include cerebral, spinal, thoracic, abdominal, and ovarian paragonimiasis. People with paragonimiasis may experience bloody sputum, cough, chest pain, and lethargy in pulmonary infections, and headache, seizure, and other neurological symptoms in the cerebral type.

Fascioliasis

Fasciola hepatica and *F. gigantica* are the responsible agents. They are contracted to humans mainly by consumption of freshwater vegetables, including watercress, containing metacercariae. However, several other modes of infection, for example, food dishes and soups made from contaminated water and infected raw liver of cattle, have been suggested (Mas-Coma et al., 2007). The parasite normally

infects the bile duct of humans and herbivorous animals and can cause cholangitis, cholecystitis, cholelithiasis, and biliary obstruction. In not a few instances, the parasite causes extrahepatic infections.

Echinostomiasis

Twenty species of the family Echinostomatidae are known to cause human echinostomiasis around the world (Chai, 2009). They constitute an important group of food-borne helminths of public health importance in Southeast Asia and the Far East (Chai et al., 2009). *Echinostoma* (*E. revolutum, E. ilocanum, E. echinatum, E. hortense, E. cinetorchis, E. macrorchis*, and *E. angustitestis*) and *Echinochasmus* (*E. japonicus, E. perfoliatus, E. fujianensis, E. liliputanus,* and *E. jiufoensis*) are the two major genera. *Acanthoparyphium tyosenense, Artyfechinostomum malayanum, A. oraoni, Echinoparyphium recurvatum, Episthmium caninum*, *Himasthla muehlensi, Hypoderaeum conoideum,* and *Isthmiophora melis* are the minor group. Various types of food animals, including freshwater fish, brackish-water fish, freshwater snails, brackish-water snails (gastropods and bivalves), and amphibians are the sources of human infection (Chai, 2009). The pathogenicity and host-parasite relationships have been studied extensively in several species. Mechanical damages by worms and their metabolites are important factors in the pathogenesis. Mucosal ulcerations and bleeding at the upper part of the duodenum or at the distal part of the stomach are encountered in gastroduodenal endoscopy of human *E. hortense* infections (Chai, 2009).

Metagonimiasis

Three species of *Metagonimus*, i.e. *M. yokogawai*, *M. miyatai,* and *M. takahashii,* are known to cause human infections (Chai, 2007; Yu and Chai, 2010). The former species, *M. yokogawai*, is distributed in the Far East, including Korea, Japan, Taiwan, and Russia. The major source of infection is a species of freshwater fish called the sweetfish, *Plecoglossus altivelis*. The other two species, *M. miyatai* and *M. takahashii,* are prevalent along the upper reaches of big rivers in Korea and Japan, where minnows, *Zacco platypus*, and carps, *Carassius auratus*, are caught for eating raw. Infected people live along the small atreams or large rivers, and experience abdominal discomfort, diarrhea, and lethargy.

Heterophyiasis

Three species, namely *Heterophyes heterophyes*, *H. dispar,* and *H. nocens* are responsible for human infections (Chai, 2007; Chai et al., 2009). They are contracted to humans via consumption of raw or undercooked brackish-water fish, including the mullet, *Mugil cephalus* and goby, *Acanthogobius flavimanus*. Two species, *H. heterophyes* and *H. dispar,* are distributed in Egypt and the Middle East, including Iran and Saudi Arabia. The other species, *H. nocens,* is found in Korea and Japan. In humans, the parasite can cause gastrointestinal discomfort, including abdominal pain, diarrhea, and anorexia.

Haplorchiasis

Five species, namely *Haplorchis taichui, H. pumilio, H. yokogawai, H. pleurolophocerca* (in Egypt), and *H. vanissimus*, are responsible for human infections, the first three being the most important and *H. taichui* being the most frequently found. Human infections are now commonly found throughout Southeast Asia and the Far East, including Thailand, Laos, Vietnam, the Philippines, Taiwan, and China (Chai, 2007; Chai et al., 2009). Various species of freshwater fish, including *Cyprinus carpio, C. auratus, Zacco platypus, Pseudorasbora parva, Rhodeus ocellatus, Gambusia affinis, Puntius* spp., *Raiamas guttatus, Mystacoleucus marginatus*, and *Henichoryhnchus siamensis* have been identified as the second intermediate hosts for these flukes. Clinical aspects of *Haplorchis* spp. infection in humans have not been well documented; however, it is presumed that they may cause gastrointestinal troubles.

Other hetrophyid infections

Other heterophyid flukes (the family Heterophyidae) that infect humans include *Pygidiopsis summa, Heterophyopsis continua, Stellantchasmus falcatus, Centrocestus armatus, Stictodora fuscata,* and *Stictodora lari* (Chai, 2007; Chai et al., 2009). With the exception of *C. armatus*, which is transmitted by freshwater fish, all others are transmitted to humans by eating raw or improperly cooked brackish-water fish, such as *Mugil cephalus, Acanthogobius flavimanus*, and *Lateolabrax japonicus*.

Plagiorchiasis

Plagiorchis muris and *P. vespertilionis* are the two species reported from human infections (Chai, 2007; Guk et al., 2007). The sources of human infection may include a wide range of animals, such as the aquatic insects (mosquito larvae), insect naiads, freshwater snails, and freshwater fish. No specific symptoms are known in human infections.

Neodiplostomiasis

Human infections with *Neodiplostomum seoulense* (the family Neodiplostomidae) has been reported in Korea (Chai, 2007). This species began to draw medical attention from 1982 when an infected young man complained of an acute abdominal pain, diarrhea and fever. The patient had eaten raw snakes seven days before admission to a hospital, and the grass snake, *Rhabdophis tigrina,* was found to carry metacercariae (Shin et al., 2008). Later, twenty-five human cases were found among soldiers who had eaten raw snakes during survival trainings. Life cycle studies have revealed that the second intermediate hosts of *N. seoulense* are tadpoles and frogs, and that the grass snake is a paratenic host. This trematode is highly pathogenic and lethal to mice (Kook et al., 1998; Shin et al., 2008).

Gymnophalloidiasis

A minute intestinal fluke, named *Gymnophalloides seoi*, unique in Korea, was first discovered in a woman with acute pancreatitis and gastrointestinal problems (Chai, 2007; Shin et al., 2008; Chai et al., 2009). The southwestern coasts and coastal islands of Korea have been identified as endemic areas. The source of human infections is the oyster, *Crassostrea gigas*, and humans and wading birds, i.e., the Palearctic oystercatcher *Haematopus ostralegus*, are natural definitive hosts. Gastrointestinal troubles, including diarrhea and abdominal pain, may develop in infected people. Gymnophalloidiasis cases, accompanied by diabetes mellitus, have been reported.

CONCLUSION AND PERSPECTIVES

For prevention and control of these food-borne trematodiases, strategies, such as use of proper diagnostic procedures and of chemotherapy are essential to improve status evaluation of each trematodiasis and reduce morbidity and mortality, respectively. Health education to avoid consumption of raw or improperly cooked food stuffs and sanitary disposal of human and animal excreta containing the trematode eggs are also highly important. However, because of local constraints, deeply-embedded cultural traits, and inadequate national priorities, it is problematic whether these strategies will be implemented sufficiently to have significant public health impact (Chai et al., 2005).

References

Chai J.Y. 2007. Intestinal Flukes. Food-borne Parasitic Zoonoses: Fish and Plant-borne Parasites (ed. Murrell KD and Fried B). World Class Parasites, Vol. 11, p 53-115. New York, USA. Springer.

Chai J.Y. 2009. Chapter 7. Echinostomes in humans. In The Biology of Echinostomes (Ed. Fried B. and Toledo R.), New York, USA. Springer, pp. 147-183.

Chai J.Y., Murrell K.D., Lymbery A.J. 2005. Fish-borne parasitic zoonoses: status and issues. Int. J. Parasitol. 35(11-12):1233-1254.

Chai J.Y., Shin E.H., Lee S.H., Rim H.J. 2009. Foodborne intestinal flukes in Southeast Asia. Korean J. Parasitol. 47(suppl.):69-102.

Guk S.M., Kim J.L., Park J.H., Chai J.Y. 2007. A human case of *Plagiorchis vespertilionis* (Digenea: Plagiorchiidae) infection in the Republic of Korea. J. Parasitol. 93:1225-1227.

Kook J., Nawa Y., Lee S.H., Chai J.Y. 1998. Pathogenicity and lethality of a minute intestinal fluke, *Neodiplostomum seoulense,* to various strains of mice. J. Parasitol. 84:1178-1183.

Mas-Coma S., Bargues M.D., Valero M.A. 2007. Plant-borne trematode zoonoses: Fascioliasis and fasciolopsiasis. *In* Food-borne Parasitic Zoonoses: Fish and Plant-borne Parasites (ed. Murrell K.D. and Fried B.). World Class Parasites, NY, USA, Springer, Vol. 11, pp. 293-334.

Narain K., Agatsuma T., Blair D. 2010. Chapter 61. Paragonimus. In Molecular Detection of Foodborne Pathogens (Liu D. ed.). Boca Raton, Florida, USA. CRC Press (Taylor and Francis Group), pp. 827-837.

Shin E.H., Guk S.M., Kim H.J., Lee S.H., Chai J.Y. 2008. Trends in parasitic diseases in the Republic of Korea. Trends Parasitol. 24:143-150.

Yu J.R., Chai J.Y. 2010. Chapter 59. *Metagonimus*. *In* Molecular Detection of Foodborne Pathogens (Liu D. ed.). Boca Raton, Florida, USA. CRC Press (Taylor and Francis Group), pp. 805-811.

HUMAN TREMATODIASES IN INDIA: PRESENT SITUATION

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More than a third of the tropical world's population is infected with helminths (trematode, cestode, nematode and acanthocephalan parasites). Of these, trematodiases caused by digenean flukes (Platyhelminthes: Trematoda) are a major public health problem worldwide. Food-borne trematodiasis (FBT) is an important cluster of neglected zoonoses that are transmitted by the consumption of raw or undercooked aquatic food contaminated with the infective larval stage (i.e. metacercariae) of the fluke. The global estimates of FBTs projected by World Health Organization during 1995 reflect rather conservative figures (i.e. > 41 million people currently infected and those at risk exceeding 750 million). Based on a systematic review and meta-analysis of electronic databases for reports about these infections covering the period of last three decades (1980-2008), Fürst et al. (2012) calculated the number of people infected with trematodiases to be about 56.2 million in 2005 and estimated the global burden of FBT to be 665352 (ranging between 479496-859051) disability adjusted life years (DALYs).

Many of trematodiases are endemic in certain areas of the old world countries and their geographical distribution, reservoir hosts and mode of transmission have been well documented in Central and Southeast Asia including the Indian Subcontinent (Table 1).

In addition to well-recognized trematode zoonoses, there are some unrecognized or lesser known ones that have emerged as major health hazards in recent years (Table 2).

In India, like most tropical countries, several parasitic infections are part of everyday life and Northeast India in particular, where traditional food practices and culinary habits of many native societies are similar to those of the neighbouring Near East countries, trematode infections of the lung and intestinal flukes are especially significant as potential zoonoses in the region.

The giant intestinal fluke, *Fasciolopsis buski*, is the only fasciolid species and the largest fluke reported to frequently infect people in the impoverished areas of the tropical and subtropical regions (Roy and Tandon, 2003). In India, morbidity due to fasciolopsiasis was recorded since 1843 when Busk for the first time recovered *F. buski* from the duodenum of an Indian sailor. In foci of parasite transmission, the prevalence of infection in children is reported to range from 60% in India and 50% in the neighboring Bangladesh to 10% in Thailand.

Artyfechinostomum sufrartyfex, commonly found in pigs, is causative of echinostomiasis in India. The first human infection of this echinostome was reported in an 8-year old girl in the erstwhile Greater Assam in the year 1915. Two other cases of infection by another species, *A. mehrai*, were reported in 1962 and 1964. Since then, the occurrence of this fluke has been reported from the states of Andhra Pradesh, Assam, Bihar, Tamil Nadu, Uttar Pradesh and among the Oraon tribes of West Bengal (Tandon et al., 2009). In the neighboring countries, the prevalence of echinostomiasis ranges from 65% in Taiwan to 5% in mainland China and from 50% in northern Thailand to 22% in Korea (Fried et al., 2004).

Of numerous heterophyid species causing human FBTs, the two most prevalent species are *Heterophyes heterophyes* and *Metagonimus yokogawai* having greatest medical importance in the Far East and Middle East countries (Kumar, 1999; Chai, 2009). In India, *H. heterophyes* infection has been sporadically reported from canid and felid hosts.

Of amphistomid flukes, *Gastrodiscoides hominis* is a potential zoonosis in India, although not considered a serious health threat in humans. Besides, there is no information on the occurrence of human infection by *Fischoederius* spp. that affects the mammalian livestock in India.

Human fascioliasis, prevalent in many countries covering all continents, is mainly caused by *Fasciola hepatica*, though in Asia including India the species implicated in infection is *F. gigantica* (Mas-Coma et al., 2007).

The Chinese liver fluke, *Clonorchis sinensis*, which is a major public health problem of several countries in East Asia, is not of common occurrence in India, though it was first reported in India in 1874. Like *Clonorchis*, species of *Opisthorchis*, especially *O. viverrini* and *O. felineus* are most prevalent human biliary parasites in South Asia and endemic in Thailand, Laos, Cambodia and

Vietnam. However, in India, *O. noverca* has been frequently reported from pigs and has plausible zoonotic implications in the northeastern region.

Eurytrema pancreaticum, a parasite of pancreatic and ducts and bile ducts of humans and livestock, occurs as natural infection of domestic ruminants in India.

Clinostomum complanatum, also known as “yellow grub”, resides in the pharynx of piscivorous birds. Human clinostomiasis, reported from countries in America, Southeast Asia and Eastern Europe, has not been reported from India so far, though the occurrence of *Clinostomum* metacercariae has been frequently recorded from freshwater fishes in many parts of the country.

Paragonimus, one of the most harmful zoonotic flukes, found in different parts of Asia, Africa and Americas, covers about 40 species that infect lungs of many mammalian hosts; of these 16 species have been reported from human beings. In northeast India, where crabs are commonly consumed as part of the traditional cuisine, particularly Manipur and Arunachal Pradesh are suspected foci of infection (Tandon et al., 2007). Besides *P. westermani*, the best-known etiological agent of human paragonimiasis in endemic regions of the world, *P. heterotremus* and *P*. *heitungensis* have also been reported to occur in the region.

Schistosomiasis is not known to occur in India, though there are a few reports of *S*. *haematobium* in western regions. In several regions of northeast India, *S. intercalatum* is a parasite of bovine hosts; besides, dermatitis caused by cercariae of non-human blood flukes has been frequently reported.

In some foci of South India, the cercaria of *Procerovum varium* (intestinal parasite of piscivorous birds) has emerged as a novel eiteological agent for ocular parasitosis (subconjunctival and posterior chamber uveitis) in children (Hoti and Tandon, 2011).

Most of the above-mentioned infections are prevalent in rural areas and linked to socio-cultural and socio-economic factors of the people in the endemic area. Epidemiologic surveys in Asia have revealed alterations in the pattern of trematode-borne diseases following changes in habits, cultural practices, health education, industrialization, and social environment. However, recent reports of increasing prevalence rates and estimates of population at risk in the region warrant in-depth investigations on the underlying causes. There are primarily four identifiable areas for potential contribution in technology development towards successful control of trematodiases: i) parasite genetics, genomics and functional genomics; ii) host-parasite interactions (pathogenesis and immunology); iii) vector/intermediate host-parasite interactions (transmission biology); and iv) scalable cost-effective diagnostics through globally coordinated programmes (Lustigman et al., 2012).

A more aggressive education programme in Southeast Asia (to discourage consumption of raw fish and improved sanitary and food hygiene practices) could produce long-term reductions in trematodiases-induced morbidity and mortality in the region.

Table 1. Common trematodiases of zoonotic potential worldwide*

Fluke (Family)	Geographical distribution	Natural reservoirs of infection	Mode of transmission	At risk population (numbers infected) (10^6)
Gastrointestinal				
Fasciolopsis buski **(Fasciolidae)**	Southeast Asia- India, Bangladesh, Pakistan, Korea, China, Taiwan, Vietnam, Cambodia, Thailand, Laos, Indonesia	Humans, pigs	Ingestion of (raw or uncooked) aquatic plants with encysted metacercarie	NK**
Gastrodiscoides hominis, Gastrodiscus	Central and Southeast Asia including India, Bangladesh, Vietnam, Thailand, China, Philippines, Russia	Humans, pigs, deer, rodents	Ingestion of vegetation growing in faecally contaminated water; frogs, tadpoles, crustaceans	NK
Watsonius watsoni **(Paramphistomida)**	Guyana, Africa, Malaysia, Japan	Monkey		
Artyfechinostomum sufrartyfex **(Echinostomatidae)**	Asia, Philippines	Humans, dogs, cats, some wild animals, birds, reptiles	Ingestion of encysted metacercarie in snail, fish, amphibians from faecally contaminated water	NK
Heterophyes spp.	Africa, Turkey, Mediterranean, Far East Asia (Korea, China, Japan, Philippines), Pacific Islands, Australia	Humans, domestic and wild carnivores, fish-eating birds	Ingestion of raw or inadequately cooked fish containing encysted metacercarie	NK
Metagonimus yokogawai **(Heterophyidae)**	Japan, Korea, China, Taiwan, Indonesia, Philippines, Balkans, Spain, Russia			
Liver/ Bile ducts				
Fasciola spp. **(Fasciolidae)**	World wide, human infections in > 60 countries	Cattle, sheep, other herbivores	Ingestion of aquatic plants (water chestnut, lotus tuber, seed pods of water caltrop) with encysted metacercarie	91.1
F. hepatica				
F. gigantica	Africa, Asia (China, India, Pakistan, Bangladesh, Burma, Thailand, Taiwan, Vietnam, Indonesia, Malaysia), Hawaii			NK
Clonorchis sinensis	Southeast Asia	Humans, dogs, cats, pigs, rats, fish-eating carnivores	Ingestion of freshwater fish with metacercariae	601.0 (35.0)
Opisthorchis felineus	Europe, Asia			79.8 (1.2)
O. viverrini **(Opisthorchidae)**				(10.0)
Dicrocoelium spp. **(Dicrocoeliidae)**	World wide	Cattle, sheep, other herbivores	Ingestion of metacercarie in ants	NK
Pancreas				
Eurytrema pancreaticum **(Dicrocoeliidae)**	Asia (Korea, India, China, former USSR, Malaysia), Madagascar, Mauritius, South America	Cattle, sheep, goat, water buffaloes	Ingestion of grasshoppers (infected with sporocysts containing cercariae) by herbivores	NK

Pharyngeal/ Lung/ Blood				
Clinostomum complanatum **(Clinostomatidae)**	Middle East	Fish-eating birds, reptiles	Ingestion of freshwater fish in contaminated water	NK
Paragonimus spp. **(Troglotrematidae)**	Southeast Asia, Philippines, Africa, Pacific Coast of South America	Humans, canids, felids, pigs, wild carnivores	Ingestion of freshwater crustaceans (crabs) containing metacercarie	292.8 (20.7)
Schistosoma spp. **(Schistosomatidae):**	Southeast Asia	Humans, dogs, cats, pigs, cattle, rodents, monkeys	Direct penetration of skin by furcocercous cercariae in contaminated water bodies	NK
S. japonicum				
S. haematobium	Africa, Middle East	Humans		
S. mansoni	Africa, South America, the Caribbean	Humans, rodents		
S. indicum	India	Cattle		

*adapted from Fried et al., 2004; data from Keiser and Utzinger, 2009; **NK- not known.

Table 2. Lesser known (or rarely reported) and emerging trematode infections in humans*

Fluke (Family)	Geographic distribution	Natural reservoirs of infection	2nd intermediate hosts/habitats harbouring infective metacercariae
Paralecithodendrum, (syn.*Prosthodendrium*) *Phaneropsolus,* **(Lecithodendridae)**	Cosmopolitan, in humans- Indonesia, Thailand, Laos	Bats, birds rarely reptiles	Odonate insects
Spelotrema (syn. of *Microphallus*) **(Microphallidae)**	Cosmopolitan, in humans- Phillippines	Birds, mammals	Crustaceans (crabs, shrimp)
Nanophyetus salmincola **(Nanophyetidae)**	Russia, USA	Mammals-Carnivora, Insectivora	Fish (salmon, trout)
Fischoederius spp. **(Gastrothylacidae)**	Africa, China, Malaysia, Singapore	Cattle	Vegetation, aquatic plants
Plagiorchis muris **(Plagiorchiidae)**	Phillippines, Indonesia, Japan, Thailand, Korea		Snails, insect larvae, arthropods
Cotylurus japonicus **(Strigeidae)**	China	Birds	Pulmonate snails
Procerovum spp. **(Heterophyidae)**	China, Japan, Philippines, Australia, Taiwan, India, Thailand	Birds, occasionally mammals	Freshwater fishes
Cercariae of blood flukes and diplostomes		Birds, mammals	Aquatic environment, fish

* adapted from Kumar, 1999; Fried et al., 2004; Hoti and Tandon, 2011

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References

Chai J.Y. 2009. Echinostomes in humans. *In*: Fried B., Toledo, R. (ed.), The Biology of Echinostomes. From the molecule to the community. Springer, New York, pp. 147-183.

Fried B., Graczyk T.K., Tamang L. 2004. Food-borne intestinal trematodiases in humans. Parasitol. Res. 93:159-170.

Fürst T., Keiser J., Utzinger J. 2012. Global burden of human food-borne trematodiasis: a systematic review and meta-analysis. Lancet Infect. Dis. 12:210-221.

Hoti S.L., Tandon V. 2011. Ocular parasitoses and their immunology. 'Review for the Parasitosis Series' of Ocul. Immunol. Inflamm. 19(6):385-396. Doi: 10.3109/09273948.2011.626141.

Keiser J., Utzinger J. 2009. Food-borne trematodiases. Clin. Microbiol. Rev. 22:466-483.

Kumar V. (Ed.). 1999. Trematode Infections and Diseases of Man and Animals. Kluwer, Dordrecht.

Lustigman S., Geldhof P., Grant W.N., Osei-Atweneboana M.Y., Sripa B., Basanez M-G. 2012. A research agenda for helminth diseases of humans: basic research and enabling technologies to support control and elimination of helminthiases. PloS Negl. Trop. Dis. 6:e1445.

Mas-Coma S., Bargues M.D., Valero M.A. 2007. Plant-borne trematode zoonoses: fascioliasis and fasciolopsiasis. In: Murrell K.D., Fried, B. (Ed.), World-class parasites, vol.11. Food-borne parasitic zoonoses. Fish and plant-borne parasites. Springer, New York, pp. 293-334.

Roy B., Tandon V. 2003. *Fasciolopsis buski*. *In*: Miliotis M.D., Bier, J.W. (Ed*.),* International Handbook of Foodborne Pathogens. Marcel Decker, Inc., New York, Basel, pp. 563-570.

Tandon V., Prasad P.K., Chatterjee A., Bhutia P.T. 2007. Surface fine topography and PCR-based determination of metacercaria of *Paragonimus* sp. from edible crabs in Arunachal Pradesh, Northeast India. Parasitol. Res.102:21-28. Doi 10 1007/s 00436-007-4.

Tandon V., Goswami L.M., Prasad P.K., Chatterjee A. 2009. Molecular characterization of intestinal fluke, *Artyfechinostomum sufrartyfex* (Trematoda: Echinostomatidae) using PCR amplification of rDNA ITS regions. In: Tandon V., Yadav A.K., Roy B. (Ed.). Current Trends in Parasitology Proceedings of the 20th National Congress of Parasitology, India; November 3-5, 2008. Panima Publishing Corporation, New Delhi, pp. 153-162.

PROTEOMIC ANALYSES OF *DIROFILARIA* SPECIES

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Introduction

Dirofilariosis is a vector borne transmitted disease caused by different species of the genus *Dirofilaria*. Among these, the most important are *D. immitis* and *D. repens*, respectively responsible of cardiopulmonary and subcutaneous canine and feline dirofilariosis. Moreover, both species can infect the human host, in which they cause pulmonary (*D. immitis*) and subcutaneous/ocular (*D. repens*) dirofilariosis, characterized by the appearance of benign pulmonary and subcutaneous nodules that can be confused with lung or cutaneous tumors (Simón et al., 2005; McCall et al., 2008).

Both the development of *D. immitis* and *D. repens* and their clinical implications are different in each of the hosts. *D. immitis* can live more than 7 years in the blood stream of dogs, producing microfilariae, while in cats, the adult worms live not more than 2 years causing amicrofilaremic infections. Moreover, the disease has a commonly chronic development in dogs but it presents an unpredictable and generally acute course in cats. In man only immature *D. immitis* worms in different decomposition stage, have been found, while live adult *D. repens* worms have been observed in near the 50% of reported subcutaneous/ocular cases. Some of these worms were mature female presenting microfilariae in the uterus. Moreover, at least 3 microfilaremic cases attributed to *D. repens* or *Dirofilaria* spp. have been reported until now (Genchi et al., 2011; Simón et al., 2012).

The knowledge of both the proteic composition and the molecular parasite/host relationships in dirofilariosis are scarce. The protein data base of the National Center for Biotechnology Information (NCBI) contains 119 results for *D. immitis*, a low number when comparing to other nematodes in which many molecular studies have been developed (*Caenorhabditis elegans*: 58336 results; *Brugia malayi*: 23263 results), but it is quite similar to other filarial species (*Onchocerca volvulus*: 270 results; *Wuchereria bancrofti*: 114 results), while there are only 8 results for *D. repens*.

In this context, the objective of present review is to increase and organize the information about the proteic composition of *D. immitis* and *D. repens* and to correlate it with the *Dirofilaria*/hosts relationship mechanisms. This can contribute to determine if, in parallel to the biological and clinical differences, there exist also differences among these two species at molecular level. The basic methodology needs the development of proteomes and immunomes by two-dimensional electrophoresis and Western blot and the identification of proteins by mass spectrometry. These techniques have allowed us to develop comparative studies of the antigenic recognition patterns of *D. immitis* in their different hosts as well as the comparison of the recognition patters of *D. immitis* and *D. repens* antigens by human hosts. Moreover, the same methodology has been applied to identify proteins with a specific function, relevant for the *Dirofilaria*/host relationships.

I. Identification of immunogenic proteins of *D. immitis* recognized by sera from their different hosts (dogs, cats and humans)

The proteome of *D. immitis* was obtained by two-dimensional electrophoresis of the soluble antigen extract of adult worms of *D. immitis* (DiSB). Proteins were stained with silver nitrate or transferred to nitrocellulose membranes for their immunoblot analysis, in which proteins were incubated with pools of sera from the different infected hosts or with healthy controls. The spots containing immunogenic proteins were manually cut off from the 2-D gels and sent to the Unit of Proteomics of the Centro Nacional de Investigaciones Cardiovasculares (Madrid, Spain) for their identification by mass spectrometry. Two-dimensional gels revealed more than 400 spots in the proteome of *D. immitis*, most of them with isoelectric points (pIs) between 5 and 8 and with a broad range of molecular weights (MWs) (10-170 kDa). The 2D-Western blot respectively revealed, in the case of analysis with sera from dogs, cats and humans with dirofilariosis, a total of 48, 105 and 66 spots of *D. immitis*, of

which 37, 72 and 61 were identified and corresponded to 19, 32 and 23 proteins. Then, the molecular function and biological process involvement of the identified proteins were assigned according to the Gene Ontology database (http://www.geneontology.org) and the Swiss-Prot/UniProt database (http://beta.uniprot.org).

The majority of immunogenic proteins identified belong to three functional groups, which are critical in parasite/host relationships on dirofilariosis: heat shock proteins (HSPs), energy metabolism and redox enzymes. Within these functional groups, cats recognize more proteins than humans, and humans recognize more proteins than the canine host (Figure 1) (Oleaga et al., 2009; González-Miguel et al., 2010a; 2010b).

II. Identification of immunogenic proteins of *D. immitis* and *D. repens* recognized by sera of patients with pulmonary or subcutaneous human dirofilariosis

The aim of this study was to identify immunogenic proteins of *D. immitis* and *D. repens* differentially recognized by serum samples from patients with pulmonary or subcutaneous dirofilariosis, using the same methodology as in the previous section. Pools of sera from patients with pulmonary and subcutaneous dirofilariosis and somatic antigen from adult worms of *D. immitis* and *D. repens* were used.

After developing the proteomes of both species a great similarity between them in number and distribution of spots was observed. Immunoblot analysis allowed us to identify a total of 23 *D. immitis* and 15 *D. repens* immunoreactive proteins. Among others, different isoforms of 6 enzymes involved in glycolysis, 3 redox proteins with antioxidant capacity and 3 heat shock proteins were recognized in the proteome of *D. immitis* by individuals with pulmonary nodule. Individuals with subcutaneous nodules recognized in the proteome of *D. repens* only 3 glycolytic enzymes, a protein involved in redox processes and a heat shock protein (Figure 1) (González-Miguel et al., 2010b).

III. Identification of plasminogen-binding proteins in the excretory/secretory products of *D. immitis*

The aim of this study was to investigate the interaction between the excretory/secretory products of *D. immitis* (DiES) and the fibrinolytic system of the host. As previously mentioned, *D. immitis* can survive for long periods of time (7 years or more) in the circulatory system of immunocompetent reservoirs, usually producing a chronic vascular disease of inflammatory nature. Moreover, the simultaneous death of groups of adult worms can trigger acute conditions characterized by the exacerbation of inflammatory reactions and the generation of thromboembolisms of varying magnitude. We hypothesized that, as in other hematologic parasites, metabolic products excreted by *D. immitis* contain molecules that control the formation of thrombi, managing to establish a net anti-thrombotic status, facilitating parasite survival. This equilibrium would shift toward a pro-thrombotic net status when death occurs in adult worms and the consequent massive exposure to its somatic antigens. The plasminogen fibrinolytic system plays a major role, being in charge of generating plasmin to remove clots. The fact that DiES extract binds plasminogen and that plasmin is generated in the presence of tissue plasminogen activator (t-PA) was demonstrated. Moreover, we established that DiES extract enhances t-PA expression in cultured vascular endothelial cells. Subsequently, we identified parasite plaminogen-binding proteins using the same methodology described above: 1) Development of the proteome of the excretory/secretory proteins of *D. immitis* by 2D-electrophoresis, 2) immunolocalization of the antigens from DiES extract capable of binding plasminogen performed by 2D-Western blotting, 3) identification of a total of 10 plasminogen-binding proteins in the DiES extract using mass spectrometry (HSP60, actin-1/3, actin, actin 4, transglutaminase, GAPDH, Ov87, LOAG_14743, galectin and P22U) (González-Miguel et al., 2012).

Conclusions

The results obtained after the comparison of the *D. immitis* and *D. repens* proteomes, the recognition patterns from the different hosts and the identified immunogenic proteins suggest the existence of differences at the molecular level in the *Dirofilaria* sp/host relationships. This fact could be related to the clinical and biological differences described above. The shortening of the life cycle of the worms of *D. immitis* in cats and humans, with respect to what happens in the dog, could be related to a greater ability of the feline and human hosts to block some key parasite proteins involved in energy generation mechanisms and evasion of the immune response with specific antibodies. Similarly, in human infections there are a larger number of proteins recognized by *D. immitis* than *D. repens*,

related to parasite survival processes like energy generation, struggle against oxidative stress and molecular repair. This could help to explain the differences in the development capacity of *D. immitis* and *D. repens* and the different frequency of appearance of lung or subcutaneous nodules in human hosts.

Finally, the application of proteomic techniques has allowed us to identify plasminogen-binding proteins. Data from this study suggest that proteins present in the DiES extract interact with the intravascular environment of *D. immitis* regulating the activation of the fibrinolytic system of the host. This fact could allow the parasite to control the formation of thromboembolisms, promoting their survival in the host's circulatory system, reducing damage to the host.

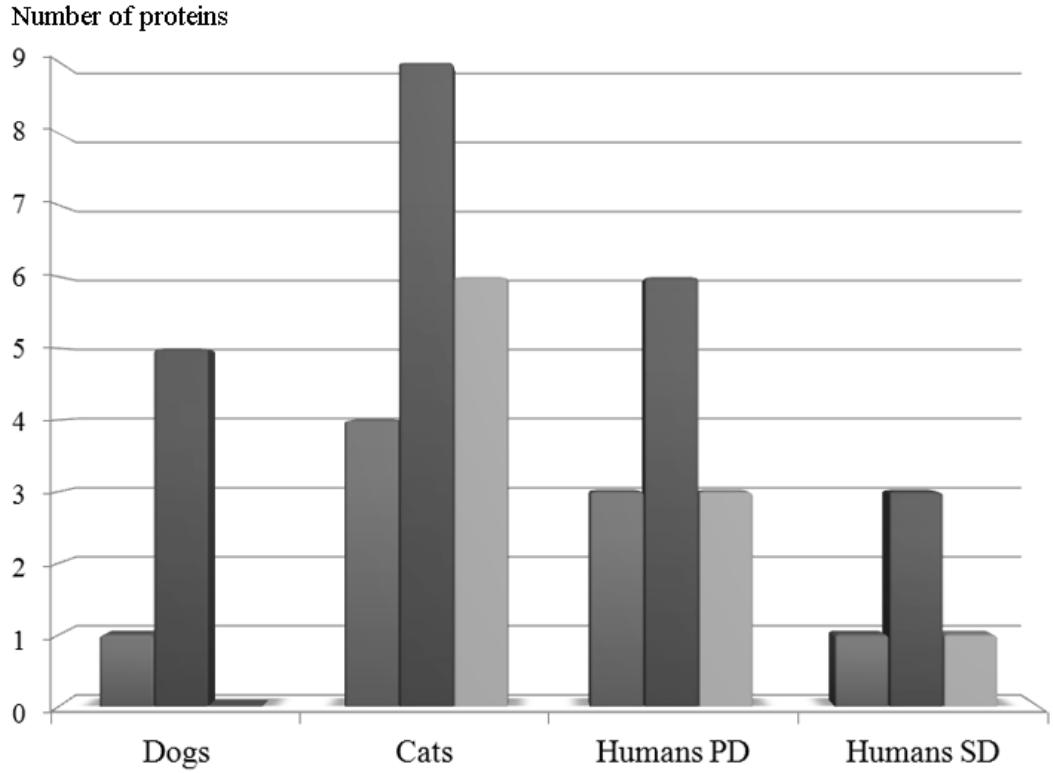

Figure 1. Comparison of the number of immunogenic proteins of the DiSB extract from *D. immitis* and of the DrSB extract from *D. repens* differentially recognized by serum samples from dogs, cats and humans infected with *Dirofilaria immitis* or by serum samples from humans infected with *Dirofilaria repens*. The immunogenic proteins are related to stress response (blue bars), energetic metabolism (red bars) or redox processes (green bars), according to Gene Ontology and Swiss-Prot/UniProt databases. Humans PD: humans with pulmonary dirofilariosis caused by *Dirofilaria immitis*; Humans SD: humans with subcutaneous dirofilariosis caused by *Dirofilaria repens*.

References

Genchi C., Kramer L.H., Rivasi F. 2011. Dirofilarial infection in Europe. Vector Borne Zoonotic Dis. 11:1307-1317.

González-Miguel J., Morchón R., Mellado I., Carretón E., Montoya-Alonso J.A., Simón F. 2012. Excretory/secretory antigens from *Dirofilaria immitis* adult worms interact with the host fibrinolytic system involving the vascular endothelium. Mol. Biochem. Parasitol. 181:134-140.

González-Miguel J., Morchón R., Siles-Lucas M., Oleaga A., Simón F. 2010a. Identification of *Dirofilaria immitis* immunoreactive proteins recognized by sera from infected cats using two-dimensional electrophoresis and mass spectrometry. Mol. Biochem. Parasitol. 174:78-82.

González-Miguel J., Rosario L., Rota-Nodari E., Morchón R., Simón F. 2010b. Identification of immunoreactive proteins of *Dirofilaria immitis* and *D. repens* recognized by sera from patients with pulmonary and subcutaneous dirofilariosis. Parasitol. Int. 59:248-256.

McCall J.W., Genchi C., Kramer L.H., Guerrero J., Venco L. 2008. Heartworm disease in animals and humans. Adv. Parasitol. 66:193-285.

Oleaga A., Pérez-Sanchéz R., Pagés E., Marcos-Atxutegi C., Simón F. 2009. Identification of immunoreactive proteins from the dog heartworm (*Dirofilaria immitis*) differentially recognized by the sera from dogs with patent or occult infections. Mol. Biochem. Parasitol. 166:134-141.

Simón F., López-Belmonte J., Marcos-Atxutegi C., Morchón R., Martín-Pacho J.R. 2005. What is happening outside North America regarding human dirofilariasis? Vet. Parasitol. 133:181-189.

Simón F., Siles-Lucas M., Morchón R., González-Miguel J., Mellado I., Carretón E., Montoya-Alonso J.A. 2012. Human and animal dirofilariasis. The emergence of a zoonotic mosaic. Clin. Microbiol. Rev. In press.

THE HISTORY OF *WOLBACHIA* ENDOSYMBIOSIS IN FILARIAL WORMS

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One hundred years ago, Paul Buchner, the father of symbiosis researches, described a remarkable array of both bacterial and fungal endosymbionts associated to arthropods (Buchner, 1912). *Wolbachia* was first identified as a rickettsia-like microorganism in *Culex pipiens*, a mosquito species by Hertig and Wolbach in 1924 (Hertig and Wolbach, 1924). Hertig formally described the genus in 1936 as *Wolbachia pipientis*.
Wolbachia pipientis actually encompasses a group of intracellular microorganisms found in arthropods, ascribed to the α-2 proteobacteria and belongs to the order of Ricketttsiales (Weiss and Dasch, 1992). In arthropods, *Wolbachia* infection is generally associated with alterations of host reproduction (Warren, 1997), including killing of male embryos, feminization of genetic males, parthenogenesis, and cytoplasmic incompatibility (CI). CI can be described as a way by which *Wolbachia* sterilizes those females that do not carry the bacterium by modifying the sperm of infected males. Because *Wolbachia* is transmitted through females, by inducing CI it increases its own fitness: infected females can mate successful with both infected and uninfected males and they will thus always transmit the infection, whereas uninfected females can mate successfully only with uninfected males (for review Bandi et al., 2001; Genchi et al., 2003).
The history of bacterial endosymbiosis in filarial worms begins more than 30 years ago, when Kozek (1971), and then Harada et al. (1975) and Lee (1975), revealed the presence of dense bodies within the reproductive organs and microfilariae of worms by ultrastructural studies. Later, McLaren et al. (1975) observed intracellular, gram-negative microorganisms, in larval stages and adults of *Dirofilaria immitis* and *Brugia phangi,* and two years later Kozek (1977) found that such microorganisms were transovarially-transmitted in adult and larval stages of *Brugia malayi*. In 1995, these microorganisms were identified as bacteria belonging to the genus *Wolbachia* by a team of scientists at Milan University working on *D. immitis* (Sironi et al., 1995). Subsequently, *Wolbachia* has been observed in representatives of several filarial pathogenic species and genera (including *Dirofilaria*, *Onchocerca, Brugia,* and *Wuchereria)* (Casiraghi et al., 2004). These endosymbiont bacteria are philogenetically close to rickettsiae and are transovarial transmitted to microfilariae.
The discovery of *Wolbachia* endosymbiosis on filarial worms opened the way to a new understanding of pathogenesis and immune response of filarial diseases in humans and animals. In fact, as a gram-negative bacterium, *Wolbachia* has the potential to play an important role in the pathogenesis and immune response to filarial diseases. The immunopathology of filarial disease is extremely complex and the clinical manifestations of infection are strongly dependent on the type of immune response elicited by the parasite. Furthermore, the fact that adult parasites can survive for years in otherwise immunecompetent hosts is likely due to the parasite's ability to avoid/modulate the immune response (for review McCall et al., 2008). Following the discovery of the bacterial nature of filarial endosymbionts and that tetracycline treatment was able to deplete bacteria from the worms leading to sterility of females, death of microfilariae and eventually death of adults (Genchi et al.,1998; Bandi et al. 1999), *Wolbachia* has been the subject of intense research not only for its role in the reproduction and survival of the filarial worms, but also for the possible role of contact between the filarial infected host and these gram-negative bacteria (see for review Taylor et al., 2005).
In this story, however, we do not have to forget that who firstly opened the way to such a studies was John W. McCall and colleagues, who in 1971, because of an serious outbreak of staphylococcal dermatitis affecting the filarial infected and non-infected Mongolian jirds (*Meriones unguiculatus*), observed that tetracycline in drinking water was able to prevent the development to adult stage of *Brugia pahangi* and *Litomosoides sigmodontis* in gerbils, but not of *Acanthocheilonema vitae* (McCall et al., 1999). In fact, more recent studies of the distribution and phylogeny of *Wolbachia* endosymbionts in filarial nematodes have shown that *A. vitae* is uninfected and that *B. pahangi* and *L. sigmodontis* are infected (Bandi et al., 1998; Casiraghi et al., 2001). It suggested that the activity of tetracycline on the latter two species was related to the presence of *Wolbachia*. The results were presented at the 48th Annual Meeting of the American Society of Parasitologists (held in Toronto,

Canada 25-29 June 1973), but none of this data was published until the end of '90s (McCall et al., 1999). McCall's empirical data were later confirmed by Genchi et al. (1998) and Bandi et al. (1999), which analyzing the uterine content of adult female of *D. immitis* from dogs with patent infection and treating them with tetracycline, observed inhibition of embryogenesis. Currently, the use of doxycycline in animals and humans was found to be able to inhibit microfilaria production by adult female filarial worms (Langworthy et al., 2000; Tamarozzi et al., 2011) and dogs when combined with ivermectin (Genchi et al., 2003; Bazzocchi et al., 2008). Furthermore, Grandi et al. (2010) have shown that doxycycline combined with ivermectin have an adulticide effect in naturally infected dogs and decrease the risk of thrombosis caused by the inflammatory reaction mediated by the endosymbiont bacteria released by dyeing worms (Taylor et al., 2005).

To note that the molecular methods currently employed in symbiosis studies have allowed to acquire new data about arthropods and its symbionts. Recently, an intracellular bacterium with the unique ability to enter mitochondria has been discovered in *Ixodes ricinus* (Beninati et al., 2004). Based on 16S rRNA and gyrB gene sequences, as well as electron microscopy (EM), in situ hybridization and other observations that confirmed the phylogenetic position of the bacterium as a divergent lineage within the Rickettsiales (Alphaproteobacteria) the name "Candidatus *Midichloria mithochondrii*" was proposed (Beninati et al., 2004; Sassera et al., 2006; Epis et al., 2008). The symbiont appears to be ubiquitous in females of *I. ricinus* across the tick's populations, while lower prevalence is observed in males. PCR and sequencing results have shown the presence of a related bacterium from two human patients (Mediannikov et al., 2004). Furtermore, amplification and sequencing of 16S rRNA showed high identity with *M. mithocondrii* in 36% of dog blood samples (Mariconti et al., 2010) and Richard et al. (2009) have identified the bacterium in bed bugs (*Cimex lectularius*), well known human parasites. These results bring for the question of whether this bacterium infects humans and mammals and whether it might be responsible for any clinical or sub-clinical pathology. Interestingly, in a preliminary study antibodies against a flagellar protein from *Midichloria* were found in more than 80% of sera from *Borrelia burgdorferi* infected patients with a positive history of *Ixodes ricinus* infestation (Mariconti, 2012, unpublished data).

References

Bandi C., Anderson T.J., Genchi C., Blaxter M.L. 1998. Phylogeny of *Wolbachia* in filarial nematodes. Proc. Biol. Sci. 265:2407-2413.

Bandi C., Dunn A.M., Hurst G.D., Rigaud T. 2001. Inherited microorganism, sex-specific virulence and reproductive parasitism. Trends Parasitol. 17:88-94.

Bandi C., McCall J.W., Genchi C., Corona S., Venco L., Sacchi L., 1999. Effects of tetracycline on the filarial worms *Brugia pahangi* and *Dirofilaria immitis* and their bacterial endosymbiont *Wolbachia*. Int. J. Parasitol. 29:357-364.

Bazzocchi C., Mortarino M., Grandi G., Kramer L.H., Genchi C., Bandi C., Genchi M., Sacchi L., McCall J.W. 2008. Combined ivermectin and doxycycline treatment has microfilaricidal and adulticidal activity against *Dirofilaria immitis* in experimentally infected dogs. Int. J. Parasitol. 38:1401-1410.

Beninati T., Lo N., Sacchi L., Genchi C., Noda H., Bandi C. 2004. A novel alpha-proteobacterium resides in the mitochondria of ovarian cells of the tick *Ixodes ricinus*. Appl. Environ. Microbiol. 70:2596-2602.

Buchner P. 1912. Studien an intracellulären Symbionten. Arch. Protistenk. 26:1-116.

Casiraghi M., Anderson T.J., Bandi C., Bazzocchi C., Genchi C. 2001. A phylogenetic analysis of filarial nematodes: comparison with the phylogeny of *Wolbachia* endosymbionts. Parasitology 122:93-103.

Casiraghi M., Bain O., Guerrero R., Martin C., Pocacqua V., Gardnerd S.L., Franceschi A., Bandi C. 2004. Mapping the presence of *Wolbachia pipientis* on the phylogeny of filarial nematodes: evidence for symbiont loss during evolution. Int. J. Parasitol. 34:191-203.

Epis S., Sassera D., Bennati T., Lo N., Beati L., Piesman J., Rinaldi L., Mccoy KD., Torina A., Sacchi L., Clementi E., Genchi M., Magnino S., Bandi C. 2008. *Midichloria mitochondrii* is widespread in hard ticks (Ixodidae) and resides in the mitochondria of phylogenetically diverse species. Parasitology 135:485-494.

Genchi C., Mortarino M., O'Neill S.L. 2003. *Wolbachia* endosymbionts: implications for the control of filarial diseases and arthropod vectors. J. Parasitol. 89(suppl. 1):S236-S244.

Genchi C., Sacchi L., Bandi C., Venco L. 1998. Preliminary results on the effect of tetracycline on the embryogenesis and symbiotic bacteria (*Wolbachia*) of *Dirofilaria immitis*. An update and discussion. Parassitologia 40:247-249.

Grandi G., Quintavalla C., Mavropoulou A., Genchi M., Gnudi G., Bertoni G., Kramer L., 2010. A combination of doxycycline and ivermectin is adulticidal in dogs with naturally acquired heartworm disease (*Dirofilaria immitis*). Vet. Parasitol. 11:347-51.

Harada R., Maeda T., Nakashima A., Sadakata M., Ando M., Yonomine K., Otsuji Y., Sato H. 1970. Electronmicroscopical studies on the mechanism of oogenesis and fertilization in *Dirofilaria immitis*. In: Sasa M. (Ed.), Recent advancement in researchers on filariasis and schistosomiasis in Japan. University Press, Baltimore, MD, pp. 99-121.

Hertig, M. 1936. The rickettsia *Wolbachia pipientis* (gen. et. sp. n) and associated inclusions of the mosquito, *Culex pipiens*. Parasitology 28:453-486.

Hertig M., Wolbach S.B. 1924. Studies on rickettsia-like micro-organisms in insects. J.Med. Res. 44:329-374.

Kozek W.J. 1971. Untrastructure of the microfilaria of *Dirofilaria immitis*. J. Parasitol. 57:1052-1067.

Kozek W.J. 1977. Transovarially-transmitted intracellular microorganisms in adult and larval stages of *Brugia malayi*. J. Parasitol. 63:992-1000.

N., Renz A., Meckenstedr U., Henkle-Duhrsen K., Bronvoort M., Tanya V., Donnelly M., Trees A.J. 2000. Macrofilaricidal activity of tetracycline against the filarial nematode *Onchocerca ochengi*: elimination of *Wolbachia* precedes worm death and suggests a dependent relationship. Proc. R. Soc. London Series B 267:1063-1069.

Lee C.-C., 1975. *Dirofilaria immitis*: ultrastruttural aspects of oocyte development and zygote formation. Exp. Parasitol. 37:449-469.

McCall J.W., Genchi C., Kramer L.H., Guerrero J., Venco L. 2008. Heartworm disease in animals and humans. Adv. Parasitol. 66:195-285

McCall J.W., Ju J.J., Bandi C. 1999. *Wolbachia* and antifilarial properties of tetracyclines. An untold story. Ital. J. Zool. 66:7-10.

McLaren D.J., Worms M.J., Laurence B.R., Simpson M.G. 1975. Micro-organisms in filarial larvae (Nematoda). Trans. R. Soc. Trop. Med. Hyg. 69:509-514.

Mariconti M., Bazzocchi C., Epis S., Rinaldi L., Pistone D., Sassera D., Bandi C. 2010. *Midichloria mithocondrii*: a novel infection agent for vertebrates. Parassitologia 52: 206.

Mediannikov O.I., Ivanov L.I., Nishikawa M., Saito R., Sidel'nikov I.N., Zdanovskaia N.I., Mokretsova E.V., Tarasevich I.V., Suzuki H. 2004. Microorganism 'Montezuma' of the order *Rickettsiales*: the potential causative agent of tick-borne disease in the Far East of Russia. Zhurn. Mikrobiol. Epidemiol. Immunobiol. 1:7-13.

Richard S., Seng P., Parola P., Raoult D., Davoust B., Brouqui P. 2009. Detection of a new bacterium related to "Candidatus *Midichloria mithocondrii*" in bed bugs. Clin. Microbiol. Infect. 15(suppl. 2): 8485.

Sassera D., Beninati T., Bandi C., Bouman E.A., Sacchi L., Fabbi M., Lo N. 2006. 'Candidatus *Midichloria mitochondrii*', an endosymbiont of the tick *Ixodes ricinus* with a unique intramitochondrial lifestyle. Int. J. Syst. Evol. Microbiol. 56:2535-2540.

Sironi M., Bandi C., Sacchi L., Di Sacco B., Damiani G., Genchi C.1995**.** A close relative of the arthropod endosymbiont *Wolbachia* in a filarial worm. Mol. Biochem. Parasitol. 74: 223-227.

Tamarozzi F., Halliday A., Gentil K., Hoerauf A., Pearlman E., Taylor M.J. 2011. Onchocerciasis: the role of *Wolbachia* bacterial endosymbionts in parasite biology, disease pathogenesis, and treatment. Clin. Microbiol. Rev. 24:459-468.

Taylor M.J., Bandi C., Hoerauf A. 2005. *Wolbachia* bacterial endosymbionts of filarial nematodes. Adv. Parasitol., 60:248-286.

Warren J.H. 1997. Biology of *Wolbachia*. Ann. Rev. Entomol. 42:587-609.

Weiss E., Dasch G,A. 1992. Introduction to the Rickettsiales and other parasitic or mutualistic prokaryotes. In: Balows A., Trüper H.G., Dworkin M., Harder W., Schleifer K.-H. (Ed.), The Prokaryotes, Springer-Werlag, New York, pp. 2401-2406.

DIROFILARIOSIS IN RUSSIA – PAST AND PRESENT

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Dirofilariasis is a vector borne transmitted diseases caused by species of the genus *Dirofilaria*. The most important *Dirofilaria* species – *D. immitis* and *D. repens*- are responsible for canine and feline dirofilariasis worldwide. *D. immitis* causes the most serious and potentially fatal disease, cardiopulmonary dirofilariasis, while *D. repens* produces subcutaneous dirofilariasis, a less serious disease in most cases. Where canine dirofilariasis exists, there also exists human dirofilariasis. In humans, *D. immitis* causes pulmonary dirofilariasis and *D. repens* causes subcutaneous/ocular dirofilariasis. In Europe, both *D. immitis* and *D. repens* infect canine populations of the Southern Mediterranean countries. Moreover, a dramatic spreading of *D. repens* from these territories to the northern areas is currently detected. On the other hand, human subcutaneous/ocular dirofilariasis caused by *D. repens* predominates over pulmonary dirofilariasis in the European population. In fact, dirofilariasis is currently considered as an emerging disease even in countries where the disease was not previously reported.

Veterinarians from Russia and neighborhood Ukraine noticed sharp increase of dirofilariasis morbidity and mortality in the dog population during the last decade. Recognition and clinical diagnosis of canine dirofilariasis is difficult, even in endemic regions, because it can be asymptomatic for a long time. It may be not recognized at its early stage or in the case of low concentration or absence of microfilariae in the blood. Data on canine dirofilariasis in Russia are scarce and scattered. Specific examination of guard/patrol police dogs training kennel in Ufa (Bashkortostan, Russia), in the dog training Centre of the Russian Ministry of Internal Affairs was performed. During the period 2009-2011, 70 (14%) out of 500 dogs arrived from more than 20 different Russian territories to the training school, were infected. The average rate of dirofilariasis in dogs is around 25-30% in the Republic of Bashkortostan but in the case of stable cage housing of the dogs and a limited kennel area especially if it is close to open water and in absence of preventive measures, the prevalence reaches 90%. In the Lower and Middle Volga region the dog infection reaches 16% and is characterized by year-round disease of dogs with remarkable seasonal epizootic additives in the April-June and October-November. In some regions, dirofilariasis is now considered a highly endemic animal disease. Overall rates of microfilaremic dogs in a highly endemic town near Rostov – Novocherkassk, increase from 38.5% in 2009 to 50% in 2011. At the same time, in the neighborhood Shakhty city less than 5% of dogs are infected. To study circulated *Dirofilaria* species we examined 76 dogs from Rostov city. *D. repens* was detected in 34 dogs (44.7%) and *D. immitis* in 23 dogs (30.3%). Mixed infections (*D. repens* + *D. immitis*) were found in 19 dogs (25%). So the rate of dogs infected by *D. immitis* alone or by its combination with *D. repens* was 55.3%. Autopsy study of 21 dogs that died as a result of a massive invasion of *D. immitis* showed that 11 out of 21 animals (52%) were co-infected with *D. repens*.

According to the Kiev (Ukraine) State Administration of Veterinary Medicine, 15 infected dogs were registered in 1999 but in 2000 130 cases were diagnosed. In the last decade in Ukraine, a consistent trend to increasing number of canine *Dirofilaria* infections was noticed. In only one veterinary clinic located in Kiev 73 cases of canine dirofilariasis were diagnosed in 2009-2010. Most infected animals were from Kiev but some dogs travelled with their owners to Kiev from other Ukraine territories. In 2010 a network of veterinary clinics from Kiev carried out a campaign: for all the owners that made blood tests for their pets they offered to make free test for dirofilariasis. The results of that study

showed that more than 50% of dogs were infected but their owners were even not been informed about this disease. Since 1999 the laboratory examination of dogs for dirofilariasis was planning to be obligatory for all the veterinary clinics of Kiev.

Different mosquitoes species incriminated in *Dirofilaria* spp. transmission in other countries, belonging to the genera *Aedes*, *Culex* and *Anopheles,* are also present in Russia. Reported prevalences of *Dirofilaria* larvae in mosquitoes of the genus *Aedes* varies from 7% to 17%, *Culex* 7%-14% and *Anopheles* 3%-6%.

Nothing was known about human dirofilariasis in Russia until 1915 when the first case was diagnosed in Krasnodar by Dr. Vladimirsky. In 1948 that first case was presented by Skryabin. In total 8 human cases (7 of them with ocular location) were reported between 1915 and 1927. Cumulative number of cases was 15 until 1956, but in 1996 already 113 human cases were published in the literature. The total number of reported cases in Russia reached 564 in 2008 and then 750 in 2011. New autochthonous cases were repeatedly reported from the northern Russian territories and from Siberia territory in the last times. Around 70% of patients are females of middle age. The rate of ocular location is around 50%. Nearly all the cases were subcutaneous but in 2002, 2 pulmonary cases from Rostov and in 2010, 1 pulmonary case from Moscow, were recognized. It should be noticed that human dirofilariasis has not been included in the list of diseases of obligatory declaration in Russia, so not all the cases are reported in publications. At the neighboring Ukraine the strict notification of every human case was introduced since 1996. Until that time only 17 cases were published since 1927. In the first 5 years (1996-2000) 41 human cases were listed; in the next 5 years (2001-2005) the number of reported cases reached 356 and in the last 5 years (2006-2010) there were 576 registered cases. The total number of human cases identified between 1996 and 2010 was 973. Considering that the nature and climate condition in the European part of Russia and Ukraine are similar it means that the real morbidity in Russia can be underestimated. Our study of anti-*Dirofilaria* antibodies prevalence among a random population living in Rostov Oblast (317 healthy blood donors) showed a seroprevalence of 10.4%. Taking into account that the population of Rostov Oblast is around 4 million inhabitants, it means that at least 400000 people are at risk of infection. Moreover, seroprevalence was 19% in the policemen professionally working in close contact with dogs in Rostov. This suggests that the close and continuous relationship with dogs in this area is a very important risk factor.

We have developed a GIS model to mapping the potential risk of dirofilariasis transmission in the territory of Russia and the neighboring countries of the former Soviet Union. Our model is based on the zoning maps for the historical climate layers that were taken from the Interactive Agricultural Ecological Atlas of Russia and the former USSR (Afonin et al, 2008). The calculation was based on estimated yearly average predicted number of *Dirofilaria* larvae generations (Genchi et al., 2005, 2009). The main factor affecting the full development of microfilariae in mosquito vectors is the accumulation of amounts of external effective temperatures above 130 °C with an effective temperature of 14 °C (Fortin and Slocombe, 1981). We took into account that estimated maximum life span of an infected mosquito is not more than 30 days (Slocombe et al., 1989; Lok and Knight, 1998). The northern boundary of potential *Dirofilaria* spread is a zone of low-risk with low summer temperature, where optimal conditions appear from time to time. In the southern regions within isolated arid areas with dry climate, the risk of dirofilariasis transmission is very high but it is patchy in nature. Such kind of focuses caused by mosquito replication only at areas that are close to the open water basins. Because these territories are highly populated, the risk of dirofilariasis spreading is generally high. In accordance with the mapping, the northern boundary of stable risk of dirofilariasis transmission is at latitude 55-57°. This boundary stretches from the west to the east across Belarus, Moscow and Nizhny Novgorod regions, crosses the southern part of Perm region, and then passes through Kurgan, Omsk, Novosibirsk Region and Altai Territory. Eastern regions, such as Chita, Amur Region, Khabarovsk and Primorsky Krai also belong to the zone of stable risk of dirofilariasis. The number of possible generations of microfilariae varies from 1 at the northern boundary to 19 at the south. This prediction model correlates well with the fragmentary reports on the incidence of canine and human dirofilariasis from those territories.

In conclusion it should be that dirofilariasis is an emerging disease in human and dog populations of Russia that is spreading to the North probably because of changing climate conditions that favors its vector transmission. Our model can be used to assess the risk of dirofilariasis as a basis for planning more detailed regional epidemiological situations.

References

Afonin A.N., Greene S.L., Dzyubenko N.I., Frolov A.N. (eds.). 2008. Interactive Agricultural Ecological Atlas of Russia and Neighboring Countries. Economic Plants and their Diseases, Pests and Weeds. As accessed in 2008 from http://www.agroatlas.ru.

Fortin J.F., Slocombe J.O.D. 1981. Temperature requirements for the development of *Dirofilaria immitis* in *Aedes triseriatus* and Ae.vexans. Mosq. News. 41:625-633.

Genchi C., Rinaldi L., Cascone C., Mortarino M., Cringoli G. 2005. Is heartworm disease really spreading in Europe? Vet. Parasitol. 133(2-3):137-148.

Genchi C., Rinaldi L., Mortarino M., Genchi M., Cringoli G. 2009. Climate and Dirofilaria infection in Europe. Vet. Parasitol. 163(4):286-292.

Lok J.B., Knight D.H. 1998. Laboratory verification of a seasonal heartworm model. Proceedings of the Heartworm Advances in Heartworm Disease Symposium, pp. 15-20.

Slocombe J.O.D., Surgeoner G.A., Srivastava B. 1989. Determination of heartworm transmission period and its use in diagnosis and control. Proceedings of the Heartworm Symposium, pp. 19-26.

WOLBACHIA IN *DIROFILARIA*

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Dirofilaria immitis, the causative agents of canine and feline heartworm disease and *D. repens*, the agent of subcutaneous dirofilariosis, harbour intracellular bacteria named *Wolbachia pipientis*. Indeed, most filarial species studied so far, with very few exceptions, contain these microorganisms which are thought to play an essential role in the biology and reproductive functions of their filarial hosts. When Sironi et al. (1995) demonstrated that *Dirofilaria immitis* harbours *Wolbachia*, the scientific community realized that a major discovery had been made, one that would likely change the way we look at filarial disease. Indeed, as gram-negative bacteria, *Wolbachia* have the potential to play an important role in the pathogenesis and immune response to filarial infection.

Wolbachia pipientis, the only species thus far identified in the genus, are gram-negative bacteria belonging to the order Rickettsiales. They are closely related to other bacteria belonging to the same group, such as *Ehrlichia* spp. and *Anaplasma* spp. (Bandi et al., 2001). Electron microscopy, histology and immunohistochemistry have offered a clear description of the distribution of *Wolbachia* in *D. immitis* and *D. repens* (Kramer et al., 2003; Kozek, 2005; Grandi et al., 2008). They are found throughout all the stages of the life cycle of the nematode although they occur in varying proportions between individual worms and different developmental stages. In adult *D. immitis*, *Wolbachia* is predominantly found throughout the hypodermal cells of the lateral cords. In females, *Wolbachia* is also present in the ovaries, oocytes and developing embryonic stages within the uteri. They have not been demonstrated in the male reproductive system, suggesting that the bacterium is maternally transmitted through the cytoplasm of the egg and not through the sperm. *Wolbachia* is necessary for the reproduction and long-term survival of those filarial worms that harbour it.

In hosts that are infected with filarial nematodes harboring *Wolbachia*, the bacteria are released following worm death through larval molt, natural attrition, microfilarial turnover and pharmacological intervention. Interaction between *Wolbachia* and the humoral immune system has been reported in dogs and cats with *D. immitis.* Circulating antibodies against the *Wolbachia* Surface protein (WSP) has been shown in dogs and cats in both experimental and natural infection models (Morchón et al., 2004; Dingham et al., 2010). The immune response to *Wolbachia* antigens can be detected as early as 2 months after infection in experimentally infected cats, before detection of specific antibodies against *D. immitis* antigens, suggesting its diagnostic potential (Morchón et al., 2004). It has recently been reported that anti-WSP antibodies are also present in the urine of infected dogs, suggesting that bacterial-derived antigens may play a role in immune-complex glomerulonephritis (Morchon et al., 2012).

It is also known that *Wolbachia* is both highly pro-inflammatory and immuno-regulatory (McCall et al., 2008). Thus, while during patent infection it appears that *Wolbachia* is involved in immune-tolerance towards the parasite (predominance of IL-10 and Treg lymphocytes), once the nematode dies, *Wolbachia*-derived antigens tend to stimulate the production of reactive oxygen species and pro-inflammatory cytokines.

The pathogenesis of canine heartworm infection has been widely studied (McCall et al., 2008). Live heartworms can cause endarteritis and muscular hypertrophy of arteriole walls especially in the caudal pulmonary arteries. If not treated, these alterations lead to pulmonary hypertension and right-sided congestive heart failure. The elimination of worms through adulticide therapy should be carried out according to specific guidelines that are aimed at limiting further pathology associated with dying worms. Specific antibiotic therapy given to infected dogs reduces the endosymbiotic bacterial population of *Wolbachia* within all life stages of *Dirofilaria immitis*. This reduction of *Wolbachia* not only severely affects the female heartworm reproductive tract, but also greatly reduces pro-inflammatory reactions to dying worms. Doxycycline treatment is able to quickly reduce antigenic mass of adult females of *D. immitis* by eliminating developing embryos within the female uterus (Kramer et al., 2011). Indeed, the loss of antigenic mass and reduction in the populations of *Wolbachia* organisms in *D. immitis* induced by doxycycline, which is normally given in association with a macrocyclic lactone (ML) is able to markedly reduce the potentially harmful effects of adulticide

therapy and thus improve health status of dogs treated for heartworm disease (Kramer et al., 2008; Kramer et al., 2011). Furthermore, the targeting of *Wolbachia* with antibiotics, combined with the nematocide effects of MLs, may represent a valid alternative to current adulticide therapy (Bazzocchi et al., 2008; Grandi et al., 2010).

Less is known about the possible pathogenic role of *Wolbachia* in subcutaneous dirofilariaosis by *D. repens*. Grandi et al. (2008) carried out immunohistochemical staining against WSP on human skin nodules and showed numerous bacteria within the intact worms and occasional positive staining within the surrounding inflammatory infiltrate. Serum samples from many of these patients resulted positive for total immunoglobulin G titers against WSP as examined in enzyme-linked immunosorbent assay. It is not currently known what effect specific antibiotic treatment has on *D. repens* and further study is necessary.

References

Bandi C., Trees A.J., Brattig N.W. 2001. *Wolbachia* in filarial nematodes: evolutionary aspects and implications for the pathogenesis and treatment of filarial diseases. Vet. Parasitol. 98:215-238.

Bazzocchi C., Mortarino M., Grandi G., Kramer L.H., Genchi C., Bandi C., Genchi M., Sacchi L., McCall J.W. 2008. Combined ivermectin and doxycycline treatment has microfilaricidal and adulticidal activity against *Dirofilaria immitis* in experimentally infected dogs. Int. J. Parasitol. 38:1401-10.

Dingman P., Levy J.K., Kramer L.H., Johnson C.M., Lappin M.R., Greiner EC., Courtney C.H., Tucker S.J., Morchon R. 2010. Association of *Wolbachia* with heartworm disease in cats and dogs. Vet. Parasitol. 170:50-60.

Grandi G., Morchon R., Kramer L., Kartashev V., Simon F. 2008. *Wolbachia* in *Dirofilaria repens*, an agent causing human subcutaneous dirofilariasis. J. Parasitol. 94:1421-3.

Grandi G., Quintavalla C., Mavropoulou A., Genchi M., Gnudi G., Bertoni G., Kramer L. 2010. A combination of doxycycline and ivermectin is adulticidal in dogs with naturally acquired heartworm disease (*Dirofilaria immitis*). Vet. Parasitol. 169:347-51.

Kozek W.J. 2005. What is new in the *Wolbachia*/*Dirofilaria* interactions? Vet. Parasitol. 133:127-132.

Kramer L.H., Passeri B., Corona S., Simoncini L., Casiraghi M. 2003. Immunohistochemical-immunogold detection and distribution of the endosymbiont *Wolbachia* of *D. immitis* and *Brugia pahangi* using a polyclonal antiserum raised against WSP (*Wolbachia* surface protein). Parasitol. Res. 89:381-386.

Kramer L., Grandi G., Passeri B., Gianelli P., Genchi M., Dzimianski M.T., Supakorndej P., Mansour A.M., Supakorndej N., McCall S.D., McCall J.W. 2011. Evaluation of lung pathology in Dirofilaria immitis-experimentally infected dogs treated with doxycycline or a combination of doxycycline and ivermectin before administration of melarsomine dihydrochloride. Vet. Parasitol. 176:357-60.

Kramer L., Grandi G., Leoni M., Passeri B., McCall J., Genchi C., Mortarino M., Bazzocchi C. 2008. *Wolbachia* and its influence on the pathology and immunology of *Dirofilaria immitis* infection. Vet. Parasitol. 158:191-5.

McCall J.W., Genchi C., Kramer L.H., Guerrero J., Venco L. 2008. Heartworm disease in animals and humans. Adv. Parasitol. 66:193-285.

Morchón R., Ferreira A.C., Martín-Pacho J.R., Montoya A., Mortarino M., Genchi C., Simón F. 2004. Specific IgG antibody response against antigens of *Dirofilaria immitis* and its *Wolbachia* endosymbiont bacterium in cats with natural and experimental infections. Vet. Parasitol. 125:313-21.

Morchón R., Carretón E., Grandi G., González-Miguel J., Montoya-Alonso J.A., Simón F., Genchi C., Kramer L.H. 2012. Anti-*Wolbachia* surface protein antibodies are present in the urine of dogs naturally infected with *Dirofilaria immitis* with circulating microfilariae but not in dogs with occult infections. Vector Borne Zoonotic Dis. 12:17-20.

Sironi M., Bandi C., Sacchi L., Di Sacco B., Damiani G., Genchi, C. 1995. A close relative of the arthropods endosymbiont *Wolbachia* in a filarial worm. Mol. Biochem. Parasitol. 74:223-227.

FROM GOATS TO MEN: SOME SELECTED ZOONOSES OF PUBLIC HEALTH IMPORTANCE

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More than 13 million of goats are bred all over Europe mainly for milk and cheese production. The biggest herds are found in Greece, Spain, France Italy and Romania but more than 80% of milk production is made by France, Greece and Spain. Goat meat consumption remains anecdotal at the European level. Though, goats can be involved in the cycle of various parasitosis such as *Fasciola hepatica* or *Echinococcus granulosus*, they are not probably the major parasitic reservoir. *Cryptosporidium* and *Giardia* have been found on intensively reared goat farms and are a potential source for zoonotic infections though never clearly demonstrated. On the opposite, goat products such as milk or cheese have been found in some occasion to be sources of human toxoplasmosis or tick borne diseases. Q fever, due to the bacteria *Coxiella burnettii* is a severe disease in humans with lung involvement and possible cardiac complications. It can be a source of abortion in pregnant women and debilitating chronic cases are possible. A huge outbreak began in the Netherlands in 2007; involved thousands of cases and surveys showed that abortion waves on dairy goat farms were the primary source of infection for humans, primarily affecting people living close to such goat farms. *Ixodes ricinus* was not a factor for the dissemination of this disease which is usually airborne. Tick borne encephalitis (TBE) is a serious viral disease highly prevalent in Central Europe which can be easily prevented in humans by an efficient vaccine. The major route of virus transmission is tick bites, but TBE also can be transmitted during consumption of non-pasteurized milk and milk products from infected goats. Goat health has to be monitored as they can be source of serious zoonosis and this approach should be efficiently addressed in a “One Health”.

TOXOPLASMOSIS IN GOATS: A RISK FOR HUMAN INFECTION?

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The cosmopolitan protozoan *Toxoplasma gondii* is a major parasite of warm-blooded animals including man, estimated to infect one third of the global population. Humans get infected by ingestion of both sexual and asexual parasite stages, i.e. from oocysts from the environment and from tissue cysts present in the muscles of infected animals. Meat animals mostly associated with a risk for human infection include pigs, sheep and goats. Among these, goats are the least studied species, which needs to be changed in line with the increased interest in goat products (both milk and meat) and increased goat industry. Importantly, in contrast to sheep, *T. gondii* may be transmitted to humans not only by goat meat but by milk as well. Although raw milk from acutely infected animals contains mostly tachyzoites, which are susceptible to pepsin digestion and therefore considered less infective, human infection due to consumption of raw goat milk has been reported and consumption of raw goat milk was associated with *T. gondii* seropositivity.
As obligate herbivores, goats get infected by ingestion of oocysts. Toxoplasmosis is an important cause of fetal loss in goats which parallels that in sheep. In pregnant goats, primary infection may lead to fetal infection, resulting in fetal death and resorption, abortion or stillbirth, or birth of weak kids. Congenitally infected kids, even if they appear normal, have persisting tissue cysts. The worldwide seroprevalence of toxoplasmosis in goats ranges widely, from 0% (small study in Pakistan) to as high as 77% in France. Interestingly, *Toxoplasma* infection in goats tends to be more prevalent in Europe than in Asia, Africa or South America. Across Europe, a seroprevalence of above 60% has been reported in Austria, Spain and the Czech Republic.
The infection reservoir in goats and the heightened market share of goat products, coupled with the current practices of “healthy” eating which often include undercooking of meat as well as the common presumption that goat milk should be consumed unprocessed, suggest the role of goats for human toxoplasmosis is likely to be increasing, and thus preventive measures to decrease the zoonotic risk are needed. Unlike for sheep, there has yet been no vaccine for goats. Until a vaccine to prevent or reduce *T. gondii* tissue cysts in meat goats becomes available, measures to improve goat health and thus reduce the risk of transmission to people include improved management of farm cats, fodder and water.

***CRYPTOSPORIDIUM*, *GIARDIA* AND GOATS**

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The protozoan parasites *Cryptosporidim* spp. and *Giardia duodenalis* have worldwide been reported in a wide range of animal hosts, including goats. Infection is primarily seen in young animals and results in diarrhea, ill thrift and impaired weight gain. Besides the clinical relevance, concerns on the zoonotic potential of ruminant infections stimulated research into the molecular epidemiology with both protozoans. Within each parasite, different species and subspecies have been identified of which some are found in both humans and animals, whereas other species or subspecies seem to be relatively host-specific to animals or humans. The host-specific (sub)species comprise the bulk of the animal infections worldwide. The prevalence of both parasites in goats has been reported with great variability among studies. Furthermore, there is little information on the species or subspecies infecting goats, and their potential relevance for human infections. New data from Greece and Belgium will be presented, as well as an overview of existing data.

TICK INFESTATION AND TICK-BORNE INFECTIONS OF GOATS FOCUSING ON THEIR ZOONOTIC IMPORTANCE

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It is known that hard ticks are obligate, blood-feeding ectoparasites of vertebrates, particularly several mammals including goats. Our knowledge about tick infestation of these small ruminants is scanty comparing with other domestic animals. The vast majority of recent studies of ticks on goats have been carried out in some countries of Asia, Africa and the Mediterranean region, indicating the importance of these parasites and goats in these areas. According to reports similar tick species of *Ixodes*, *Dermacentor*, *Haemaphysalis*, *Hyalomma* and *Rhipicephalus* genera infest the grazing goats as sheep and cattle. However, it was observed that significantly more goats harboured ticks than sheep in the same pasture, probably due to pasture differences and most likely in association with the browsing habit of goats. The economic impact of these ectoparasites on goats can manifest in a number of ways. If infestation levels are heavy, they can result in direct tick damage (e.g., anaemia, skin damage). Tick paralysis is associated with several of species and it is especially common in kids which are very susceptible because of their low body weight. Goats such as other mammals are also affected by tick-borne pathogens (TBPs) which are less studied than those of other mammals. Amongst the known TBPs of goats tick-borne encephalitis virus (TBEV), Crimean-Congo hemorrhagic fever virus (CCHFV) and *Anaplasma phagocytophilum* are the most important zoonotic agents. The TBEV is transmitted by the *I. ricinus* tick in Europe. Most infected goats are generally asymptomatic but they develop circulating antibodies to TBEV. The major route of the virus transmission to humans is tick bites, but TBEV can be transmitted during consumption of nonpasteurized milk and milk products from infected animals when they are viraemic. The most common vector of CCHFV, *Hyalomma marginatum* often infest goats in the Mediterranean region where several animals were found to be antibody-positive for the virus. From epidemiological point of views, the role of infected goats in human infection has not been known yet. *Anaplasma phagocytophilum* causing a few cases of human granulocytic anaplasmosis in some European countries is also transmitted by *I. ricinus* to goats. After patent bacteraemia with mild clinical or subclinical manifestations the animals may remain persistently infected "carriers".

NEGLECTED HELMINTH ZOONOSES AND GOATS

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It is worldwide recognized that one of the main threats on the outdoor breeding of goats is parasitism caused by protozoa, helminths and arthropoda. Parasites are ubiquitous, highly prevalent, and responsible for major economic losses in goat production in terms of product quantity and quality. In particular, infections by helminths (caused by different genera of nematodes, e.g., *Teladorsagia*, *Haemonchus*, *Trichostrongylus* and *Oesophagostomum*) as well as by trematoda (e.g., *Fasciola hepatica, Dicrocoelium dendriticum*) still today remains one of the main constraints to goat production both in temperate and tropical countries. Therefore monitoring and surveillance of parasitic infections in goats is of great importance, with the final goal of setting up sustainable and effective control strategies. Surveillance of helminth infection in goats is very important also from a public health point of view, because many of these organisms can also cause zoonoses considered neglected so far. However, most epidemiological data on the distribution and prevalence of zoonotic helminthes are available for developing areas, whereas in developed countries, these helminthes are usually not notified and few data are reported in the literature mostly related to immigrants. Few data are available regarding the presence, prevalence and distribution of these zoonotic parasites among human population living in (or around) the goat farms. Monitoring human infections in the context of the animal farming represent an imperative need of modern society due to the possibility of transmission of the infection, through the food chain, even to the inhabitants of urban areas.

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ECHINOCOCCUS GRANULOSUS COMPLEX IN OVINE AND CAPRINE HOSTS

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Cystic echinococosis (CE) is a zoonotic disease caused by the cystic stage (metacestodes) of the tapeworms belonging to the *Echinococcus granulosus* complex. The adult worms, inhabiting the small intestine of the definitive hosts species (mainly dogs), shed their eggs into the environment during the host defecation and contaminate the pasturelands. Intermediate host species (mainly livestock such as sheep and goats) accidentally ingest infective eggs which develop into a larval stage (metacestodes) in different organs (e.g., liver, lungs and kidneys).
CE is a major human and veterinary public health problem in areas where extensive livestock production provides suitable conditions for the cyclic transmission between dogs and livestock animals. A preliminary estimate of the annual global burden of CE has suggested approximately 1 million DALYs (disability-adjusted life years) are lost due to this disease, with perhaps 1 million individuals currently suffering from CE globally. In addition the financial burden of CE on the global livestock industry is considerable with up to $2 billion lost annually.
In the past 25 years, molecular studies based mainly on mitochondrial DNA sequences have identified 10 genotypes (from G1 to G10) within the *E. granulosus* complex. This classification supports the genotypes G1 (sheep strain), G2 (Tasmanian sheep strain) and G3 (buffalo strain) as *E. granulosus* sensu stricto. The G4 genotype (horse strain, *Echinococcus equinus*) and the G5 genotype (cattle strain, *Echinococcus ortleppi*) were confirmed to be good species. The genotypes G6 (camel strain), G7 (pig strains) and G8 (cervid strain) were thus grouped under the species name *Echinococcus canadensis*.
G1, G2 and G3 genotypes are responsible of the majority of the human infections and sheep and goats are their reservoirs. Moreover, in some South American regions, goats seem to be the reservoir of the G6 genotype

PARASITIC DISEASES: EPIDEMIOLOGICAL CONSIDERATIONS ON RELATIONSHIPS PARASITE – HOST IN HUMAN MEDICINE

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Parasitic infectious agents represent the outcome of a long evolutionary process, mirroring the survival efforts of species that loosed their capacities of existing as autotrophic species. The more complex such parasites are, the more difficult is to understand why they ended by be(com)ing parasites, shifting from autotrophy towards the parasitic style of life, losing their autonomy, their capacities of self-surviving. The most important and impressive is the detrimental impact this event has on the human host, exploited by many parasite species, with selfish benefits in the favour of parasites. This is an unfair relationship, if compared with the mutual advantageous relationship parasite-host so many microorganisms have with human body.

Parasitic infections represent a particular group of infectious diseases in human pathology, for many reasons:

a. The aetiology includes both micro- and macro-organisms, both categories being adapted to a parasitic relationship between the human host and the sub-human species of parasite. How similar this aspect is in the sub-human host species? If it is – how useful its more profound knowledge for all existing epidemiologic and clinic concerns?

b. The reservoir of infections is diverse and heterogeneous, human and nonhuman natural hosts being involved in most of parasitic infections. This remains one of the very important and sensitive issues from epidemiological point of view.

c. The pathogenesis is complex because of the complex interrelationships between parasite and host. The deeper the phylogenetic gap would be the most dramatic clinical picture and final outcome the infection could have. The level of immunocompetence of the human host, the co-existence of different abnormal conditions, the microbiological balance or disequilibrium within the human host, her capacity of reacting against the infection, the quality of immune response, the influence of simultaneous non-parasitic infections, the available curative resources - all are just some rings of the chain featuring the complexity of epidemiology of parasitic infections in humans.

d. The outcome of host-parasite relationship is a typical example of multifactorial causal relationship, each of the two partners in this cohabitation being involved with multiple factors of vital importance to the final result. This explains why we can identify all three kind of clinical/epidemiological situations: contamination, infection, or disease. How much could we blame the paradigm of host polymorphisms and sensitivities? (Lederberg, 1997).

e. The surveillance of zoonotic parasites, anthropophilic or not, is a complex and difficult task to be accomplished through a complex strategy by a professional partnership of human and veterinarian epidemiologists (epidemiologists and epizootologists). All efforts made by epidemiologists while trying to implement and making operational a surveillance system of parasitic diseases, tailoring strategies and tactics of preventing and controlling such diseases in humans, do have weaknesses and failures, merely because of lack of appropriate knowledge on similar topics in epizootology.

f. It is more and more obvious that the prevention and control of parasitic infections would have to be the corollary of a multi-professional approach, exploiting the competence of qualified experts in the domains of parasitology, veterinary medicine, clinical epidemiology, anti-parasitic therapy, environmental decontamination etc.

g. At least but not last, all the issues of above are asking for a clearer and deeper approach of parasite - host relationship, a better understanding of the meaning and relevance of parasite – host interrelationship and its importance for the most pragmatic consideration of pathogenesis, diagnosis, prevention and therapy of such infectious diseases.

The very large prevalence of digestive parasitic infections makes this group one of the most important of all infectious diseases, particularly for paediatric population. The most relevant example is the case of giardiasis; the high frequencies of diagnosis and subsequently prescribed therapy are well known. All practitioners ignore or disregard the even much higher frequency of asymptomatic infections, diagnosed incidentally, plus the also well-known high prevalence of presence of *Giardia lamblia* cysts in both dwells- and tap- drinking waters. Where is the border between curative and preventative needs to be prescribed? Who takes such decision, and based on which evidences? How could someone be sure that the cause of

diarrhea in a child is the giardiasis, at the age of the highest incidence of all-causes enteric infections, taking place in a highly contaminated / populated organ (the gut) by 10 times higher number of (microbial) cells than the whole number of component cells of the human body? How could be incriminated / sustained exclusively the giardiasis as clinic and aetiological diagnosis to be targeted by specific therapy, as long as the common rate of aetiological diagnosis of all enteric infections is below 40% of cases in common field conditions?

The closer to human species a parasitic agent is, the more predictable the clinical outcome of the relationship would be. The case of trichinosis is so appropriate to illustrate the potential catastrophic clinical result of such interrelationship. *Trichinella spiralis* is also a microscopic parasite, but he is so much more aggressive than the much smaller *Giardia*; the question is why? If crossing the border from microscopic dimensions towards macroscopic ones, is the gravity of such relationships increasing? Let us take a look on *Echinocosus granulosus* and everyone would be impressed. What about *Taenia saginata* or many other worms?

To the end, where is the border between tolerance and intolerance, between symbiosis and disbiosis, and who is the guiltiest in such circumstances – the aggressive infecting parasite or the aggressed infected host? How should we approach this obviously existing problem from both ends -human patient and physician? Who else must be involved as co-actor in this game, why and what for? Who is the guiltiest – the parasite, the infected human host, the parasitologist who performed the diagnosis, and who has to be sentenced to what?

Allow me reminding you a 15-years old statement coming from CISET (NSTC-CISET Working Group on Emerging and Re-emerging Infectious Diseases, 1996): "CISET* recommendations for addressing global infectious disease threats":

1. Concerted global and domestic surveillance and diagnosis of disease outbreaks and endemic occurrence. This must entail the installation of sophisticated laboratory capabilities at many centres now lacking them.
2. Vector management and monitoring and enforcement of safe water and food supplies; and personal hygiene (e.g., Operation Clean Hands).
3. Public and professional education.
4. Scientific research on causes of disease, pathogenic mechanisms, bodily defences, vaccines, and antibiotics.
5. Cultivation of the technical fruits of such research, with the full involvement of the pharmaceutical industry and a public understanding of the regulatory and incentive structures needed to optimize the outcomes.

*Committee on International Science, Engineering and Technology Policy of the National Science and Technology Council.

If we ended by being impressed, even overwhelmed by the complexity of such parasite-host relationship, the following questions look so reasonable (Lederberg, 1997; Bocşan, 2006; Hoberg et al., 2008):

- How well do we understand the host – parasite coevolution?
- How close we are to the integrated surveillance concept (system?) in this group of epidemiologic and clinic concern, of diseases of parasitic aetiology?

An improved and larger homogenous approach in these respects will reward us by a much better and more pragmatic understanding of the complexity of parasitic diseases, both in veterinary and human medicine.

References

Bocşan I.S. 2006. Basic Epidemiology. Ed. Med. U.M.F. Cluj-Napoca, pp. 27-60.

Hoberg E.P., Polley L., Jenkins E.J., Kutz S.J., Veitch A.M., Elkin B.T. 2008. Integrated Approaches and Empirical Models for Investigation of Parasitic Diseases in Northen Wildlife. Emerg. Infect. Dis. 14(1):10-17.

Lederberg J. 1997. Infectious Disease as an Evolutionary Paradigm. Emerg. Infect. Dis. 3(4):417-429. Norman R. 1996. Pace and ASM News. ASM News 62(9):464.

NSTC-CISET Working Group on Emerging and Re-emerging Infectious Diseases. 1996. Infectious disease – a global health threat. Washington DC. The Group.

PET INTESTINAL WORMS: A CONTINUING THREAT IN A CHANGING PARASITIC WORLD

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Human beings and domesticated small animals have started their relationships about 15000 years ago, leading to the dispersion of pets all over the World, along with their pathogens. Some of these pathogens are common and zoonotic, thus the interest on their sanitary impact, and on prevention and control methods is still kept high. Indeed, the Scientific Community has recently focused attention on extra-intestinal nematodes affecting dogs and cats, e.g., *Dirofilaria* spp., *Angiostrongylus vasorum* and *Aelurostrongylus abstrusus*. The distribution of these parasites is increasing where they are already endemic and, also, they are emerging in several countries and spreading into regions previously free. One of the likely culprits of this epidemiologic change is global warming. Indeed, climate changes are influencing the ecology of helminths with multiple hosts and different transmission routes. This is particularly true for invertebrate-borne nematodes (e.g., filariae, eye worms, heartworms, lungworms). Such scenario, together with the availability of broad spectrum drugs sold often over-the-counter, has likely caused the minimization of the importance of the "common intestinal worms". There could be the misconception that routine use of anthelmintics may control and reduce diffusion and impact on animal health and welfare of intestinal nematodes affecting dogs and cats. Indeed, several pet intestinal nematodes are zoonotic and endemic globally, and also their spread may be favoured by current global warming, given that these parasites have periods of development and survival in the environment, which are often at the basis of transmission routes in sapro-zoonoses. Specifically, present climate changes are likely at the basis of quicker egg embryonation and increased over-wintering of environmental infectious elements. These changing biological features will likely increase the spread of intestinal nematodes infecting dogs and cats in several areas of the World. Indeed, virtually 100% of pets, from the cosseted kitten or puppy, to the stray and feral animal, have been or will be in contact with intestinal worms, or, at least, are at risk of infection. The most common are intestinal roundworms (i.e., ascarids), hookworms (i.e. ancylostomatids) and whipworms (*Trichuris* spp.). These nematodes can be recognized as the most important parasites affecting dogs and cats, for their dispersion and distribution, pathogenic impact and risk for animal and human health.

Toxocara canis and *Toxocara cati* are the two major ascarids globally infecting dogs and cats, respectively, while *Toxascaris leonina* affects both species. The biological cycle of these species relies on different pathways of larval migrations and transmission, depending upon the ascarid and host species, the source of infection and the animal age. Animals may become infected *via* transplacentar and/or lactogenic routes, and by ingesting environmental infectious eggs or paratenic hosts harboring tissutal larvae. With regard to ancylostomatids, *Ancylostoma caninum* and *Ancylostoma tubaeforme* are species-specific for dogs and cats respectively, while *Ancylostoma braziliense*, *Ancylostoma ceylanicum* and *Uncinaria stenocephala* affect both species. While *A. caninum*, *A. tubaeforme* and *U. stenocephala* are spread especially in warm countries and in colder areas of temperate and subarctic regions, the other species are most often present in sub-tropical and tropical regions. The major infectious hookworms stage is represented by filariform larvae present in the soil, which parasitize a suitable host by actively penetrating the skin (especially for *Ancylostoma* spp.) and/or *via* the oral route (i.e. *Ancylostoma* spp., *Uncinaria* spp.). Also, other infection routes are nursing and ingestion of paratenic hosts, while transplacentary transmission is still debated. Among whipworms, dogs and canids in general are preferential hosts of the worldwide distributed *Trichuris vulpis*, while species affecting felids, including the domestic cat, are *Trichuris felis*, *Trichuris campanula* and *Trichuris serrata*. Indeed, the identity of cat whipworms is questioned and it is unclear whether they are separate species or there is a sort of synonymy between *T. felis* and the other species. Also, these worms are absent in most countries of the World, being confined in few areas of the Americas and few other regions, where they occur extremely seldom. Animals become infected by whipworms by ingesting larvated infectious eggs contaminating the environment.

In summary, several factors make intestinal nematodes the most common endoparasites affecting pets in all corners of the World. The possibility of puppies and kittens being infected by their dam by transmammary and/or transplacental route/s for some ascarids and/or ancylostomatids is a powerful host-finding strategy. Also, animals may shed relevant amounts of roundworm and whipworm eggs for weeks, thus causing a high environmental contamination. Whipworm and ascarid eggs can survive for

years in extreme conditions thus are available for ingestion at any time. Infected paratenic hosts are ubiquitous, being a constant source of infection especially for cats, given their hunting instinct.
It is often thought that “intestinal worms” are only a health problem of puppies and kittens and that adult animals are, instead, free of these parasites. Indeed, this is not true. Pets are exposed to intestinal endoparasites throughout the year and for all their life. Even though parasitic burdens, egg output and infection rates are higher in puppies and kittens especially for roundworms, it is nowadays established that patent intestinal infections may occur in dogs and cats of all ages for all major nematodes.
Adult dogs have the same susceptibility for patent infections by *T. canis* as naïve patients when later re-exposed and even when repeatedly exposed to the parasite and having circulating antibodies versus roundworm surface antigens. Animals ageing more than 3 years often show patent ascaridosis even after ingestion of few larvated ova and, additionally, nursing bitches may present heavy patent infections by about 4 weeks after delivery. As another key example, cats of all ages may develop patent intestinal infections by *T. cati* for both ingestion of prey and of environmental larvated eggs by their self-grooming. Interestingly, the typical biological features make the infection by *T. leonina* much more common in adult animals than in young patients.
There is not a clear relationship between host age and prevalence of *Ancylostoma* spp., although a significant higher prevalence of hookworms in young dogs is often detected. Also, prevalence of *A. caninum* in dogs < 11 months of age can be significantly lower than infection rates in dogs aged 1-6 and > 6 years, and infection rates by *U. stenocephala* can be higher in dogs of more than 3 years of age than in puppies of less than 4 months. Given that *A. tubaeforme* is not transmitted *in utero* or *via* the milk, the infection can be present in cats of all ages and not only in kittens and, interestingly, there are studies that have shown an increasing trend of infection rate in 1-5 year old cats rather than in kittens.
Eggs are a constant source of infection for dogs living in contaminated environments, thus the incidence of trichurosis is higher in adult dogs, which also have higher parasitic burdens than youngsters. The prevalence rates of intestinal trichurosis in adult dogs may be also due to the absence of a transplacental and/or transmammar transmission, to its long pre-patent period and to a likely inability to elicit a protective immune response.
Roundworms, hookworms and whipworms have a well-known and recognized pathogenic potential for their animal hosts but it is noteworthy that they may cause disease in humans as well. Specifically, several animal ascarids and ancylostomatids may infect people, causing different syndromes, e.g., *visceral larva migrans*, *ocular larva migrans* or covert toxocarosis (ascarids) or cutaneous, muscular and intestinal damages (ancylostomatids). Albeit hypothesized, the zoonotic potential of *T. vulpis* is debated and questioned and, currently, not recognized.
Different parasiticide classes are available for treatment and control of intestinal nematodes, being (pro-)benzimidazoles (e.g., febantel, fenbendazole), tetrahydropirimidines (e.g., pyrantel), cyclooctadepsipeptides (i.e. emodepside) and macrocyclic lactones (e.g., ivermectin, selamectin, moxidectin, milbemycin oxime) the most used.
Veterinarians have a plethora of formulations that can be selected according to each individual possible *scenario* and owner and animal compliances. The use of antiparasitic molecules should be programmed according to several factors, related to the nematode biology and their epidemiological features in different regions. Key points are the geographical spread of these parasites, their clinical importance in both human and veterinary medicine, and the high resistance of infectious stages in the environment regardless season or climate. Regular “de-worming” or “worming”, an imprecise term but common in daily language today, is the basis for an effective chemoprophylaxis irrespective the age of the pet.
Owners and veterinarians should always thoroughly follow manufacturer’s indications for each of the selected parasiticides administered to bitches, queens, puppies and kittens. The US Companion Animal Parasite Council (CAPC) and the European Scientific Counsel Companion Animal Parasites (ESCCAP) have published guidelines for treatment and control of major parasites affecting companion animals. The CAPC suggests treatment of puppies and kittens at two, four, six and eight weeks of age, followed by monthly treatments as soon as label recommendations allow, possibly using molecules effective in preventing heartworm infections and having efficacy against intestinal worms as well. Hence, a lifelong preventative program, using a administrations at 4-week intervals, in accordance with the pharmacokinetics of the molecule used, is indicated to exclude any risk of infection. Conversely, the ESCCAP advises that pups should receive a parasiticide at 2 weeks of age, then at fortnightly intervals until two weeks after weaning. Thereafter, puppies should undergo monthly treatments until six months old. Fortnightly treatment of kittens can start at 3 weeks of age and should be repeated fortnightly until two weeks after weaning, then monthly for six months. The

ESCCAP also states that a treatment frequency of at least 4 times per year, or at intervals not exceeding 3 months, or even a monthly treatment, are general recommendations, according to different scenarios, e.g., real zoonotic risks, presence of children in the pet owners family, pregnancy of dams, housing conditions. Also, when a year-round-control is not performed, regular faecal examinations are considered a feasible way of evaluating the re-occurrence of intestinal nematodes.
Worthy of mentioning is that frequent use of anthelmintics in companion animals could lead to a decrease in efficacy of parasiticides. Indeed, the abuse of anthelmintics caused the global spreading of livestock and horse parasites resistant to one or more drug classes. The same concern could emerge for resistant populations of pet nematodes, especially due to long-term indiscriminate use of parasiticides, which have been on the market over a long time. At the moment only resistance to pyrantel has been found in *A. caninum* but a high level of attention should always be maintained to detect any hint of drug resistance.
In conclusion, given the clinical importance of intestinal nematodes affecting pets, their ubiquitous dispersion and sanitary relevance, public education is crucial for reducing risk exposure in both humans and companion animals. At the same time pet owners and, in general, the public opinion should maintain a self-confidence that keeping a pet is safe and a positive experience. In fact, it is established that direct contact with dogs and cats infected by intestinal nematodes does not present any relevant risk in the transmission of infections and there is no association between ownership and parasitoses. Hence, owners should have confidence that ownership of any companion animal is beneficial and safe as long as their pets are healthy.
On the other hand, guard on the potential risks of infections originating from companion animals should always be kept high. This has become even more so in recent years, when sociological changes have influenced the relationships between physicians and veterinarians. The major goal of the re-discovered “One Health Program” (i.e., “*the collaborative work of multiple disciplines to help attain optimal health of people, animals, and our environment*”) demonstrates the key role of a strict relationship between the human health experts, veterinarian and public opinion. Veterinarians should always inform their clients on potential risks, with a special focus on some categories (e.g., immunocompromised, children, elderly) in order to allow pets to remain integral members of household and families and, at the same time owners should trust on the effects of a lifespan control program based on routine faecal examinations and frequent worming.

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References

Bowman D.D., Montgomery S.P., Zajac A.M., Eberhard M.L., Kazacos K.R. 2010. Hookworms of dogs and cats as agents of cutaneous larva migrans. Trends Parasitol. 26:162-167.
Companion Animal Parasite Council. www.capcvet.org.
Epe C. 2009. Intestinal nematodes: biology and control. Vet. Clin. North. Am. Small Anim. Pract. 39:1091-1107.
European Scientific Counsel Companion Animal Parasites. www.esccap.org.
Lee C.Y., Schantz P.M., Kazacos K.R., Montgomery S.P., Bowman D.D. 2010. Epidemiologic and zoonotic aspects of ascarid infections in dogs and cats. Trends Parasitol.26:155-161.
Robertson I.D., Thompson R.C. 2002. Enteric parasitic zoonoses of domesticated dogs and cats. Microbes Infect. 4:867-873.
Traversa D. 2011. Are we paying too much attention to cardio-pulmonary nematodes and neglecting old-fashioned worms like *Trichuris vulpis*? Parasit. Vectors 4:32.
Traversa D. 2012. Pet roundworms and hookworms: a continuing need for global worming. Parasit. Vectors. In press.

IS *ALARIA ALATA* A HARMFUL PATHOGEN OR JUST A CAUSE OF SIGNIFICANT ECONOMIC LOSS?

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Alaria alata mesocercaria is a typical life cycle stage of the intestinal trematode *Distomum musculorum suis* found in different canids including dogs. *Mesocercariae* are usually found in amphibians but also snakes, rodents and mammals that are used as paratenic hosts. Quite frequent background findings of *Alaria alata* mesocercariae during ordinary *Trichinella* inspection as reported in several European countries pose many questions that were still not answered but also stress the importance for the reassessment of this potential foodorne pathogen. Death cases caused by *Alaria americana* and clinical investigations in occasionally infected people clearly demonstrate that mesocercariae could be dangerous if consumed through intermediate or paratenic hosts tissue. The autopsy of the dead person showed the presence of mesocercariae in the stomach wall, lymph nodes, liver, myocardium, pancreas and surrounding adipose tissue, spleen, kidney, lungs, brain and spinal cord. Intraocular and pulmonary infection was also described. It is important to stress that there is also a clear evidence of transmammary transmission of another American species *Alaria marcianae* in different mammals including primates. Recently a new more accurate migration method for the detection was optimized. German authors clearly showed that the ordinary artificial digestion for the detection of *Trichinella* muscle larvae is inapplicable. The same impose a fact that probably a high number of cases of alariosis in wild boars were not reported. Since alariosis is not a reportable disease in Croatia in order to understand the current situation a questionnaire for *Trichinella* analysts was prepared. The questionnaires were sent to 100 laboratories that routinely performs artificial digestion. The analysis showed a very good knowledge on the morphology and biology of the trematode which clearly prove the high incidence of infected muscle tissue wild boars. Furthermore ecoparasitological investigations were also performed that included various species of amphibians. The screening was performed by the artificial digestion and migration technique of tongues of frogs. The prevalence in frogs varied from 26 up to 60% depending on the species and site of collection. Hunted boars from different regions of Croatia were also found infected. The prevalence ranged from 15 up to 100% depending on the region. Mesocercariae of *Alaria alata* were also sporadically found in domestic pigs. Since due to the current Ordinance in Croatia parasitized meat is unfit for human consumption, a huge numbers of infected wild boars represent an important source of economic loss. Besides that improper disposal of infected carcasses by hunters significantly increase the risk of transmission to final hosts.

INTEGRATING GENETIC, MORPHOLOGICAL AND ECOLOGICAL DATA FOR THE CHARACTERIZATION OF CRYPTIC SPECIES OF ANISAKID NEMATODES: IMPLICATIONS FOR THEIR EPIDEMIOLOGY, FOR THE HUMAN AND THE ECOSYSTEM HEALTH

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The combination of genetic, morphological and ecological data for the characterization of cryptic species of anisakid nematodes, has greatly contributed to the knowledge on their epidemiology, on the definition of their zoonotic role to humans, as well as on the possible use of these parasites as indicators of ecosystem health (Mattiucci and Nascetti, 2008).
The recent results achieved by the combination of sequences analysis of multiple loci in the systematics and in "species delimitation" of this group of parasites, will be presented. Moreover, the discovery of diagnostic morphological characters accompanied the genetic detection of cryptic species supporting, with morphological evidences, the results obtained with genetic/molecular approaches. The combination of genetic and morphometric data sets by multivariate analysis, readily distinguishing among cryptic species of anisakids, will be shown.
Marked differences in the occurrence of different cryptic species of anisakid nematodes in various host species, including fish, as a result of differential host-adaptation and different life-history path-ways speculated by anisakid species in sympatric areas, will be presented.
Population genetic structure and patterns of phylogeography, inferred from mitochondrial DNA markers, indicating distinct geographical distribution patterns in some anisakid species, will be given. High genetic diversity values, at both nuclear and mitochondrial genes, have been generally observed in anisakid populations and species from Austral regions. The possible use of genetic diversity estimates obtained at different markers in anisakid populations as an indicator of stability of marine food webs will be discussed. Thus, the estimation of the genetic diversity of anisakid nematodes and their abundance levels in hosts from different geographical areas, over time and space, as a tool for monitoring the stability of marine trophic webs, will be also suggested.
Finally, the implications resulted from the application of molecular/genetic markers in the diagnosis of human anisakidosis, which greatly advanced the knowledge on the zoonotic role of these parasites, will be reported.

THE IMPORTANCE OF PARASITES IN TROUT FARMING

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Romanian lacustrin bioproductive potential is summarized on 70 lakes with a total water surface of 9552 ha of which, 19 oligothrophic mountain lakes. In parallel with the growth and development of aquaculture industries thorough some man-made lakes like Bicaz (Nt) (32 km long, 3000 ha, 92 m depth) and Vâlcea, diseases problems on trout farming including hazards caused by parasitic problems are presented in this paper.

RECENT DATA ON HUMAN DIPHYLLOBOTHRIOSIS

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Tapeworms of the genus *Diphyllobothrium* are widely distributed all around the world, with some being agents of human diphyllobothriosis, one of the most important fish-borne zoonosis caused by a cestode parasite. *Diphyllobothrium* cestodes are commonly present in the wildlife among fish, mammal and bird hosts that make an important reservoir. The human parasitosis is frequently reported from Japan, South Korea, the Baltic countries, Scandinavia, Western and Eastern Russia, and North America (Pacific Northwest). Recent surveys indicate that human diphyllobothriosis is emerging or re-emerging in some European countries, particularly in Italian and French speaking sub-Alpine regions although in South America and other countries sporadic cases have been reported so far. To date, a dozen of species have been reported as human pathogens and can be easily identified by molecular studies. Various parts of the genome of species of this genus have been identified and sequenced: 18S rDNA; cytochrome c oxidase subunit I (COI), ITS. We proved years ago that COI sequences were very discriminative between *D. latum*, *D. nihonkaiense*, *D. ditremum* and *D. dendriticum*. Genotyping of eggs or proglotids from patients permitted to identify, in addition to the autochthonous *D. latum* still present in Europe, *D. nihonkaiense* (locally acquired after consumption of raw imported Pacific salmon), *D. dendriticum* (imported). In addition, eggs of *Bothriocephalus achelognati* were identified in human stools. Molecular studies also allowed proving the identity of *D. klebanovski* and *D. nihonkainense* and of *Diplogonoporus balaenopterae* with *D. stemmacephalum*. A study on *D. latum* DNA repetitive sequences could provide a tool to study a possible intraspecific variation. The parasite is still present in the lake of Geneva as proven by a recent survey in *Perca fluviatilis* and *Esox lucius*.

UNDERSTANDING GIARDIASIS AT THE MOLECULAR LEVEL

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The protozoan parasite *G. intestinalis* (syn. *G. lamblia, G. duodenalis*) is the causative agent of giardiasis in a wide range of vertebrates, including humans. The parasite is estimated to cause 280 million cases of human giardiasis per year (Ankarklev et al., 2010). The disease is characterized by bouts of diarrhea, bloating, flatulence and malnutrition, and is especially troublesome in children living in low-income countries where stunted growth and poor cognitive function have been correlated with the disease (Ankarklev et al., 2010). Asymptomatic *Giardia*-infections are common, where the host may act as a reservoir for transmission of the disease. Both the adaptive and innate immune responses are involved in clearance of the parasite. Innate immunity involved in combating giardiasis, first of all includes the initial contact with the excyzoite/trophozoite and the intestinal epithelium. Roxström-Lindquist et al., (2005) showed that CXCL1-3, together with CCL2 and CCL20, were upregulated in the human intestinal epithelial cell (IEC) line, Caco2, upon interaction with *Giardia* trophozoites. We have verified this data using different IECs, different *Giardia* strains and RNA sequencing and real time RT-PCR. The up-regulation of these chemokines starts after 30 min of interaction and the RNAs are down-regulated already after 120 min. The parasites do not need to be in direct contact with the IECs and the inducing factor is secreted and sensitive to proteolysis. After the induction of chemokines in the IECs dendritic cells (DCs) are most likely attracted to the mucosa (Kamda et al., 2012). The DCs process giardial antigens, which leads to activation of the adaptive immune system, including secretion of IgA. The main antigens are variable surface proteins (VSPs), alpha giardins and enzymes involved in arginine metabolism (Palm et al., 2003; Rivero et al., 2010).
Arginine plays essential roles in growing individuals and under non-physiological conditions/disease and is therefore referred to as a conditionally essential amino acid (Morris, 2006). Interference with host cell arginine-usage has been described for a variety of pathogens, mainly with regards to arginine as a nitric oxide (NO) synthase substrate, whereas consequences of reduced arginine-availability on host cell physiology have only been addressed to a limited extent. The intestinal parasite *Giardia* actively consumes arginine and secretes an arginine-consuming enzyme, arginine deiminase (ADI, Ringqvist et al., 2010). We have shown that IEC proliferation is reduced upon arginine-withdrawal and in an arginine-dependent manner upon interaction with *Giardia* or addition of *Giardia* ADI. The observed reduction of cell proliferation coincided with a reduction in levels of the polyamine spermine and with upregulation of cell cycle inhibitor genes. We think this can be a general mechanism of intestinal microbes since arginine-consuming pathogens in the gastrointestinal tract, such as *Giardia,* can reduce NO production and also affect intestinal tissue homeostasis through reduction of IEC replication, thereby promoting colonization of the epithelium. Citrulline can replace arginine in the interaction system but it cannot be used by the parasite so it might be a good nutritional suplement during diarrhea.
Eight different *G. intestinalis* genotypes or assemblages have been described (A-H), where assemblages A and B infect humans and other mammals and assemblages C through H are more host-specific (Lebbaad et al., 2011). Recent data suggest that *Giardia* assemblage A and B can actually be two different species (Franzen et al., 2009) and several studies have recently shown associations between assemblage type and specific symptoms (Lebbad et al., 2011). We have performed whole genome sequencing of two sub-assemblage AII isolates, recently axenized from symptomatic patients, to study the genetic diversity within assemblage A and to identify new assemblage A-specific genotyping targets. Several biological differences between the assemblages A isolates were identified, including a difference in growth medium preference. The two AII isolates were of different sub-assemblage types (AII-1 (AS98) and AII-2 (AS175)) and showed size differences in the smallest chromosomes. The amount of genetic diversity was characterized in relation to the genome of an assemblage AI isolate (WB). Our analyses indicate that the divergence between AI and AII is approximately 1%, represented by about 100000 single nucleotide polymorphisms (SNP). Moreover, SNPs are homogeneously distributed over the chromosomes with an enrichment in regions containing surface antigens and non-coding sequences. The level of allelic sequence heterozygosity (ASH) in the two AII isolates were found to be 0.25-0.35%, which is 25-30-fold higher than in the WB

isolate. 37 protein-encoding genes, not found in the WB genome, were identified in the two AII genomes. The large gene families of variant-specific surface proteins (VSPs) and high cysteine membrane proteins (HCMPs) showed isolate-specific divergences of the gene repertoires. Certain genes, often in small gene families with 2 to 7 members, showed high sequence diversity between the assemblage A isolates and they could have important roles in host-parasite interactions. A subset of the variable genes was used to develop new genotyping methods for assemblage A isolates.
The protozoan parasite *G. intestinalis* and the pathogenic bacterium *Helicobacter pylori* are well known for their high prevalences in human hosts world-wide. The prevalence of both organisms is known to peak in densely populated, low resource settings and children are infected early in life. Different *Giardia* genotypes/assemblages have been associated with different symptoms and *H. pylori* with induction of cancer. Despite this, not much data are available from sub-Saharan Africa with regards to the prevalence of different *G. intestinalis* assemblages and their potential association with *H. pylori* infections. We studied fecal samples from 427 apparently healthy children, 0-12 years of age, living in urban Kampala, UgandaThe samples were analyzed for the presence of *H. pylori* and *G. intestinalis*. *G. intestinalis* was found in 86 (20.1%) out of the children and children age 1 < 5 years had the highest rates of colonization. *H. pylori* was found in 189 (44.3%) out of the 427 children and there was a 3-fold higher risk of concomitant *G. intestinalis* and *H. pylori* infections compared to non-concomitant *G. intestinalis* infection, OR=2.9 (1.7-4.8). No significant association was found in the studied population with regards to the presence of *Giardia* and gender, type of toilet, source of drinking-water or type of housing. A panel of 45 *G. intestinalis* positive samples was further analyzed using multi-locus genotyping (MLG) on three loci, combined with assemblage-specific analyses. *Giardia* MLG analysis yielded a total of five assemblage AII, 25 assemblage B, and four mixed assemblage infections. The assemblage B isolates were highly genetically variable but no significant association was found between *Giardia* assemblage type and *H. pylori* infection. This study shows that *Giardia* assemblage B dominates in children in Kampala, Uganda and that the presence of *H. pylori* is an associated risk factor for *G. intestinalis* infection.

References

Ankarklev J., Jerlström-Hultqvist J., Ringqvist E, Troell K., Svärd S.G. 2010. Behind the smile: Cell biology and disease mechanisms of *Giardia*. Nat. Microbiol. 8:413-22.

Franzén O., Jerlström-Hultqvist J., Castro E, Sherwood E., Ankarklev J., Reiner D.S., Palm D., Andersson J.O., Andersson B., Svärd S.G. 2009. Draft genome sequencing of *Giardia intestinalis* assemblage B isolate GS: is human giardiasis caused by two different species? PLoS Pathog. 5(8):e1000560.

Kamda J.D., Nash T.E., Singer S.M. 2012. *Giardia duodenalis*: dendritic cell defects in IL-6 deficient mice contribute to susceptibility to intestinal infection. Exp. Parasitol. 130(3):288-91.

Lebbad M., Petersson I., Karlsson L., Botero-Kleiven S., Andersson J.O., Svenungsson B., Svärd S.G. 2011. Multilocus genotyping of human *Giardia* isolates suggests limited zoonotic transmission and association between assemblage B and flatulence in children.PloS Negl. Trop. Dis. 5:e1262.

Morris S.M. 2006. Arginine: beyond protein. Am. J. Clin. Nutr. 83:508S-512S.

Palm J.E., Weiland M.E., Griffiths W.J., Ljungstrom I., Svärd S.G. 2003. Identification of immunoreactive proteins during acute human giardiasis. J. Infect. Dis. 187(12):1849-59.

Ringqvist E., Palm D., Skarin H., Hehl A.B., Weiland M., Davids B.J., Reiner D.S., Griffiths W.J., Eckmann L., Gillin F.D., Svärd S.G. 2008. Release of metabolic enzymes in response to interaction with intestinal epithelial cells. Mol. Biochem. Parasitol. 159:85-91.

Rivero F.D., Saura A., Prucca C.G., Carranza P.G., Torri A., Lujan H.D. 2010. Disruption of antigenic variation is crucial for effective parasite vaccine. Nat. Med. 16(5):551-557.

Roxström K., Ringqvist E., Palm D., Svärd S. 2005. *Giardia lamblia*-induced changes in gene expression in differentiated Caco-2 human intestinal epithelial cells. Infect. Immun. 73:8204-820.

GIARDIA – POLYPARASITISM AND ZOONOTIC TRANSMISSION: SOME OBSERVATIONS BASED ON RECENT STUDIES IN LAOS AND VIETNAM

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A growing number of enteric parasites are considered to have zoonotic potential. Their clinical impact varies and in many cases is poorly defined, particularly in developing countries where mixed infections predominate. Similarly, the epidemiology of such infections, particularly the role of non-human hosts, requires further study. We have recently completed community surveys of over 2000 humans in rural communities in northern Laos and Vietnam, as well as cohabiting dogs and pigs. *Giardia* was just one of a multitude of parasites identified in this population. Its endemicity is discussed with emphasis on polyparasitism, clinical impact and zoonotic potential.

CURRENT AND NEW DRUGS AGAINST *GIARDIA* AND GIARDIASIS

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Despite recent reductions in all-cause mortality in children, diarrhea remains the second leading cause of death among children under 5 years of age (You et al., 2010), and 8.8 million children still die every year before reaching their 5th birthday (Liu et al., 2012). Almost two- thirds died of infectious causes, nearly all of which were preventable (Johnson et al., 2010). Recently Fischer-Walker and colleagues (2012) had estimated that diarrhea was responsible for 31.3% of deaths among children 1-59 months of age compared to the multi-cause estimate of 26.1% in South-east Asia. In Africa they estimated that diarrhea accounted for 25.2% of deaths as compared to multi-cause estimate of 25.3% of deaths in this same age group. These results provide a justification for the continued effort to scale-up diarrhea prevention and treatment interventions targeted to children less than 5 years.
Giardiasis, a leading cause of parasitic diarrheal illness in humans and a wide variety of livestock and companion animals that is caused by the protist *Giardia duodenalis*, causes a clinical burden of 2.5 million cases per year (WHO, 2006). Epidemiological studies have shown that its prevalence varies between the population studied and geographically, from 2 to 5% in developed countries to 20-30% in the developing countries. This infection is a self-limited clinical illness which is also characterized by abdominal cramps, bloating, weight loss, and malabsorption; asymptomatic infection also occurs frequently (reviewed by Escobedo and Cimerman, 2007).
Giardia infection is normally transmitted by the faecal-oral route and results from the ingestion of *Giardia* cysts through the consumption of contaminated food or water or through person-to-person and to a lesser extent, animal-to-person transmission. The risk of exposion to this infection increases if travellers visit-endemic areas, for children in child care centres, and who may have close contact with infected persons or relatives who take care for them. Also infection may occur if persons swallow contaminated recreational water or who consume unfiltered, untreated water or who do not have good hygienic habits and are in contact with infected animals, and also this infection also occurs among homosexuals.
Up to now six drugs have been commonly used for the treatment of giardiasis these include 5-nitroimidazole compounds (metronidazole, tinidazole, ornidazole and secnidazole), acridine derivative (quinacrine), nitrofuran derivative (furazolidone), aminoglycoside (paromomycin), benzimidazole carbamates (albendazole and mebendazole), and a 5-nitrothiazolyl derivative (nitazoxanide) which is the first new drug specifically developed for treatment of giardiasis (Gardner and Hill, 2001). Most of these compounds exhibit different mechanisms of action. Metronidazole, furazolidone and nitazoxanide have a 5-nitro group which has to be reduced by enzymes like pyruvate-ferredoxin oxidoreductases, NADH oxidases and nitroreductases (GlNR1) respectively to display most of their cytotoxic effects. In the case of quinacrine, this drug may interact with DNA hence interfering with its replication and rearrangements while paromomycin interferes with protein translation by interacting with ribosomes (Gardner and Hill, 2001). When the *in vitro* efficacy of these representative compounds has been evaluated in the reference strain WB using the minimal lethal concentration (MLC) as parameter and techniques such as subculture in liquid medium as a gold standard, albendazole is the most effective compound followed by nitazoxanide while metronidazole, furazolidone and quinacrine have comparable efficacies (Argüello-García et al., 2004).
Based on reports in scientific literature (NCBI database), metronidazole is reported as the drug most commonly used at 5-10 days regimes in adults and children, with a median efficacy of 89%. Severe secondary effects in patients (metallic taste, nausea, headache, vertigo and leukopenia) are associated with these regimes and even using shorter ones, since a longer duration of therapy reduces patient compliance and increases the possibility of side effects. This fact has limited the time life of this agent for treatment of giardiasis hence other semi-synthetic compounds have been tested. Metronidazole is followed in its use to treat children with *Giardia* infections by tinidazole, another 5-nitroimidazole of single dose regime (50 mg/kg) that exhibits a better tolerance, comparable efficacy and sometimes is efficient when metronidazole fails. Furthermore in a recent report it was described that tinidazole has particular advantages in a resource poor setting due to its characteristics (well

tolerated and treatment requires only a single dose) thus it has the potential to improve compliance. It is also slightly cheaper than metronidazole per treatment (Chandy and McCarthy, 2008).
Albendazole and mebendazole, two former anthelminthic benzimidazoles that show an efficacy of 50-95% in giardiasis when used at single, twice or three-dose regimes, have been mostly used at single-dose regimes in 2- or 6-monthly intervals in communitarian deworming programs in some regions in the World such as Bangladesh and Mexico where giardiasis is endemic (Rousham, 1994; Flisser et al., 2008). Particularly mebendeazole can be used when first-line drugs have failed, or these were not tolerated or the drugs are not available. Also this drug can be taken in combination with other anti-giardial drug thus cure rates may be higher (Escobedo et al., 2011). However the long-lasting impact of these treatments for control of protozoa like *Giardia* needs further evaluation since compelling information is not yet available. Further longitudinal studies with a post-treatment follow-up should be carried out.
Nitazoxanide, one 5-nitrothiazole of more recent introduction for giardiasis in children, has shown an overall efficacy of 75% at a regime of 7.5 mg/kg for 3 days (Escobedo and Cimerman, 2007). Other less used compounds are secnidazole, ornidazole, chloroquine and paromomycin.
Although the use of these drugs is effective, there are some disadvantages in their use which include resistance to their effect, the duration of the treatment, suboptimal effectiveness in some regimes and side-effects that may affect compliance. Thus, new studies are needed to develop or design new drugs for giardiasis.
Several studies on new anti-*Giardia* drugs have been performed. In particular, experimental studies to test the effectiveness of bacitracin zinc chloroquine, DL-propranol, propolis, and ozonized sunflower oil with promising results for treatment of *Giardia* infections. In a representative trial, children infected with *G. duodenalis* were randomly assigned to treatment with propolis and given aminosidine-sulfate for 10 days, metronidazole for 10 days or 30% propolis for 10 or 20 days. Cure rates (assessed by fecal examination of each child 7, 14 and 21 days after treatment completion) with propolis were better in the group treated with propolis over 20 days (79.8%), similar to that of metronidazole (79.3%); however, the best result was found with aminosidine-sulfate (91.5%) (Núñez et al., 2004).
Regarding drugs that have a direct effect on trophozoites several candidates have been experimentally tested (reviewed by Tejman-Yarden and Eckmann, 2011). These include components that were derived from modifications of existing drug cores, such as 5-NI, benzimidazoles, or the nitro drug nitazoxanide but with activities that are often more potent than the original anti-giardial compounds (Upcroft, 2006; Valdez et al., 2009; Navarrete-Vazquez et al., 2011). Another approach has been the generation of hybrid molecules that combine characteristics of different active molecules. An example is the synthesis of hybrids between a quinoline and isoxyls which showed up to 500-fold increased antigiardial activity compared with metronidazole (Nava-Zuazo et al., 2010).
Natural compounds have also been tested for antigiardial activity. These include the weed creeping woodsorrel (*Oxalis corniculata*) from which a novel galacto-glycerolipid with antigiardial activity similar to metronidazole has been obtained (Manna et al., 2010). Another example is the alkaloid called osyrisine obtained from the sandalwood *Osyris alba* which has activity against *Giardia* with no toxicity against human cells (Al-Jaber et al., 2010). Likewise, garlic derivatives (allyl sulfides, allyl cysteines and allyl thiosulfinates) are promising anti-*Giardia* agents because they seem to interact cysteine-containing molecules, which are abundant in this microaerophilic parasite. In particular, allicin could be a good candidate because it was able to eliminate trophozoites from the intestine of infected gerbils (Argüello-García et al., unpublished).
The identification of other drug candidates in large compound libraries by high throughput technologies has provided promising data. The analysis of a 1600-compound library of known drugs and other bioactive molecules revealed 12 compounds with significant activity against *Giardia* (Gut et al., 2011). In another screen 4096 compounds were tested and it was found that 43 had selective activity against *Giardia* (Chen et al., 2011). Although these compounds need to be tested as antigiardial drugs they offer the advantage over newly synthetized molecules since these are safe in humans.
Finally, the identification of potential key targets together with the design of specific inhibitors for these molecules has been recently approached. In this two metabolic enzyme targets which include arginine deiminase (ADI) and the fructose-1, 6-biphosphate aldolase have been studied. Inhibitors of the catalytic mechanisms of ADI have been shown to be effective against the parasite *in vitro* (Li et al., 2009). The fact that humans do not have a homolog of AID, makes this enzyme a promising drug target. In the case of the fructose-1, 6-biphosphate aldolase its active site and catalytic mechanism

differ from those of human enzyme making it a good target for specific inhibitors (Li et al., 2011). Also the proteins disulphide isomerases PD12 and PD14, as well as GINR-1 nitroreductase, have been reported as promising targets for new drugs.

Additionally, *in vitro* studies have revealed that some HIV-protease inhibitors, mainly ritonavir, inhibit G*iardia* growth at therapeutic plasma concentrations (Müller et al., 2007; Dunn, et al., 2007).

Based on the current research on anti-*Giardia* drugs, their use will remain as the major tool to control *Giardia* infections in adults and children for the next years – and possibly for decades. In this context, *Giardia* proteomic research is a promising approach for designing drugs that interfere with parasite metabolism, but cause little damage to human cells. These studies will also provide useful information for elucidating mechanisms of drug resistance. Is worth mention that in treating and controlling this infection children should be considered as a special group since preventing *Giardia* infections in this population would contribute to their better physical and cognitive development in endemic areas.

References

Al-Jaber H.I, Mosleh I.M., Mallouh A., Abu Salim O.M., Abu Zarga M.H. 2010. Chemical constituents of *Osyris alba* and their antiparasitic activities. J. Asian Nat. Prod. Res. 12:814-820.

Argüello-García R., Cruz-Soto M., Romero-Montoya L., Ortega-Pierres G. 2004. Variability and variation in drug susceptibility among *Giardia duodenalis* isolates and clones exposed to 5-nitroimidazoles and benzimidazoles *in vitro*. J. Antimicrob. Chemother. 54:711-2.

Chandy E., McCarthy J. 2008. Evidence behind the WHO guidelines: hospital care for children: what is the most appropriate treatment for giardiasis? J. Trop. Pediatr. 55: 5-7.

Chen C.Z., Kulakova L., Southall N., Marugan J.J., Galkin A., Austin C.P., Herzberg O., Zheng W. 2011. High-throughput *Giardia lamblia* viability assay using bioluminescent ATP content measurements. Antimicrob. Agents Chemother. 55:667-675.

Dunn L.A., Andrews K.T., McCarthy J.S., Wright J.M., Skinner-Adams T.S., Upcroft P., Upcroft, JA. 2007. The activity of protease inhibitors against *Giardia duodenalis* and metronidazole-resistant *Trichomonas vaginalis*. Int. J. Antimicrob. Agents 29:98-102.

Escobedo A.A., Almirall P., Robertson L.J., Franco R., Hanevik K., Mørch K., Cimerman S. 2010. Giardiasis: the ever-present threat of a neglected disease. Infect. Disord. Drug Targets. 10:329-48.

Escobedo A.A., Cimerman S., Almirall P. 2011. An old drug against giardiasis: mebendazole as a treatment option. Option. Infect. Disord. Drug. Targets. 11:94-95.

Escobedo A.A., Cimerman S. 2007. Giardiasis: a pharmacotherapy review. Expert Opinion. Pharmacother. 8:1885-1902.

Fischer-Walker C.L., Aryee M.J., Boschi-Pinto C., Black R.E. 2012. Estimating diarrhea mortality among young children in low and middle income countries. Plos One 7:1-7e29151.

Flisser A., Valdespino J.L., García-García L., Guzman C., Aguirre M.T., Manon M.L., González González G., Gyorkos T.W. 2008. Using national health weeks to deliver deworming to children: lessons from Mexico. J. Epidemiol. Community Health 62:314-17.

Gardner T.B., Hill D.R. 2001. Treatment of giardiasis. Clin. Microbiol. Rev.14:114-128.

Gut J., Ang K.K., Legac J., Arkin M.R., Rosenthal P.J., McKerrow J.H. 2011. An image-based assay for high throughput screening of *Giardia lamblia*. J. Microbiol. Methods 84:398-405.

Johnson H.L., Liu L., Fischer-Walker C., Black R.E., 2010. Estimating the distribution of causes of death among children age 1-59 months in high-mortality countries with incomplete death certification. Int. J. Epidemiol. 39:1103-1114.

Lalle M. 2010. Giardiasis in the post genomic era: treatment, drug resistance and novel therapeutic perspectives. Infect. Disord Drug. Targets. 10(4):283-94.

Li Z., Liu Z., Cho D.W., Zou J., Gong M., Breece R.M., Galkin A., Li L., Zhao H., Maestas G.D., Tierney D.L., Herzberg O., Dunaway-Mariano D., Mariano P.S. 2011. Rational design, synthesis and evaluation of first generation inhibitors of the *Giardia lamblia* fructose-1,6-biphosphate aldolase. J. Inorg. Biochem. 105:509-17.

Li Z., Kulakova L., Li L., Galkin A., Zhao Z., Nash T.E., Mariano P.S., Herzberg O., Dunaway-Mariano D. 2009. Mechanisms of catalysis and inhibition operative in the arginine deiminase from the human pathogen *Giardia lamblia*. Bioorg. Chem. 37:149-161.

Liu L., Johnson H.L., Cousens S., Perin J., Scott S., Lawn J.E., Rudan I., Campbell H., Cibulskis R., Li M., Mathers C., Black R.E. 2012. Global, regional, and national causes of child mortality: an updated systematic analysis for 2010 with time trends since 2000. Lancet 6736(12)60560-1.

Manna D., Dutta P.K., Achari B., Lohia A. 2010. A novel galacto-glycerolipid from Oxalis corniculata kills Entamoeba histolytica and *Giardia lamblia*. Antimicrob. Agents Chemother. 54:4825-4832.

Müller J., Wastling J., Sanderson S., Müller N., Hemphill A. 2007. In vitro effects of thiazolides on *Giardia lamblia* WB clone C6 cultured axenically and in coculture with Caco2 cells. Antimicrob. Agents Chemother. 51:1979-1986.

Navarrete-Vazquez G., Chávez-Silva F., Argotte-Ramos R, Rodríguez-Gutiérrez M. del C., Chan-Bacab M.J., Cedillo-Rivera R., Moo-Puc R., Hernández-Nuñez E. 2011. Synthesis of benzologues of Nitazoxanide and Tizoxanide: a comparative study of their in vitro broad-spectrum antiprotozoal activity. Bioorg. Med. Chem. Lett. 21:3168-3171.

Nava-Zuazo C., Estrada-Soto S., Guerrero-Alvarez J., León-Rivera I., Molina-Salinas G.M., Said-Fernández S., Chan-Bacab M.J., Cedillo-Rivera R., Moo-Puc R., Mirón-López G., Navarrete Vazquez G. 2010. Design, synthesis, and in vitro antiprotozoal, antimycobacterial activities of N-{2-[(7-chloroquinolin-4-yl)amino]ethyl}ureas. Bioorg. Med. Chem. 18:6398-6403.

Núñez F.A., Escobedo A. A., Finlay C.M. 2004. Eficacia de varios esquemas de tratamiento para la infección por *Giardia lamblia* en niños. Rev. Panam. Infectol. 6:17-20.

Rousham EK. 1994. *An increase in Giardia duodenalis* infection among children receiving periodic Anthelmintic treatment in Bangladesh. J. Trop. Pediatr. 40:329-33.

Tejman-Yarden N., Eckmann L. 2011. New approaches to the treatment of giardiasis. Curr. Opin. Infect. Dis. 24:451-456.

Upcroft J.A., Dunn L.A.,Wright J.M., Benakli K, Upcroft P., Vanelle P. 2006. 5-Nitroimidazole drugs effective against metronidazole-resistant *Trichomonas vaginalis* and *Giardia duodenalis*. Antimicrob. Agents Chemother. 50:344-347.

Valdez C.A., Tripp J.C., Miyamoto Y., Kalisiak J., Hruz P., Andersen Y.S., Brown S.E., Kangas K., Arzu L.V., Davids B.J., Gillin F.D., Upcroft J.A., Upcroft P., Fokin V.V., Smith D.K., Sharpless K.B., Eckmann L. 2009. Synthesis and electrochemistry of 2-ethenyl and 2-ethanyl derivatives of 5-nitroimidazole and antimicrobial activity against *Giardia lamblia*. J. Med. Chem. 52:4038-4053.

World Health Organization. 2006 World Health Report, Genève, Switzerland.

You D., Wardlaw T., Salama P., Jones G. 2010. Levels and trends in under-5 mortality, 1990-2008. Lancet 375:100-103.

ROMANIAN COUNTY-MAP DESIGN OF HUMAN PARASITIC DISEASES: FINDINGS FROM THE CLINIC-FOCUSED SPECIALTY PRACTICE

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Parasitic Diseases Clinic from Colentina Universitary Hospital Bucharest correlates with the current trend in hospital models development, respectively that of hospital-focused specialty practice, being single human parasitic diseases (HPD)-dedicated medical unit in Romania. In 2009, the clinic has celebrated 15 years of activity.
1. To drawn a national mapping for the most encountered HPD;
2. To assess the multiannual comparative dynamics of hospitalized-based evidence of HPD;
3. To outline the clinical spectrum of HPD inside man pathology.
Over the last 5 years, 11921 cases have been admitted, with an annual rampant raise: 3597 cases during 2006-2008 period versus 8324 cases from 2009-2011, respectively. The nosologic portfolio has 91.2% tissular versus only 8.7% intestinal HPD. Among the tissular HPD the most prevalent are cystic echinococcosis, toxocariasis, toxoplasmosis and cysticercosis with a declined of trichinellosis (mainly as individual or cluster familiar cases and not as community outbreaks). The polymorphic clinical features of HPD consist in allergic disorders, sight deficiencies, neurologic abnormalities, Crohn syndrome, irritable or hyper permeability bowel syndrome, materno-fetal illness, autoimmune ailments. Also, increasing involvement of new or neglected parasites such as *Cyclospora, Dientamoeba* and *Blastocystis* as well as the emergence of rare (dirofilariasis, borreliosis, anisakiasis) or tropical parasitic diseases (filariasis, leishmaniasis) were noticed. The limits of HPD diagnosis and surveillance emerge from the lack of standardized antigens mostly, for tissular parasites, the relatively high costs of specific antiparasitic tests (uncovered by the national health system) and the absence of new active antiparasitic drugs.
Due to parasites ability in clinical travesty, the real magnitude of HPD inside pathology is underestimated. Also, the risky behaviours of modern lifestyle (food, pets, travel) of Romanian people adds an new challenge for the co-endemicity and polyparasitism phenomenon inside the global changing ecology of parasites, Romania included.

Keywords: parasitic diseases, comparative dynamics, hospital practice.

DETECT, PREVENT AND PROMOTE PARASITIC INFECTIONS ALERT IN ROMANIAN MIGRANT

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Due to free population movement, for many Romanians, mainly from rural areas, emigration is an opportunity for socio-economical rescue. According to the available statistics, the number of temporary and (at fewer extents) permanent Romanian citizens' emigration is estimated to be between 2.5-2.7 millions. On the other side, even at a modest magnitude inflow, Romania is attractive for foreigners' natives from developing world.
1. To review the current epidemiological and clinical status of human parasitic diseases (HPD) in migrant citizen/patient; 2. To further refine the Romania-linked migrant`s profile and risk assessment for HPD transmission, particularly their relevance to the EU health policy.
The survey has been conducted from 2008-2011 at the Parasitic Diseases Clinic - Colentina Universitary Hospital and Parasitology Department of Cantacuzino Institute, Bucharest. Data were collected per migrant seeking medical assistance, using a standard form including demographic characteristics, travel destination, purpose and stay duration as well as the relevant clinical features.
Of a total 392 patients with migrant-exposure risk and health-related complains, 335 were Romanian migrants/travellers and 57 immigrants. Demographically, 58% were male with a mean age of 34 years. The most common travel destination was southern Europe, then Middle East and Asia. At immigrant cohort, Middle East, Asia (mainly, China), Eastern Europe (Moldova, Ukraine) and Africa (Western and Central) were the source countries. As a travel purpose criterion, 71% traveled for work, 18% for leisure, 11% students. As sojourn duration, 7% stayed for short period (under 1 month), 3% long-period (> 6 months), 38% (1-3 months) and most (52%) for 3-6 months. Immigrants in Romania were mainly (85%) medium-period residents. The detected HPD were large spectrum: vector-borne (malaria, leishmaniasis, filariasis, myasis, and African trypanosomiasis), food/water borne (amoebiasis, anisakiasis, cyclosporosis, giardiasis, fasciolosis, taeniasis) and soil-transmitted (ascaridiasis, larva migrans).
The Romanian migrant profile is not well known, generally featured as a middle-aged, low-income, rural native male, temporary traveling for work purpose in Europe. The vector borne- HPD are the most frequent acquired, while the "exported" HPD were with autochthonous endemic parasites (*Giardia lamblia, Echinococcus granulosus*). Cross-border parasites represent a global increased threat for public health with an urgent need in specialized parasitic medical advice of both migrants and medical professionals.

Keywords: parasites, migrant health policy.

MEDICAL PARASITOLOGY - A NEGLECTED DISCIPLINE: TOWARDS EXPANDED CURRICULA INSIDE GLOBAL INTERNATIONAL HEALTH CONCEPT

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Parasitic Diseases emerge as a major public health issue in the industrialized world, Romania, included. Disapprovingly, the medical schools throughout this globalised world offer a limited interest to Parasitology in their teaching programs. Using a comparative multifactorial-guided analysis, we present an appraisal of Medical Parasitology Curricula and Training Programs at the undergraduate level inside Romanian medical schools.
Ten public medical schools were selected for collecting information on Parasitology education: number of lectures hours, number of practical classes hours, placement inside universitary year time-table, autonomously or concurrently course taught, use of computer-assisted facilities, use of clinical cases/ own teaching manuals/ handouts/ demo materials, exams evaluations, teachers` qualifications (clinicians or non-clinical).
Parasitology discipline is heterogeneously studied among Romanian medical schools, with an overall high-majority reduced less than 20 hours of lectures as independent courses. Majority (60%) taught Parasitology concurrently with Microbiology during the second or third year of undergraduate training and scarce Parasitology topics occur in Infectious Diseases modular Curricula. Tropical and Clinical Parasitology are mainly (80%) optional courses. The computer-assisted lectures are generalized methods of training, using mostly personal teaching manuals and handouts. The MCQs, macroscopic parasites identifications, quizzes and clinical or web-based virtual microscopy of specimens are in various degrees applied. The heterogeneity is also present in teachers` qualifications (Medicine or Biology background), majority (70%) with non-clinical practice, but all involved in basic or applied research in public or private institutions.
Even achieved the EU standard for teaching Parasitology, its current curricula among medical schools in Romania is reduced, devoted to respond to the real practice of Medicine in a changing world. As relevance and interest inside global international health concept, Medical Parasitology status should be imperatively upgraded.

Keywords: medical parasitology, curricula, education

STRONGYLOIDASIS - AN INTESTINAL HELMINTIASIS WITH POLYMORPHIC CLINICAL PICTURE

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Strongiloidasis is a nematode infection due to *Strongyloides stercoralis*, manifested through general and digestive disorders, chronic evolution, without the spontaneous tendency by the host for removal of the parasites, but on the contrary, with maintenance of parasitism and slow deterioration, which can be lethal in immunosupresed patients.
Although more common in warm countries, strongyloidasis is present also in Romania, where the climate of tropical type with hot summers, warm and moist, made possible the transmission of the infection, and where it is relatively difficult to implement control of the pollution of the soil, to prevent human contamination.
From April 2000 to April 2012, in the Parasitic Diseases Department of the Universitary Hospital Colentina, Bucharest, a number of 21 patients were diagnosed and treated for intestinal strongyloidasis.
The clinical picture of strongyloidasis is characterised by non-specific, polymorphic symptoms, which, accompanied by eozinophylia, evokes only a helminthiasic infection, without further specification. The emergence and persistence of digestive symptomatology for a long time in most patients, associated with cutaneous manifestations (present at 80.95% of cases), pulmonary disorders (present at 61.90% of cases) and psychic troubles (present at 71.42% of cases), have suggested the hypothesis of an infection with *Strongyloides stercoralis*.
Diagnosis of certitude has been established in all patients by direct coproparasitologic exams, as well as by stool cultures on charcoal.
Antiparasitic drugs were administered as follows: Thiabendazol in 38.09% of cases; Ivermectin at 14.29% of cases; Albendazole, followed by Thiabendazol in 47.61% of cases.
Diagnosis in strongyloidiasis represent a real challenge, due to diversity in clinical features and because the existence of particularities in the parasite biological cycle (autoinfection, with common long time persistence in the human host).

PUBLISHING IN VETERINARY PARASITOLOGY: AIMS AND SCOPE OF THE JOURNAL

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Veterinary Parasitology is concerned with those aspects of helminthology, protozoology and entomology which are of interest to animal health investigators, veterinary practitioners and others with a special interest in parasitology. Papers of the highest quality and novelty dealing with all aspects of disease prevention, pathology, treatment, epidemiology, and control of parasites in all domesticated animals, fall within the scope of the journal. Papers of geographically limited (local) interest which are not of interest to an international audience are usually not be accepted. However, authors who submit papers based on local data will have to indicate why their paper is relevant to a broader readership.
Parasitological studies on laboratory animals fall within the scope of the journal only if they provide a reasonably close model of a disease of domestic animals. Additionally, the journal considers papers relating to wildlife species where they may act as disease reservoirs to domestic animals, or as a zoonotic reservoir. Case studies considered to be unique or of specific interest to the journal, are also considered on occasions at the Editors' discretion. Papers dealing exclusively with the taxonomy of parasites do not fall within the scope of the journal.
Studies on bacterial diseases (such as caused by *Ehrlichia*, *Anaplasma*, *Eperythrozoon* and *Borrelia*) are considered for publication in Veterinary Parasitology, but only if the paper deals with vector transmission of these organisms to domesticated animals, or if zoonotic. Studies on Rickettsia *per se* are not accepted.
Studies dealing with parasite control by means of natural products, both in vivo and in vitro, fall within the scope of the journal, but only if well documented and with therapeutically relevant minimum inhibitory concentrations of the active compound(s) being clearly demonstrated.
Circumstances relating to animal experimentation must meet the International Guiding Principles for Biomedical Research Involving Animals as issued by the Council for International Organizations of Medical Sciences. (Obtainable from: Executive Secretary C.I.O.M.S., c/o W.H.O., Via Appia, CH-1211 Geneva 27, Switzerland).
The manuscripts must be original and submitted to the journal in electronic format (http://ees.elsevier.com/vetpar), following carefully the instruction for authors. At the end of the text, under a subheading "Conflict of interest statement" all authors must disclose any financial and personal relationships with other people or organisations that could inappropriately influence (bias) their work. Examples of potential conflicts of interest include employment, consultancies, stock ownership, honoraria, paid expert testimony, patent applications/registrations, and grants or other funding.
The manuscripts are assigned to one of the assigned Co-Editors for a preliminary assessment and then submitted to 2 or 3 reviewers for comments. Do not forget, it is possible to suggest some reviewers for your manuscript. The final decision it taken by the Co-Editor. When you resubmit a revised manuscripts, a cover letter must be attached where, point by point, your replay to the reviewers has to be noted showing clearly if the comment was accepted and where the suggested modifications have been included. In case, check again the reference list to be sure that all the references have been included following the journal system.
Finally, submitting a manuscript to Veterinary Parasitology you have to keep in mind that it is an international journal, the journal has a well educated international readership and your study should add some novelty to what already known about the topic you are working on. Veterinary Parasitology is one of the journals that each year increases their Impact Factor and a lot of manuscripts are received each day by the Journal Manager and by the Co-Editor in Chief for the Preliminary Assessment. It is a hard job and you should do your best to submit a well organized and interesting manuscript.

HISTORY AND DEVELOPMENT OF THE KOREAN JOURNAL OF PARASITOLOGY

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The Korean Journal of Parasitology (KJP) is the official organ of The Korean Society for Parasitology. It was first published in 1963 and will have the 50th anniversary in 2013. At the beginning, KJP was issued only once a year but the number of issues increased to biannual (twice a year) during 1964-1987 and quarterly since 1988 to date. We are now planning to issue bimonthly (6 times a year) from 2013. One of the turning points for the development of KJP occurred at 1989 when it began to be indexed by worldwide abstracting journals and websites, such as Index Medicus, Medline (now PubMed), Excerpta Medica, Tropical Diseases Bulletin, and Helminthological Abstracts. KJP became more or less advertised but some articles were still published in Korean language. Therefore, in 1993, we decided to make KJP a full English journal. This was another big event for the development of KJP. Now KJP is indexed by CAB International, Chemical Abstracts, Scopus, Google Scholar, and Science Citation Index (SCI). The SCI impact factor for 2010 was 0.963 and we expect it to be over 1.0 for 2011. The *h*-index of KJP during 2008-2011 was 6.0-9.0. Until 2000, most manuscripts were submitted from domestic researchers. However, since 2001 many manuscripts are submitted from international researchers and institutions, not only Asian countries, including China, Japan, Thailand, and Taiwan but also from other parts of the world, including Iran, Saudi Arabia, Turkey, Germany, Egypt, Nigeria, USA, Canada, Mexico, and Brazil. We publish mini-reviews, original articles, case reports, brief communications, book reviews, and letters to the editor on parasites of humans and animals, vectors, host-parasite relationships, zoonoses, and tropical medicine, and we welcome manuscripts from any parts of the world.

EDITING PARASITOLOGY IN ASIA

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Parasitology from a long time ago has been one of the most important fields in terms of human diseases. Not only too many people in developing countries are infected with parasitic diseases but also in some cases, developed countries are involved as such. For example the global burden as for malaria, is 45 million DALYs (disability-adjusted life years). Asia as the largest and oldest continent has to encounter this issue in many aspects. Knowledge translation in this field in Asia more or less has been implemented but the process needs to be appraised. In this category, a bird's eye view has clarified the different features in context of editing Parasitology in Asia. Two important databases including Thomson ISI and The SCImago Journal & Country Rank (Affiliated to SCOPUS) have been utilized to appraise this topic. Five specific journals released in Asia are indexed by Web of Science (ISI) including Parasitol Int, Korean J Parasitol, Iran J Arthropod-Bor, Iran J Parasitol, and Trop Biomed encompass an Impact Factor as 2.259, 0.963, 0.647, 0.605, 0.581, respectively according to the last released dataset. They belong to Japan, South Korea, Iran, Iran, and Malaysia in that order. From 1996-2010, Japan with 2,802 papers, Thailand with h-index of 53, Japan with 26166 citations, India with 8.233 self-citations, and Vietnam with 22.03 Citations per Document, have occupied the highest rate. More data in terms of analytical comparison with countries of Western and Eastern Europe, and global zone will be illustrated in detail. As a conclusion remark, the benchmarking profile on this topic in Asia sounds acceptable more or less, but in parallel with increasing developments and public desires more and more efforts should be implemented to meet the needs of a proper management of public health affairs.

Keywords: parasitology, journalism, Asia, paper, article.

PARASITOLOGY JOURNALS IN ROMANIA: PAST, PRESENT AND FUTURE

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The presence of members of prestigious international journals publishing in the field of Parasitology in particular, and Veterinary Medicine in general, aims to broaden the horizon of Romanian researchers in terms of publishing the scientific results obtained in impact factor ISI publications. The cooperation between Romanian and international researchers in Parasitology aims to significantly increase the number of ISI scientific publications, as well as the number of publications (books, studies, articles) published by prestigious scientific publishing houses abroad with an increase of the rigor of the scientific activity. It is imperious that we maintain and improve national and international recognition of Romanian Parasitology by increasing its international visibility.
We also aim to create a priority list of the directors in Parasitology research in Romania, because scientific research is an essential part of the academic activity.
Participation in international programmes and collaboration with universities abroad ensures that students, PhD students and young researchers enter into the international circuit (they can study at foreign universities, participate in international events, conduct training sessions in laboratories and research centres of international universities). On the other hand, the outcome of these collaborations can also lead to an increase of the quality of the results obtained by implementing new working methodologies and by broadening the horizons for research.
The organization of high level scientific events at regular intervals, to which relevant scientific and academic international partners take part, and the support of joint publications (editing, co-edited volumes and books), joint editing of international rated journals and periodicals may lead to the international spreading and acknowledgement of Romanian Parasitology.
At present, publishing in the field of Parasitology in Romania is represented by the following journals: Revista română de parazitologie (The Romanian Journal for Parasitology) and *Scientia Parasitologica*.

CURRENT PROBLEMS FACED BY EDITORS OF PARASITOLOGICAL JOUrnals

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The pressures of being the editor of a parasitological journal in the 21st Century arise from numerous sources, although these vary considerably from journal to journal. Among these are technology, expectations of the publisher, citation indices, refereeing, competition, unscrupulous authors and an increased requirement for language editing. Some of these, often interrelated, problems include:
On-line editorial management systems, which may be a boon to editors of large journals, but make it too easy for referees to opt out of reviewing a paper – in some cases this greatly restricts an editor's options. Furthermore, some specialists, especially older ones, find it difficult to negotiate these systems and so decline to review. Consequently, for many saying 'no' rather than 'yes' is becoming the norm.
Finding good reviewers is problematical, especially for the editors of general journals unfamiliar with specialist areas. This is also difficult for specialist journals where potential reviewers are few and their average is increasing. Also, although some reviewers do an excellent job, whereas others produce perfunctory reports of little value. Consequently, there is a tendency to overuse good referees, putting them under pressure, and to ignore or overlook the poor ones.
The fashionable, but flawed, use, of impact factors is a problem to smaller journals. Such is the pressure to publish in high impact journals that these can cherry-pick articles, avoiding scientifically valuable, but non-topical, works which are unlikely to be well cited during the short period utilized by the ISI index. This applies especially to taxonomic papers.
In recent years there has been a significant increase in submissions from non-English-speaking countries, especially from Asia and South America. A large proportion of these need extensive language editing. An editor has either to do this himself or rely on the publisher, which may mean that it may be carried out in countries where English is not the mother tongue; this sometimes results in a publication in pigeon English!
Another problem relates to taxonomic papers, where fewer authors, referees and editors have a classical education or are familiar with the rules of the International Code of Zoological Nomenclature. There are therefore an increased number of mal-formed names for new taxa, many of which require correction under the Code.
Other points of contention include unjustified multiple authorships, huge repetitive 'Materials and methods' sections in molecular papers, and dishonest authors (plagiarism, double submission, fictional data, etc.).

MAIN ACTUALLY TRENDS IN PARASITIC PROTISTS RESEARCH; SOME EDITING CONCERNS IN ROMANIA

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Protists are eukaryotes with a unicellular level of organization, without cell differentiation into tissues. Sizes are small (micron range) and because of this, from van Leeuwenhoeck (1657) up to the 21st century the only available criterion for the assessment of higher taxa was the morphologicaly-based similarity of organisms. New ultrastructural work (since 1970') allowed new insights revealing the huge diversity within the unicells, as well as the supposition of independent genealogical linneages. The results were incorporated in the classification of Levine et al.,1980 but the last classification of Adl et al., 2005 incorporates results both ultrastructural research and molecular phylogenetic studies. Proved that data from modern morphological approaches, biochemical pathways and molecular phylogenetics are generally complementary and this has resulted in a classification scheme, identifying several monophyletic lineages within the protists. Today ***modern protistology*** targets a wide range of research issues including taxonomy, phylogeny and evolution, biodiversity, biogeography, immunology, molecular epidemiology, ecology of aquatic and soil protists; general morphology and cell biology of protists; molecular biology, genetics and genomics, proteomics of protists; symbiosis involving protists; parasitic protists, with special emphasis on physiology, biochemistry and molecular biology of Microsporidia (more than 1200 spp. infecting all animal phyla including humans-14 spp.). We think it is an urgent need for such concerns and recent results to be included in Romania in the new specialized university textbooks, in various monographs, as in reviewed articles published in the field journals.

Association of Romanian Parasitologists includes all specialists in parasitology (general, human, veterinary, phytoparasitology) and publishes "Revista Română de Parazitologie" (Journal of Romanian Parasitology) founded in 1991, published regularly (1 volume containing 2 issues annual). The scientific papers are written in Romanian (English or French too) accompanied by abstracts in English. From 2007 Abstracts are available on web page of APR. The Journal aims to showcase valuable new results of Romanian parasitologic research, with clinical, laboratory or experimental data, general reviews with scientific news in different parasitology domains, but also to inform about activities of APR or other similar societies in the country or abroad and also of the international bodies. Special volumes have been devoted to a theme, event, homage volume etc. In Romania there are no journals profiled a group of parasites or parasitology special chapters.

PUBLISHING STRATEGIES IN IMPACTED JOURNALS

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Oral Presentations

SY15

PROSTAGLANDIN E_2 AS A CESTODES IMMUNOMODULATOR

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Parasitic worms can survive in the host body for a long time due to their ability to control the immune response. Prostaglandins synthesized by parasites form an important class of immunomodulators. The mechanisms underlying immunoregulation of the host body with prostaglandins are poorly studied, and there is no evidence of prostaglandin functioning in cestodes. Prostaglandin E_2 (PG E_2) is the most important immunomodulator among prostaglandins. The present study investigated distribution of PG E_2 in the organism of *Diphyllobothrium dendriticum* and influence of PG E_2 on the cell culture of sticklebacks head kidney leukocytes.
D. dendriticum plerocercoids have been withdrawn from Baikal omul. The sections of plerocercoids were incubated with monoclonal antibodies against PG E2 and α-tubulin, than with secondary antibodies. This was followed by examination with the aid of laser scanning confocal microscope.
We have analyzed the frequency of leukocyte subsets (granulocyte to lymphocyte ratio) and production of reactive oxygen species (ROS), of head kidney leukocytes (HKL) isolated from sticklebacks following *in vitro* stimulation with PG E2 and with *S. solidus* conditioned cell culture medium.
PG E2-IR was revealed in cortical parenchyma cells with long processes passed through subtegument and tegument, and formed bulbar-shape protrusion at the surface of the distal cytoplasm. Co-localization of the PG E2-IR and α-tubulin-IR was found in tegument, subtegument and cortical parenchyma cells. A specific α-tubulin-IR was revealed in cells with long processes directed to the tegument. These processes are penetrated distal cytoplasm and formed bulbar-like protrusions on the tegumental surface. A specific PG E2-IR lay inside of the α-tubulin-IR loci.
PG E2-like immnoreactivity of cells having IR against α-tubulin has been determined for the first time. Judging from their location and terminal frontal glands, these elements fulfil an immunoregulatory function; their glands synthesize prostaglandin E2 and release it into host cells.
PG E2 has strong influence on HKL both immediately and after 4 days of incubation. It suppressed respiratory burst alone and with extracts of *S. solidus* and LPS. It decreases the leucocyte number, mostly lymphocytes. As compared with extracts of *S. solidus* and LPS, PG E_2 has inverse action.
We can see that PG E2 influence the sticklebacks HKL. But in any cases they have effect not similar to *S. solidus*. However, from another hand, extract of *S. solidus* is a mix of different biologically active substances. Therefore we need in more detailed investigation of biochemical composition of Cestodes parasiting in fishes.

RELATIONSHIP BETWEEN AN INFLAMMATORY MUCOSAL T CELL RESPONSE AND SUSCEPTIBILITY OF SHEEP TO *TELADORSAGIA CIRCUMCINCTA* INFECTION

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Teladorsagia circumcincta is the most economically important abomasal parasite of sheep in temperate areas. Resistance to the worm has been linked to a T-helper (TH2) response and susceptibility to TH1. However, ambiguity in the defined TH1/ TH2 dichotomy with different host and helminth species suggests that immuno-regulatory response is predictable with continuous re-infection with the worm. It is hypothesised that the development of a mature immune response in host protection against *T. circumcincta* involves cytokines and markers other than those linked to TH2 type of cells and that this response may be correlated with phenotype. It is also hypothesised that genetic variations in

these transcripts are linked to differential disease susceptibility. A total of 55 lambs aged 6-10 weeks and born from parents with known variability of resistance to gastrointestinal nematodes were either sham-dosed (non-infected control, n=10) or trickle-infected (infected resistant/susceptible, n=45) with infective larvae over three months to characterize the worm burden, body weight and IgA antibody levels. Lambs with a range of susceptibilities to *T. circumcincta* were identified, as assessed by faecal egg count (FEC), from zero or low FEC (resistant) to high FEC (susceptible). Histopathology showed only mild pathological changes in the abomasal mucosa of resistant lambs but gross lymphoid infiltration and inflammation in the mucosa and sub-mucosa of infected susceptible animals. Relative expression reverse transcription-quantitative real time polymerase chain reaction (RT-qPCR) of a range of cytokines and cell markers associated with the TH1, TH2, TH17 and T-regulatory (TREG) subsets identified IL6 and IL21 as significantly increased in abomasal lymph nodes (ALN) of the five most susceptible lambs in comparison to the five most resistant. IL6 and IL21 were also significantly increased and EBI3 and IFN; were significantly reduced in the susceptible vs. control comparison. FOXP3 was significantly increased in both the resistant and susceptible vs. control comparisons. Copy number RT-qPCR assays on the ALN of all 45 infected lambs revealed a significant positive correlation between IL6, IL21 and IL23A transcript levels with adult worm count and FEC, while IL23A was also significantly negatively correlated with IgA antibody levels. Significantly positive correlation of TGF levels with adult worm count and FEC were also seen in the abomasal mucosa. These data support the hypothesis that susceptibility to *T. circumcincta* is linked to the activation of the inflammatory TH17 T cell subset.

PARASITES AND CANCERS: PARASITE ANTIGENS AS POSSIBLE TARGETS FOR CANCER IMMUNOTHERAPY

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A negative correlation between certain parasite infections and malignancy in human population has been reported by different research groups. Anticancer activity of some parasites such as *Trypanosoma cruzi, Toxoplasma gondii, Toxocarac canis*, *Acantamoeba castellani* and P*lasmodium bergei* has been shown in experimental animals. *In vitro* investigations also revealed that some parasites such as *Trypanosma cruzi*, hydatid cyst protosclieces, and *Toxoplama gondii* show anticancer activities. Moreover it has been shown that cancer-associated mucin-type O-glycan structures are expressed by parasites, so cancers and parasites have common antigens. Finally it has been shown that some anticancer drugs such as Cisplatin have antiparasitic effects. In this paper anticancer activities of some parasites have been reviewed and discussed.
Keywords: Parasite, cancer, immunotherapy, common antigen.

SY26

GENETIC VARIABILITY IN B-TUBULIN ISOTYPE 1 IN BENZIMIDAZOLE RESISTANT/SUSCEPTIBLE *HAEMONCHUS CONTORTUS* FROM SHEEP POPULATION IN RAWALPINDI, PAKISTAN

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Nematodes infections affect the productivity of livestock industry all over the world. They cause production losses each year and result in mortality in extreme conditions. There has always been search for methods to control parasitism especially helmenthiosis. Use of synthetic drugs is being practiced from long time and various groups of anthelmintic drugs are available in market.

However, there is widespread emergence of anthelmintic resistance to almost all groups of anthelmintics available. There have been reports from various parts of world about resistant strains emergence especially in *Haemonchus contortus* a highly pathogenic nematode. Benzimidazole is among prominent anthelmintic group against which resistance is emerging very fast. The mechanism of benzimidazole resistance appears to be most common in many species ranging from fungi to nematodes and involve alteration in gene encoding β tubulin isotype1. Present study was carried out to find out the variation existing in β tubulin gene isotype 1 which is directly involved with drug binding capacity involving microtubules polymerization. Adult nematode *H. contortus* were subjected to DNA extraction, amplification and sequencing. Out of 50 individuals analyzed 37 showed benzimidazole susceptible gene while 13 were resistant indicating single nucleotide mutation at amino acid 200 TTC/TAC. In addition 12 organisms showed several regions of consistent difference indicating single nucleotide polymorphism (SNPs) at various positions in coding region. This was first study carried out in area to find the trends about emergence of resistance.

RESISTANCE TO SEVERAL CLASSES OF ANTIHELMINTICS IS NOT ASSOCIATED IN PARASITIC NEMATODES: WHY SHOULD IT BE?

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Each of the three commercially available anthelmintic classes target different mechanisms in the nematode. Thus, resistance to one drug would not be expected to influence resistance to another. However, synergy between drugs has been recorded in experimental and field studies. The hypersensitivity of resistant Caenorhabditis nematodes to different drug classes is indicative of an association of resistances to anthelmintics. The efficacy of a benzimidazole and of levamisole used against human hookworm infections was largely enhanced when both drugs were combined, providing additional support to sustain the hypothesis of synergy between drugs. One may also suspect that farming practises which favours resistance to one drug may also influence the build up of resistance to another. Specifically, frequent treatments with the same anthelmintic, their use in the absence of worms in refugia, and absence of quarantine add a high probability of introducing resistance. There is a real need to establish the independence or synergy to anthelmintics in field conditions. This study tested the hypothesis that resistance against each of the three main anthelmintics (benzimidazoles, levamisole and avermectins) were independent of each other. Data was analysed from 16 published surveys of sheep, goat or cattle gastrointestinal infections regrouping data from more than 1000 farms. An anthelmintic resistance status was assumed when the faecal egg reduction test was below 90%. The predicted calculations were as follows:, based on independence of probabilities for single resistance: the calculated associated resistances between Bz (Benzimidazoles) and Lev (Levamisole) resistance was equal to the frequency of Bz resistance in farms multiplied by the frequency of Lev resistance in farms. For example, in one survey these resistances were 56% and 53% respectively, thus making an associated resistance of 30% (56/100 x 53/100 = 30/100). This was comparable to the actual observed value of 35%. These calculations were based on the theorem of composed probabilities: i.e., event A is independent of event B if the probability of A is independent of B; in that case the probability of A and B is the result of the multiplication of the probabilities of A and of B. The differences between the expected and observed frequencies were statistically evaluated; they never were significant for all set of data. The relationship between observed and calculated associations of anthelmintic resistances was high and demonstrates that anthelmintic resistances in the field are indeed independent of each other. This means that combination of drugs could be sustainable in field conditions, when resistance status is not exactly known.

COMPARATIVE EVALUATION OF DRUG RESISTANCE TESTS IN EQUINE STRONGYLIDOSIS

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Equine strongylidosis study is a world - wide highly important topic due to the serious consequences of this disease and drug resistance. In this context, research conducted between 2002 and 2011 on equines populations in Romania, focused on identification through in vivo and in vitro tests of strongyls resistance. Identification of strongyls species obtained by fecal cultures coming from 1992 equines (26 horses populations, from 13 counties) revealed dominance of *Cyatostomum* genus (82.10-93.03%) followed by *Strongylus vulgaris* (4.17-10.10%). *In vivo* analysis by fecal eggs count reduction test - FECRT of resistance to benzimidazole, conducted between 2004-2011, on 992 horses belonging to 22 populations, revealed installation of resistance phenomenon in 66.66% of cases. *In vitro* strongyls resistance to anthelmintic drugs was detected by use of egg hatch assay (EHA) and larval development assay (LDA). Strongyls resistance at benzimidazoles was detected in 77.28% of the populations taken in to study. Statistical interpretation of data was carried by use of a mathematical resistance evaluation model devised in such a manner as to be able to determine the following characteristics: egg-hatch or larval development percentage, lethal concentration (LC); minimal inhibitory concentration (MIC) and resistance factor (RF). Our analyses establish that the correlation between FECRT and in vitro tests was 86.25%, quantified through biomathematical model. EHA and LDA tests data interpretation needed identification of equines populations that were never provided anthelmintic treatment. Moreover, even if the equines were not treated, they must not have been exposed to contamination risk with possible resistant strongyls from other treated equines. Such an equines population has been identified in the Danube Delta Natural Reserve, from Romania.

SY25/1

LABORATORY MICE EXPERIMENTAL INFESTATION WITH *TRICHINELLA SPIRALIS* LARVAE FROM FROZEN PORK MEAT

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The research was made at the Parasitical Diseases Clinic of FMV Iaşi, between november 2011 – february 2012, aiming to verify the viability of *Trichinella spiralis* larvae in pig and wild boar meat which had been frozen for long periods of time, through the experimental infestation in laboratory mice. The samples of infested meat came from D.S.V.S.A. Vaslui, having been frozen at -18^{0}C, for a variable period of time (8- 33 months 2009- 2011). The mice were separated in three groups of three subjects each, which received the same care and feeding throughout the experiment.
According to the experimental protocol, each group received 20 grams of thawed infested meat/ day, for three days, as follows: group I received infested pig meat which had been frozen for 33 months and 17 days, group II received infested pig meat frozen for 10 months and 22 days, and group III received wild boar meat, frozen for 8 months and 23 days.
The experiment was ended after 80 days, by euthanising the mice with chloroform and taking samples of muscle tissue from the abdominal, intercostal, diaphragmatic and dorsal muscles. The samples were preserved in 10 % formaldehide, after which they were processed for hystopathology, cut in 5 µm slices and coloured HEA and MGG. These were examined and photographed on the Motic optical microscope, oc. 10 x ob. magnifier, 10, 40.

The results showed the presence of T. *spiralis* cysts in the groups of experimentally infested mice, which proves the extraordinary resistance and viability of *T. spiralis* larvae in pig and wild hog meat which had been frozen at -18^{0}C, for 8-33 months.
The experimental reproduction of infestation with *T. spiralis* in mice, through ingestion of pig and wild hog meat, frozen for a long time, and the postexperimental identification of trichinellic cists in the muscles of infested mice, are signalled for the first time in our country.
Keywords: laboratory mice, experimental infestation, *T. spiralis,* frozen pig meat, viability.

DEEPENING THE MOLECULAR EPIDEMIOLOGY OF HUMAN AND PIG ASCARIASIS

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The question as to whether *Ascaris lumbricoides* from humans represents a different species to *Ascaris suum* from pigs has been a controversy for many years. Several hypothesis have been proposed to explain their taxonomic status and origin: a) *A. suum* and *A. lumbricoides* are two valid species; b) *A. suum* is the ancestor of *A. lumbricoides*; c) *A. lumbricoides* is the ancestor of *A. suum*; d) *A. suum* and *A. lumbricoides* are conspecific. Here, the genetic variation at nuclear and mitochondrial target regions (ITS and cox1, respectively), within and among Ascaris population from human and pig hosts, has been investigated from a range of different geographical regions, in order to infer the epidemiological implications of the phylogenetic relationships among samples. A total of 130 Ascaris worms from pigs and humans were examined (77 from Italy and 53 from Eastern European countries) using a PCR-RFLP approach of nuclear ITS rDNA that allows the differentiation of the two Ascaris species (from human and pig origin). A representative geographical sub-samples were also analysed by sequencing cox1 mtDNA, to compute variability al population level. Data were compared to GenBank retrieved sequences from endemic regions (Brazil, Japan, Zanzibar and China), to infer pattern of genetic differentiation using phylogenetic and phylogeographic approaches. The overall results revealed no fixed differences between human and pig Ascaris. The RFLP analysis confirms pigs as a significant source of human infection in non-endemic area and the presence of hybrid genotype between the two species, both in pigs and in humans hosts. The analysis of molecular variance shows that the "endemic-non endemic" origin is more relevant than "host-affiliation" in shaping variability. The phylogeographic networks seem to indicate a genetic differentiation of the Slovak sample with respect to the other area of collection and to database retrieved sequences. These results are in agreement with previous evidences, suggesting the existence of gene flow between the two taxa, with relevant implications on the systematics, transmission and control programs.

CLINICAL SIGNS AND CLINICOPATHOLOGICAL FINDINGS IN DOGS WITH *UNCINARIA STENOCEPHALA* INFESTATION

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The aim of this study was the investigation of clinical signs and clinicopathologiocal findings in dogs with *Uncinaria stenocephala* infestation.
The case files of 88 dogs, naturally infested, were reviewed. Fecal samples were collected and examined before administration of any anthelminthic therapy. Fecal parasitological examination was performed using a flotation method with special gravity 1,2. Blood samples were taken from those dogs, found positive for *Uncinaria stenocephala* eggs (mixed infestation not included in this study).
In a total of eighty-eight infected dogs (17 pure and 61 mixed breeds, 46 males and 42 females, 40 less than 6 months and 48 up to 6 months of age), sixty-nine dogs did not present any clinical sign, in 11 (12.50%) cases there was pallor of the mucous membranes, while in 11 (12.50%) dogs diarrhea was reported by the owners, also 5 (5.68%) exhibited generalized weakness, 4 (4.55%) had a dull, hair coat, and 7 (7.95%) had skin lesions, and finally, one dog (1.14%) was presented with vomiting.
Clinicopathological screening showed in a total of 88 blood samples taken, 58 of them were in normal value for hematocrit (HCT), anemia in 28 (31.82%) and increased hematocrit in 2 (2.27%) dogs. In 28 (31.82%) cases there was a decrease of hemoglobin concentration (HgB) and peripheral eosinophilia was recorded in 12 (13.64%) dogs.
Concerning total proteins (TP), in 56 (63.64%) dogs had low while in 4 (4.55%) had increased concentrations. Serum albumins (Alb) were subnormal in 48 (54.55%) dogs while increased in 3 (3.14%) dogs. With respect to alanine aminotransferase (ALT), 2 (2.27%) dogs had low while in 5 (5.68%) dogs had increased concentrations. Serum aspartate aminotransferase (AST) had low in 2 (2.27%) dogs and in 5 (5.68%) had increased Urea.
The results found in infected dogs by *Uncinaria stenocephala* showed that most of them (78.41%) were asymptomatic and diarrhea was the most prevalent sign in the remaining symptomatic animals. Common clinicopathological changes included a decrease in serum Total proteins and Albumins concentrations, 63.64% and 54.55% respectively. Anemia and decreased hemoglobin concentration were the most frequent alteration appearing in 31.82% of examined dogs.

THE USE OF DELTAMETHRIN ON FARM ANIMALS: OUR EXPERIENCE ON FLEA CONTROL OF SMALL RUMINANTS

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Ectoparasites reduce significantly animal production and welfare. They cause nuisance, anaemia, irritation and transfer of pathogens of important diseases, often leading to animal death. Furthermore, ectoparasites may attack humans and threaten public health. Their effective control is largely based on the use of chemicals. Deltamethrin is a synthetic pyrethroid with strong properties to repel or kill arthropods infesting livestock, such as biting midges, nuisance flies, ticks, lice, certain mites etc. Results from many field trials demonstrated the high efficacy of this compound to protect ruminants from midges, i.e., *Culicoides* spp., for periods over 4-5 weeks, even if the animals became wet several times. It has been found to be effective against ticks, including all developmental stages, mosquitoes and many others. The purpose of this pilot study was to evaluate under field practise the effect of deltamethrin against fleas infesting small

ruminants in Greece. Fleas pose a significant problem in dairy sheep and goat farms of the country, since they attack not only animals but farmers as well. Insecticide residues in milk, when treatment is applied during milk production, are a restraining factor. Twenty (15 goat and 5 sheep) farms were identified and deltamethrin (Butox®, MSD) was applied to all animals at the recommended dose rate. Herds/flocks consisted of 100-200 head of local dairy breeds. Information was collected regarding the management system of the farms, particularly on manure handling. Animals within each farm were randomly inspected every week for a minimum period of one month. No fleas were found during the post-treatment period. The main flea species identified was *Ctenocephalides felis*, which is known to be very common and widespread. Deltamethrin (Butox®, MSD) was found to an effective tool to control flea infestation in dairy farms, even during milking period, in Greece. In conclusion, deltamethrin (Butox®, MSD) can be successfully used for animal protection in control programmes against fleas and other arthropods.

EVIDENCE FOR THE VERTICAL TRANSMISSION OF *BABESIA CANIS* IN A LITTER OF CENTRAL ASIAN SHEPHERD PUPS - THE CASE STUDY

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Tick-borne diseases constitute an emerging health problem among dog populations worldwide. Recently, one of the most recognized in Poland is canine babesiosis caused by *Babesia canis* parasite vectored by *Dermacentor reticulatus* tick. The disease caused by this parasite leads in some cases to sever symptoms like anemia, liver, kidney and cardiac failure and even death, especially in young and elderly individuals. To the best of our knowledge, two routes of this piroplasm transmission to dogs were determined – by tick bite and blood transfusion.
In autumn 2011 three cases of babesiosis were diagnosed in a litter of 6-week-old puppies of a Central Asian Shepherd. The day following clinical manifestation of the disease in a first puppy, blood samples were collected from all pups in the litter (n=10) and from the female. The presence of *B. canis* DNA was detected in three puppies and their mother. Two of those puppies presented symptoms of babesiosis in following 24-48 hours after first disease occurred. The isolates derived form the pups and from the asymptomatic mother – 520bp 18S rRNA gene fragment – were compared and analyzed. All isolates were identical and showed 100% homology with *B. canis* group B (EU622793), supporting the same source of infection. Before female's treatment, the ultrasound of abdomen were performed and showed moderate splenomegaly.
With this case study we provide strong evidences for vertical transmission of *B. canis* in dogs. It shows the need of careful examination for tick-borne diseases of female dogs before mating, especially in endemic regions. The influence of chronic babesiosis on dogs' reproduction needs further investigation.

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IMMUNOLOGICAL CHANGES IN EXPERIMENTALLY INDUCED ACUTE EIMERIOSIS OF LAMBS

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Coccidiosis in young lambs at pasture has become a significant problem particularly with increased stocking densities and reduced availability of pasture for sheep. An animal's resistance to coccidia infection can be reduced by stress due to adverse conditions such as prolonged travel, dietary or environmental changes, or severe concurrent infection. The gravity of the disease is related to the number of parasites the young lamb picks up before being immune competent to fight the disease.
The study aimed to define the immunological influence of acute experimental *Eimeria* spp. infection in lambs.
Two batches of lambs (A-infected and B-control), aged 40 to 50 days, were subjected to an anti-stress treatment with Ursocyclin and Zoodin (days 0 to 5 of the experiment) and divided into four groups (A, B – unsuppressed and AS, BS- suppressed). Immune suppression was induced by administration of hydrocortisone, 25mg/day/lamb, between days 7 and 11. Animals of batch A were challenged on days 11, 13, 15 with 150,000 oocysts of *E. ovinoidalis* (50%), *E. crandalis*, *E. parva* (15%), *E. bakuensis* (7%), *E. ahsata* (5%), *E. intricata* (5%) and *E. faurei* (3%). Clinical examination, parasitological and immunological tests were performed on days 0, 5, 10 (prior to infection) and 25, 35 (post infection). Immunological changes were monitored by total leukocyte counts and N/L ratios, *in vitro* blast transformation in PHA M stimulated cultures, quantified by glucose consumption, phagocytic activity against *Staphylococcus albicans* and lysozyme levels, monitored by radial diffusion test against *Micrococcus lysodeicticus*.
The results indicated a decreased response to PHA in acute eimeriosis, more pronounced in group AS (-39.9% when compared to initial values) than in group A (-9.37%) or BS (-16.58%). There was a statistically significant (p<0.001) decrease in the phagocytic index 16 days pi in group AS (-70.9%), as opposed to BS (-33.04%). The non-specific humoral response was also lowered, lysozyme levels dropping by 53.7% în group AS compared to 22.10% in group BS. The N/L ratio used as a stress index, decreased 3.45 times in AS group as opposed to 2.88 times in BS group. It was concluded that acute experimental eimeriosis had a stronger negative effect on non specific cell-mediated and humoral immunity than on adaptive immunity. Environmental stressors, mimicked by corticosteroid treatment in this experiment, associated with a high number of infective oocysts, could negatively influence the clinical outcome and prognosis of naturally occurring disease due to impeded first line of defense.

HEMATOLOGICAL AND BIOCHEMICAL PARAMETERS IN CARPATHIAN GOATS THAT WERE DIGESTIVE AND PULMONARY INFESTED ON PASTURE

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Investigations were conducted in 2011, on a herd of 1450 Carpathian goats, private property, from the Costeşti-Botoşani area, in order to reveal the impact of digestive and pulmonary infestation on hematology, biochemistry and thus on growth performance and production. Blood samples were taken from the jugular vein in tubes with and without EDTA. From the same subjects stool samples were collected in order to identify eggs and larvae through qualitative (Willis, Vajda) and quantitative (Mc.Master, Euzeby) techniques, analyzing the intensity (OPG, LPG) and extensity (E %) of digestive and pulmonary infestation.
Hematological parameters included the number of leukocytes, erythrocytes, platelets, hemoglobin, hematocrit, MCV, MCH, and MCHC, which were measured with the automatic analyzer MS 4.5.
Biochemical parameters included alanine aminotransferase (ALT), aspartate aminotransferase (AST), Ca, Mg, urea, creatinine, albumin, globulin, total protein and were analyzed by photocolorimetric method using the automatic analyzer Accent 200, recently calibrated.

The results were statistically analised with the Student t test, calculating the Pearson correlation index (r) between hematological parameters and parasitic infestation.
Coproscopical comparative results (in the stable - grazing) showed very significant differences ($p \leq 0.001$) for the genus *Eimeria,* in lactating goats and adult male goats, very significant differences ($p \leq 0.001$) for the fam. *Trichostrongylidae* in adult lactating goats, and distinctly significant differences ($p \leq 0.01$), for cestodes in male goats.
Biochemical parameters were generally within normal range, except for calcium which had values of 7.27 ± 039 mg / dL in adult lactating goats, 8.07 ± 07 mg / dL in one year olds, and 8.53 ± 0.28 mg / dL in male goats, compared to the values of 9 to 11.6 mg / dL used as a reference.
The index of correlation between pasture infestation and the parameters analyzed showed a strong correlation (r = 0.917) between Hb and cestodes in one year old, pregnant goats, a strong correlation (r = 0.749) between Hb and cestodes in one year old goats which had recently calved, strong correlation (r = 0.882) between erythrocytes and *Eimeri*a genus; a strong correlation (r = 0.777) between erythrocytes and cestodes in one year old goats which had recently calved. We also found a strong correlation (r = 0.910) between MCV and *Trichostrongyilidae* in goats which had recent calved. Digestive and pulmonary parasites, spoliate the body with a direct impact on hematology, biochemistry and thus the production level.
Keywords: goats, endoparasites (digestive, pulmonary), hematological and biochemical parameters.

SY08

TRICHOBILHARZIA REGENTI: NEUROPATHOGENIC EFFECT OF THE MOST HARMFUL SPECIES OF BIRD SCHISTOSOMES

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Trichobilharzia regenti, similarly to other bird schistosomes, represents the causative agent of skin inflammatory reaction, cercarial dermatitis. Moreover, *T. regenti* is known as a parasite with neurotropic mode of its behaviour. Due to migration through the host nervous tissue, *T. regenti* infections of specific bird and accidental mammalian hosts could represent a more serious health risk than those by other species of bird schistosomes.
Ducklings of *Anas plathyrynchos* f. *domestica* (specific final hosts) and mice of BALB/c and SCID strains (unspecific hosts) were each exposed to freshly emerged cercariae of *T. regenti*. One group of mice was re-exposed to the same dose of cercariae. The mice were deeply anesthetized at several intervals post infection (p.i.) and transcardially perfused with saline, followed by 4% paraformaldehyde. The ducks were sacrificed at required intervals p.i. The animals were dissected; *nervus ischiadicus*, spinal cord and brain were removed and placed in paraformaldehyde. The tissues were processed for histology and immunohistology by embedding in paraffin.
Histological observation revealed that the presence of schistosomula in the duck CNS triggered an influx of immune cells (heterophils, CD3 lymphocytes, macrophages), and proliferation and activation of astrocytes. Schistosomulum migration caused disruption of axons and subsequent degeneration of neurons. Parasites were frequently observed in the subarachnoidal space of the spinal cord and, contrary to the persisting inflammation, schistosomula survived longer in this area than in gray or white matters of the spinal cord.
Similarly to the situation in ducks, the infections of mice showed that schistosomula migration caused an axonal damage. Presence of the parasite in the CNS initiated an infiltration of the surrounding tissue by immune cells (CD3 lymphocytes), and activation of microglial cells and astrocytes in the vicinity of schistosomula. The immune reaction was accompanied by formation of inflammatory lesions around the worms. The main role in the destruction of the parasite belonged to microglia/macrophages. Schistosomula destruction in the nervous tissue depended on the host immune status. Absence of T-cell response in immunodeficient SCID mice led to slower elimination of the parasite, if compared to the infection of immunocompetent BALB/c mice. Particular factors/antigens responsible for glial cell activation and parasite destruction are unknown, but their characterization is in focus.

Knowledge of the pathological effects of *T. regenti*, and characterization of the host immune cell responses within the CNS could contribute to assessment of potential health risks for birds and humans.

The experiments were supported by the Czech Science Foundation (Grant No. 502/11/1621) and the Charles University in Prague (the projects PRVOUK and UNCE 204017).

PATHOGENIC IMPACT OF BIRD SCHISTOSOME *TRICHOBILHARZIA REGENTI* ON IMMUNOMODULATED MURINE HOST

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Trichobilharzia regenti is a nasal neurotropic bird schistosome, migrating in birds and mammals via the peripheral nerves to spinal cord and brain. Birds (waterfowl), the definitive hosts, as well as mammals, abnormal/nonspecific hosts are infected by direct penetration of infective larvae (cercariae) through the skin. The routes of migrating larvae (schistosomula) are described in detail for definitive host (bird), as well as for abnormal mammalian host (mice). In mice, the survival of parasites is shortened, the migration is incomplete and the life cycle interrupted. However, the worms reach central nervous system and cause serious pathogenic changes in nervous tissues, which can be manifested by neurological symptoms.
The present study extends the knowledge on pathogenic impact of *T. regenti* on mammalian host. For experimental infections, BALB/c mice were immunomodulated by complete and incomplete Freund's adjuvant 7 days prior to exposition to cercariae (subcutaneous injection of 100 µl of adjuvant; subsequent infection by immersion of foots into the water containing 1000 cercariae). Control mice remained uninfected (BALB/c), or were infected without previous immunomodulation (BALB/c; SCID). Blood samples were collected 1-15 days post infection (p.i.) and mice were either anesthetized and transcardially perfused, or euthanized on days 1, 3, 5, 7 and 15 p.i. The spinal cord and brain was fixed with 4% paraformaldehyde and embedded in paraffin or JB4® embedding medium. Other organs/tissues were searched for parasite presence under binocular microscope. The migratory route/parasite location and local immune cell reaction were studied by methods of histology and immunohistochemistry, systemic immune response was characterized by ELISA and flow cytometry.

The research was supported by Czech Science Foundation (grant No. P302/12/P548) and Charles University in Prague (research programs PRVOUK No. P25/LF1/2 and UNCE No. 204017).

SY23/1

HUMAN CRYPTOSPORIDIOSIS IN FRANCE: CASE NOTIFICATION AND GENOTYPING DURING THE 2009-2011 PERIOD THROUGH THE ANOFEL CRYPTOSPORIDIUM NETWORK

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The ANOFEL *Cryptosporidium* National Network (ACNN) was set up in France upon request of health public authorities to provide them with informations on the incidence and epidemiology of human cryptosporidiosis in France. This Network was constituted on a voluntary basis by the French Association of Medical Parasitologists (ANOFEL) with the support of the National Institute of Disease Surveillance (INVS). ACNN includes 39 hospital Parasitology laboratories having a common working field on cryptosporidiosis. Each member is committed to notify new cases of

human cryptosporidiosis, collect stool samples for genotyping and related clinical/epidemiological data.
From January 2009 to December 2011, 283 cases of *Cryptosporidium* were notified (135 cases in 2009, 70 cases in 2010 and 78 cases in 2011). No group cases were identified during this period. As previously described, a seasonal peak was observed in summer-autumn. The M/F ratio was 1.37 in 2009, 1.8 in 2010 and 1.06 in 2011. The highest rates were reported among young children (0-4 years) and immunocompromised adults. Data analysis according to immune status shows that one third of patients had no immune deficiency, mainly young children and adults <29 year-old. The remaining two third patients were immunocompromised patients. HIV-infection was present in 25% of patients in 2009, 29% of patients in 2010 and 17% of patients in 2011 and previous history of transplantation was observed in 21% of patients in 2009, 23% of patients in 2010 and 26% of patients in 2011.
Genotyping of isolates revealed a major proportion of *C. parvum* and *C. hominis* both in immunocompromised and immunocompetent patients but other zoonotic species such as *C.meleagridis, C. felis, C. canis* and *C. cuniculus* were also identified. These data provide useful informations on the cryptosporidiosis burden in France confirming the prevalence in young children and showing a growing incidence in organ transplant recipients, mainly after renal transplantation.

The ANOFEL Cryptosporidium National Network. Eurosurveillance, 2010; 15(33): pII=19642.

TOXOPLASMA GONDII GENOTYPES IN HUMANS AND ANIMALS IN SERBIA

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Toxoplasma gondii has an unusual population structure, characterized by three main clonal lineages designated as type I, II and III. However, atypical and recombinant strains have been increasingly recognized, particularly in South America and Africa. In Europe and North America types I and II are the most frequent, with type II being largely predominant, although type III has been shown in Greece, Cyprus and Portugal. The aim of this study was to assess *T. gondii* genotypes in Serbia.
Genotyping was attempted both from human and animal materials, which included 160 human samples (blood, cord blood, amniotic fluid, ocular fluid, cerebrospinal fluid and bronchoalveolar lavage fluid) from 127 patients serologically suspected of toxoplasmosis, and blood and tissues from 144 rats (brains) and 32 pigeons (hearts). The protocol included serology for specific IgG (by direct agglutination), microscopic detection of tissue cysts (in rat brains) and/or bioassay in mice (human materials) and detection of *T. gondii* DNA, followed by bioassay of PCR-positive tissue in mice (pigeon hearts). Rat brains were analyzed directly, whereas pigeon hearts were first digested with trypsin. DNA was extracted using commercial kits, and *T. gondii* DNA was detected by Real Time PCR targeted at the 529-bp repetitive element (AF146527).
In the human samples, *T. gondii* DNA was detected in 52 (35.6%). Of 128 bioassay experiments, 32 were positive for cysts and/or serologically. In the rats, *T. gondii* DNA was detected in 15 (10.4%) while cysts were found in 11 (7.6%) animals. However, all bioassays were negative. Conversely, of the five (15.6%) seropositive pigeons, hearts from four animals were positive for *T. gondii* DNA, of which three were further bioassayed and all three bioassays were positive (two with high cyst burdens).
Genotyping of the parasite strains was performed by direct PCR amplification and RFLP analysis of markers including SAG1, SAG2, GRA6 and GRA7 in all PCR-positive samples. However, due to generally low concentrations of DNA in clinical samples, and poor harvest of brain cysts in many bioassay experiments, genotypes of only five human isolates have been determined, four as type II and one as type I. Genetic characterization of *T. gondii* strains from animals was successful from two pigeons, genotyped as type II and III, respectively.
These first data on the *T. gondii* population structure in the Western Balkans contribute to the molecular epidemiology map of *T. gondii* in Europe.

ANATOMO-CLINICAL PICTURE OF THE TRICHINELLOSIS OF THE DOMESTIC PIG EXPERIMENTALLY INFECTED WITH *TRICHINELLA BRITOVI*

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In this experimental paper the results of the anatomo-clinical exam are presented, as peculiarities which define a picture of the Trichinellosis manifestations obtained through infection with *Trichinella britovi*.
The working materials consisted of nine gruntlings, aged three months and an average weight of 34 kg, which were infested with mice meat containing 400-500 *T. britovi* larvae per gram of meat, 4000-5000 self- isolated in cysts larvae being calculated for 10 grams of mice; thus were calculated 110-120 in cyst larvae for every kilogram live weight of the gruntlings.
The mice that constituted the source of infection for the pigs were infested 45-50 days previously and under the trichinelloscopical control, a complete isolation in the cyst of the *T britovi* larvae was found, which were able to infest other animals.
The results obtained are described on the entire observation period (60 days) and detailed with numbers, graphics, 11- points photographic images, regarding: the appetite, skin modifications, the body temperature, enteritis and diarrhea, the presence of *T britovi* in the feces on a period of 5-15 d.p.i, manifestations of myositis,the result of the biopsy of the auricular and coccigian muscles, congestive-hemorrhagic lesions of the cloven hooves, Pneumonia lesions, neurological manifestations, located at SNC (world premiere in pig *Trichinellosis*, observed in human *Trichinellosis)* and the negative effects of growing fat.
The paper ends with conclusions:
From a clinical point of view, there is a real similarity between the human Trichinellosis and the one we made, experimentally, in the domestic pig.
The clinical manifestations presented in this paper and exemplified iconographical, are:

1. the decrease or disappearance of the appetite-anorexia-in the period between 3-45 days post infection, difficulties in gripping, chewing, in this period dominated by diarrheas, the appetite for liquid is high;
2. at six post infection days (d.p.i) passive hyperaemia phenomena appear through accumulation of venous blood, characterizing a stasis hyperaemia obvious at the head level, especially of the ears and eyelids;
3. the fever, increases, in average, since the second week to 39.5, then, weekly, at 40.9, 41.3, 39.5, in the 5^{th} week when it decreases at 38.4, 38.2 in the 6^{th}-8^{th} weeks;
4. the enteritis, accompanied by serous, serous-mucous diarrheas, sometimes with blood streaks;
5. the clinical manifestations of myositis, heavy walking, extended decubitus, muscular pains when pressing with the hand, hoarse squealing, kyphosis position, difficulties in swallowing
6. congestive-haemorrhagic lesions at the hooves level;
7. pneumonia lesions located at the apical and diaphragm lobes;
8. neurological manifestations, dromomanie,blindness;
9. the losses in the weight increase on the whole period of the disease evolution.

Keywords: Experimental Trichinellosis, *T. britovi*, gruntlings, anatomo-clinical manifestations.

This paper is a part of the PhD thesis entitled “The cytho-hysto-pathological and hysto-chemical study of the musculature of the pig infested with *Trichinella spiralis*” in the project “Doctoral scholarships for raising the quality of the young researchers’ formation in the field of Agronomy and Veterinary Medicine, contract code POS-DRU/88/1.5/S/52614.

SY06

SERODIAGNOSIS OF EXTRA-INTESTINAL AMOEBIASIS: VALIDATION OF A NEW ELISA KIT IN NON-ENDEMIC SETTING

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Extra-intestinal amoebiasis is a rare event in non-endemic setting and occurs in immigrants and tourists travelling in endemic regions. The direct detection in stool and abscesses is frequently negative, making the diagnosis difficult. Serological assays are thus an essential tool. Current methods include indirect immunofluorescence (IFI), indirect hemmaglutination (IHA), latex particle agglutination (LAT) and ELISA.
The objective of this study was to evaluate the performance and reproducibility of a new ELISA kit for the detection of serum specific anti-*E. histolytica* antibodies (IgG).
Reagents were provided by Bordier Affinity Products Company. Wells were coated with a soluble antigen derived from an axenic culture of *E. histolytica*. IgG were detected using protein A linked to alkaline phosphatase.
We studied 170 sera of patients hospitalised or consulting in Grenoble Teaching Hospital:

- 52 with an extra-intestinal amoebiasis;
- 7 with an intestinal amoebiasis;
- 71 non-amoebic patients (amoebiasis suspected but ruled out with certainty);
- 40 patients with other parasitic diseases;
- 99 blood donors as control group.

The results were compared with the performances given in the datasheets of 5 commercial ELISA tests.
Sensitivity of this test was 100%, specificities were respectively 89% (63/71: non-amoebic group) and 96% (95/99: blood donors' group). Other ELISA reagents currently marketed had lower sensitivities (95% on average) and lower specificities (95% on average with blood donors). The negative predictive value (NPV) of the test was 100%.
This test was as successful as other techniques routinely used in Grenoble Hospital (IFI and LAT) while possessing the advantages of ELISA format. A 100% NPV is very useful in non-endemic countries in order to exclude this disease.
We showed that this kit is a new valuable tool; it will be useful in diagnosis strategy of European patients. Similar studies are rare in European countries, revealing the originality of this work. Since its format is similar to other kits supplied by the same manufacturer, it will be possible to carry out jointly the serological diagnosis of several parasitic diseases.

CLINICAL AND LABORATORY STATUS OF ENTERIC SYMPTOMATIC AMEBIASIS IN PATIENTS

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Amebiasis is endemic in Iran and its infection rate is about 8-40%. Considering the diagnostic problems, studies on amebiasis have been sparse and little study has been performed compared

to other countries. This study was done with the aim of assessing the clinical and laboratory characteristics of enteric symptomatic amebiasis in one of Tehran's hospitals.
This cross-sectional analytical survey was performed on 159 patients who referred to the GI clinic of one of Tehran's hospitals who were selected by Census sampling method, between March and June 2009. Stool exam and serologic assessment was performed for all included patients. Ten days after treatment, stool and serology tests were requested again with the same sampling conditions. Data was analyzed by descriptive statistical methods and Chi square test with SPSS 14 software.
Highest and lowest frequency of clinical symptoms belonged to dysentery and vomiting. According to the first stool exam, 37 patients were infected with *Entamoeba histolytica/dispar*. Among them, 18 patients had cysts, 12 patients had trophozoites and 7 patients had both. After treatment, stool exam was positive only in two patients who had cysts.
Stool exam is a very poor diagnostic test for *Entamoeba histolytica* and more accurate methods such as serology or antigen test or PCR are recommended for the diagnosis of *Entamoeba histolytica* which is the only pathogen type.
Keywords: Amebiasis, Stool Exam, Serologic Exam, Clinical Symptoms, Laboratory Finding.

SY24

CHRONIC INTRAPERITONEAL HELMINTH INFECTION DISRUPTS SHORT-TERM MEMORY AS WELL AS NEUROTRANSMITTER AND CYTOKINE LEVELS IN THE HIPPOCAMPUS

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Helminthic infections are important causes of morbidity and mortality in many developing countries, where children bear the greatest health burden. The ability of parasites to cause behavioural changes in the host has been observed in a variety of host-parasite systems, including *Taenia crassiceps*-mouse model. In murine cysticercosis, mice exhibit a disruption in sexual, aggressive and avoidance predator behaviours. The present study was undertaken to characterize short-term memory, depressive status, neurotransmitters and cytokine levels in the hippocampus of cysticercotic male and female mice. Chronic cysticercotic infection induced a decrease in short-term memory in male and female mice; however, the effect was more pronounced in female mice. Interestingly, females showed f a significant increase in forced swim tests but a decrease in the mobility test. In addition, male mice showed an increment in total activity and ambulation tests. The levels of neurotransmitters, such as 5-HT, measured in hippocampus decreased by 40% in infected female animals. Interestingly, NE showed a significant increase in infected male mice when comparing to control male mice. The expression of IL-6, IFN-γ and TNF-α in hippocampus was markedly increased in infected mice of both sexes; however, expression of IL-4 was increased in infected male mice, but decreased in infected female mice. IL-1 show a slight decrease in either infected male or female mice when comparing to control mice. Our study provides clear evidence that chronic infection with cysticercus leads to persistent deficits on tasks dependent on hippocampal function. The object recognition memory task is particularly relevant because humans, particularly during childhood, in parasite endemic areas, have been reported to show deficits on recognition memory tasks. Thus, our findings support the key role of neuroimmune network in controlling short-term memory behaviors of *T. crassiceps* male and female infected mice.

SY14/1

EPIDEMIOLOGY OF CUTANEOUS LEISHMANIASIS AND TRANSMISSION CYCLE OF *LEISHMANIA TROPICA* IN SOUTHEASTERN TUNISIA

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Leishmania tropica zymodeme MON-8 (syn *L. killicki*) is the causative agent of Cutaneous leishmaniasis (CL) in Tataouine region, southeastern Tunisia, North Africa. The purpose of our work was to define the epidemiologic and clinical features of the disease and to explore *L. tropica* transmission cycle in this region.
The study area is situated in the northeastern part of the Sahara Desert; it covers four districts of the Tataouine governorate, which is located in a mountainous area under arid climate.
An Epidemiological survey performed between October 2008 and September 2009, recorded 102 confirmed CL cases. Species identification was performed using ITS1-PCR-RFLP analysis. *Leishmania tropica* and *L. major* were identified in 47% and 53% of cases respectively. Most lesions caused by *L. tropica* were single, face or limb-localized and had an insidious course. On the other hand, *L. tropica* CL cases were geographically scattered, suggesting that CL caused by *L. tropica* might be a zoonosis. The North African gundi which is extremely abundant in natural and peri-domestic environments of the study area is the putative reservoir host.
In July–September 2009 and 2010, 2546 sandflies were collected by CDC light traps in and around houses of *L. tropica* CL cases and in the habitats of gundi. Sandflies were identified according to morphological characters and by a comparative sequence analysis of cytochrome b gene to distinguish between *Phlebotomus (P.) chabaudi* and *P. riouxi*. Diversity analysis showed that *P. sergenti* was the most abundant sandfly species inside houses whereas *P. riouxi* was the most abundant in the natural habitat of gundis. Both *P. sergenti* and *P. riouxi* were equally present around houses in anthropogenic and semi-anthropogenic habitats. This distribution was consistent with the possibility of two transmission cycles: *P. riouxi* transmitting *L. tropica* among wild populations of rodent and *P. sergenti* transmitting the same parasite strains to humans within or near their houses.
In September-October 2009 and 2010, attempts were conducted to isolate *Leishmania* parasites from sandflies and gundis. *L. tropica* was identified after a dissection and culture in *Phlebotomus sergenti* caught inside the house of CL patient and by a real-time PCR in five gundis. Alignments of the ITS-1 DNA sequences and 7S-HRM curves analysis indicated that similar genotypes were present in humans, a sandfly and gundis from the same region.

CANINE VISCERAL LEISHMANIASIS IN PALESTINE: A NATION-WIDE FIELD SURVEY USING ELISA AND ITS1-PCR

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Canine visceral leishmaniasis (CVL) is caused by *Leishmania infantum* in all Mediterranean countries and transmitted by bites of sand fly vectors. The infection rate among domestic dogs in the West Bank, Palestine, was investigated by examination of parasites in culture from buffy coat, serology using ELISA and by PCR targeting the internal transcribed spacer 1 (ITS1), and cysteine protease B (CPB) loci. Samples were collected from seven districts in the Palestinian West Bank.

Of the 215 dogs examined for Leishmania infection, 36 (16.7%) were positive by at least one method. Twenty three dogs (11.2 %) were positive for *Leishmania* DNA, whereas, ELISA and culture revealed 16 (7.4 %), and 4 (1.9 %) positive dogs respectively. CPB-PCR on one of three culture-positive isolates revealed *L infantum* as the causative agent for CVL. Our study showed an association in the distribution of human visceral leishmaniasis (HVL) and CVL in some districts, suggesting that domestic dogs play a major role in the spread of *L. infantum* in the West Bank, Palestine.

Keywords: Sero-molecular diagnosis, Leishmania, domestic dogs, West Bank, Palestine.

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EPIDEMIOLOGICAL AND CLINICAL FEATURES OF VISCERAL LEISHMANIASIS IN THE EMERGING FOCUS OF KAIROUAN, CENTRAL TUNISIA

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Visceral leishmaniasis (VL) is an emergent disease in Tunisia since the early 90's. The aim of this study was to update the current epidemiological and clinical features of VL in Kairouan, the most affected governorate, and to analyze the factors of bad prognosis.
Data of 147 VL cases diagnosed from 2004 to 2009 in the pediatric department of Kairouan hospital were collected from the medical records. All patients were treated with Meglumine antimoniate as recommended by WHO. Epidemiological, clinical and biological parameters were analyzed using SPSS program.
Children less than 5 years old were the most affected (94.6% of cases with mean age 2 years and 1 month). No difference was found according to sex (Sex Ratio M/F=0.98). The geographical distribution of cases confirmed the recent spread of the disease to the Central and even Southern districts of the governorate. Diagnostic delays (mean of 36 days) have considerably shortened compared to previous reports.
The most observed clinical symptoms were spleen enlargement (98%) and fever (91.2%). Biological disturbances concerned mainly anemia (hemoglobin level < 9g/100 ml in 86.6%), leukopenia (46.6%) and the increase of γ globulin level (42.4%).
PCR, bone marrow aspirates exam and Serology (ELISA), showed respective sensitivities of 100%, 87.1% and 96.7% in VL confirmation. Iso-enzyme typing of 39 isolated strains identified *Leishmania infantum* species in all cases. The proportions of zymodemes MON-24 (28.2%) and MON-80 (17.9%) were surprisingly higher than those in other Mediterranean endemic regions.
The course of the disease allowed the patients classification in healed (n=135), relapsed (n=5) and died (n=7), ie a lethality rate of 4.8%. Bad prognostic factors were hemorrhage (p=0,002), severe thrombocytopenia (<100000/mm³) (0,003), severe anemia (p=0,015), low age (p=0, 02) and hepatic transaminases superior 4 folds to normal values (p=0,023).
The emergence of VL in central Tunisia is probably associated to the promotion of water restraints and to the development of agriculture in this arid area. Compared to previous reports, the disease seems to be better managed. However, efforts should be maintained to reduce more diagnostic delays and to improve the disease prognosis.

POLYMORPHISM IN HASPB REPEATS (K26) OF EAST AFRICAN *LEISHMANIA DONOVANI*: POTENTIAL EFFECT ON VACCINATION AND DIAGNOSIS

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Visceral leishmaniasis (VL) caused by *Leishmania donovani* is a major health problem in Ethiopia. Parasites in disparate regions are transmitted by different vectors, and cluster in distinctive genotypes. Recently isolated strains from VL and HIV-VL co-infected patients in north and south Ethiopia were characterized as part of a longitudinal study on VL transmission. Sixty-three *L. donovani* strains were examined by polymerase chain reaction (PCR) targeting three regions: internal transcribed spacer 1 (ITS1), cysteine protease B (cpb), and HASPB (k26). ITS1- and cpb - PCR identified these strains as *L. donovani*. Interestingly, the k26 - PCR amplicon size varied depending on the patient's origin. Most strains from northwestern Ethiopia (36/40) produced a 290 bp product with a minority (4/40) giving a 410 bp amplicon. All of the latter strains were isolated from patients with HIV-VL co-infections, while the former group contained both VL and HIV-VL co-infected patients. Almost all the strains (20/23) from southwestern Ethiopia produced a 450 bp amplicon with smaller products (290 or 360 bp) only observed for three strains. Sudanese strains produced amplicons identical (290 bp) to those found in northwestern Ethiopia; while Kenyan strains gave larger PCR products (500 and 650 bp). High-resolution melt (HRM) analysis distinguished the different PCR products. Sequence analysis showed that the k26 repeat region is comprised of polymorphic 13 amino acid motifs unique to *L. donovani*, as well as polymorphic 14 amino acid motifs, some which appear in *L. infantum*. The number and order of the repeats in *L. donovani* varies between geographic regions. HASPB repeat region (k26) shows considerable polymorphism among *L. donovani* strains from different regions in East Africa. This should be taken into account when designing diagnostic assays and vaccines based on this antigen.

This work was supported by a grant from the Bill and Melinda Gates Foundation.

CHARACTERIZATION OF THE HUMAN AND MOUSE IMMUNE RESPONSES TO *LEISHMANIA* EXCRETED/SECRETED PROTEINS

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Leishmania excreted/secreted proteins (ES) probably play an essential role in the establishment of infection and may thus constitute new potential vaccine candidates and drug targets. A previous work in our laboratory has led to the identification of *Leishmania* ES proteins. Here, we characterized the functional and immunological aspects of four ES proteins (EF1α, AAA-ATPase, P15 and P23).
We synthesized in *E. coli* and purified the recombinant proteins. Then, we have characterized the expression in different parasite stages of the native proteins using corresponding specific polyclonal antibodies by Western Blot. In addition, proteins localization in the parasite was done using immunofluorescence microscopy. We have also evaluated the effects of immunization with EF1α and P15 against *L. major* challenge in susceptible BALB/c mice. Finally, we have analyzed

the cellular immune responses induced by EF1α, AAA-ATPase, P15 and P23 in 15 asymptomatic Visceral Leishmaniasis (VL) individuals using proliferation and cytokine assays.
The indirect immunofluorescence using specific polyclonal antibodies anti-P15 and anti-P23 showed that P15 seems to be localized in the cytoplasm of *L. major* parasite with the presence of a dot close to the flagellar pocket. P23 displayed two localizations: in cytoplasm and flagella. Interestingly, vaccination with EF1α and P15 resulted in a significant increase in footpad swelling than with control vaccinated mice (with CpG adjuvant or with PBS) and a protective antigen identified in the laboratory. These results show that the two antigens exacerbate the disease.
The analysis of the cellular response against EF1α, AAA-ATPase, P15 and P23 in asymptomatic VL individuals showed that interestingly, EF1α and AAA-ATPase were able to induce a dominant Th1-type cytokine response, unlike the P15 protein that induced a mixed Th1/Th2 response.
Here, we characterized the proteins EF1α, AAA-ATPase, P15 and P23 by:
-The characterization of native P15 and P23 in *L. major* metacyclic promastigotes and their localization in *L. major* by immunofluorescence.
-The vaccination of susceptible BALB/c mice with proteins EF1α and P15 which induced an exacerbation of the disease progression compared to controls after a challenge with virulent *L. major*.
-The analysis of the human cellular responses against EF1α, AAA-ATPase, P15 and P23 in immune VL individuals which showed that both EF1α and AAA-ATPase induce a dominant Th1-type cytokine response suggesting that these proteins could constitute potential vaccine candidate. The immunogenicity of P23 should be confirmed with higher number of individuals.

USE OF FOURIER TRANSFORM INFRARED SPECTROSCOPY FOR CHARACTERIZATION OF *E. COLI* AND *B. BURGDORFERI* SENSU STRICTO SPECIES

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The aim of this study is to characterize and investigate differences between *Escherichia coli* and *Borrelia burgdorferi* sensu stricto by Fourier Transform Infrared Spectroscopy. This biophysical method is fast, sensitive, with low expenses and simple. FT-IR provided structural features of biological molecules such as proteins, nucleic acides, carbohydrates and lipids that are essential structural components of a bacteria cell.
The spectra were obtained in the wavenumber range of 450-4000 cm^{-1} using 2 mg of cell mass were made pill with 200 mg of KBr.
Our results provide spectral differences between *E. coli* and *B. sensu stricto* samples. Main differences have been observed in the following spectral windows: (1) 3250-3650 cm^{-1} in this region gave rise to very characteristic absorption bands due to hydroxy or amino groups. In this wavenumber range two differences can be seen, a peak at ~3291 cm^{-1} attributed to polysaccharides and amino acides O−H symmetric stretching vibration that appear only at *E. coli* and a significant absorption can be observed at ~3426 cm^{-1} only in *B. sensu stricto* spectrum*;* (2) 1500-1200 cm^{-1} is the mixed region containing information from proteins, free amino acides, polysaccharides, DNA/RNA and phospholipids. Comparing the obtained spectra of the *E. coli* and *B. sensu stricto* in this range the results show that the band which can be assigned phosphate double bond asymmetric stretching vibration of phosphodiesters appear at ~1231 cm^{-1} in the spectrum of the *B. sensu stricto*, while in the spectrum of the *E. coli* appear at ~1241 cm^{-1}. The shoulder at ~1258 cm^{-1} assigned to the C−O stretching of *O*-acetyl ester bonds only seen in *B. sensu stricto* species. The weak band at ~1339 cm^{-1} which was only seen in the spectrum of *E. coli* appear because the acetate is oxidised by tricarboxylic acid cycle. The band at ~1315 cm^{-1} represents amide III, clearly identifiable in the spectrum of the *B. sensu stricto*, but less visible in the *E. coli*; (3) 1200-900 cm^{-1} the bands in this region indicate the presence of the carbohydrates within the wall cell. The band at ~1120 cm^{-1} due to vibration stretching (CC) skeletal *trans* conformation DNA and RNA backbone can be seen only in *B. sensu stricto* spectrum.
This study suggested that FT-IR allows a noninvasive investigation of biological samples being able to identify main functional groups of bacteria.

SY14/2

RISK OF EXPOSURE TO TICKS AND CERTAIN TICK-BORNE DISEASES IN RECREATIONAL AREAS IN CENTRAL EUROPE

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The studies were conducted in the vicinity of Kozłowa Góra Reservoir (Silesian Province, Poland). It was built in 1939 on Brynica River and serves as potable water reservoir. It is widely used for different forms of recreation, there is a park located on its west shore. It is also inhabited by numerous species of animals, especially aquatic birds.
Questing ticks were collected by flagging in 2008 and 2010. Some environmental conditions were recorded for each sample: season, air temperature, relative air humidity, type of vegetation, insolation and wind exposition. Ticks were investigated by PCR, after DNA isolation by ammonia method, towards the presence of *Borrelia burgdorferi* sensu lato, *Anaplasma phagocytophilum*, *Babesia* sp. PCR-RFLP method with TasI enzyme was applied for detection of most common *Borrelia* genospecies. Tick-borne encephalitis virus (TBEV) was investigated in nymphs pooled in 10 individuals. RNA was isolated by columns. Real time PCR with confirmation of positive samples by pirosequencing, nested PCR and direct sequencing was applied for detection of viral genome. Studies were partially supported by grant from Iceland, Lichtenstein and Norway through the EEA Financial Mechanism and the Norwegian Financial Mechanism, No PL0343.
The total number of 1430 *Ixodes ricinus* ticks was collected by flagging. Larvae were dominant instars in 2008, while nymphs in 2010. Ticks were active from March to October with activity peaks in spring (May/June) and autumn (September). Tick activity correlated particularly with air humidity exceeding 70% in 2008. It was less distinct in 2010, with only one larval activity peak registered in autumn. Ticks were most numerous in deciduous and mixed forests with low or medium insolation and wind exposition. The most prevalent pathogen was *A. phagocytophilum*, present in 26/32 (81, 25%), then *B. burgdorferi* s.l. in 14/32 (43, 75%) and *Babesia* sp. in 1/32 (3, 12%). There were coinfections detected: *Anaplasma* with *Borrelia* in 11/32 (34, 37%), *Anaplasma* with *Babesia* sp. 1/32 (3, 12%). One tick was infected with all pathogens (3, 12%). Among genospecies of *Borrelia* complex, the most prevalent was: *B. burgdorferi* s.s. 14/14 (100%), then *B. afzelii* 4/14 (28, 57%), *B. garinii* 1/14 (7, 14%). Mixed infections were present: *B. burgdorferi* s.s. with *B. afzelii* in 3 ticks and all three genospecies in 1 tick. TBEV was recorded in 1 pool out of 21 investigated (0,48%).
The highest risk of exposure to ticks, especially *I. ricinus*, around Kozłowa Góra Reservoir exists in spring in forests. Among tick-borne pathogens, *A. phagocytophilum* and *B. burgdorferi* present the highest health threat for people and animals in this area.

PROSPECTIVE STUDY ON THE TRANSMISSION RISK OF *BORRELIA BURGDORFERI* SENSU LATO FROM *IXODES RICINUS* TICKS TO HUMANS IN ROMANIA

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There are few studies worldwide that have analysed ticks removed from bitten persons. The aim of our study was to identify ticks collected from humans, evaluate the prevalence of *Borrelia burgdorferi* sensu lato (Bb) infection in such ticks and investigate the clinical and serological outcome of the patients. Benefit of chemoprophylaxis after tick bite was also investigated.
All ticks collected from patients who presented to the Clinic of Infectious Diseases Cluj Napoca (01.04.-07.09.2010) were prospectively studied. Ticks were morphologically identified (species, developmental stage and sex) and investigated for Bb infection using Real Time–PCR targeting a fragment of the *hbb* gene. All patients bitten by Bb infected ticks and a control group (matched individuals bitten by Bb negative ticks, seronegative for Bb at the moment of tick bite) were asked for follow up one year later. A questionnaire was completed for each person. For investigating Bb antibodies two-tiered testing was performed (ELISA as screening, Western Blot as confirmation).
532 ticks were examined: 518 *Ixodes ricinus*, 10 *Dermacentor marginatus* and 3 *Haemaphysalis* spp. ticks, one unidentified tick. Out of 389 DNA extracts, 43 were positive for Bb (mainly *B. afzelii*, but also *B. garinii*, *Bb* sensu stricto - for the first time identified in Romania -, *B. spielmanii/B. valaisiana* and *B. lusitaniae*). 39 persons were identified as being bitten by the positive ticks. Out of them 20 persons presented for the follow up, and 20 matching persons were included in the control group. 5% of the persons developed manifestations of acute Lyme borreliosis and 12,5% seroconverted, with no difference regarding clinical and serological outcome between persons bitten by Bb positive versus Bb negative ticks (p>0.05). Comparing the group of patients with seropositive versus seronegative results, there was no difference regarding occurrence of clinical symptoms (p=0.39), results that provide evidence for unexplained symptoms in general population. No difference was found regarding clinical and serological outcome between individuals that took/didn't take chemoprophylaxis (p>0.05).
This is the first study in Romania that has analyzed humans after *Ixodes ricinus* tick bite with regard to Bb infection. The majority of ticks examined belong to genus *Ixodes*, the vector of Lyme borreliosis and all relevant human pathogenic genospecies of Bb were identified. Our results underline the importance of clinical follow up of the patient, with no benefit of testing for Bb in detached ticks. Benefits of chemoprophylaxis after the tick bite in our region could not be proven.

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LYME BORRELIOSIS: CLINICAL ASPECTS AND DIAGNOSIS

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Introduction: Borreliosis or Lyme disease (LD) is the most common tick-borne disease in Northern hemisphere, produced by at least three species of bacteria belonging to genus *Borrelia* spp. *(B. sensu stricto, B. afzelii, B. garinii*). LD is transmitted by the bite of infected tick belonging to genus *Ixodes.*

Objectives: define a better strategy to diagnose LD using serological screening and confirmation tests (indirect immunofluorescence - IIFT and Western blot - WB) and to correlate these methods with the clinical aspects. Samples were collected from patients supposed with LD, admitted in Eco-Para-Diagnostic, Medical Center from Bucharest, between October 2010 – November 2011. Tests were performed using commercial kits.
Results: a number of 627 blood samples have been tested for specific antibodies against *Borrelia* species, 305 using IIFT and 322 using WB. The total IIFT screened samples (162 for IgG and 143 for IgM), showed a positivity for IgG in 79 (48.77%) and for IgM in 38 (26,57%) cases. The samples of 53 patients with positive IgG and some with positive IgM were selected, evaluated and analyzed using differentiated WB (108 tests). The results showed: 2 (1.85%) WB *Borrelia afzelii* IgM positive, 14 (12,96%) WB *Borrelia afzelii* IgG positive, 1 (0.92%) WB *Borrelia garinii* IgM positive, 8 (7.40%) WB *Borrelia garinii* IgG positive, 7 (6.48%) WB *Borrelia sensu stricto* IgM positive and 4 (3.70%) WB *Borrelia sensu stricto* IgG positive (5 patients were infected with more than one species). The 53 patients with positive serology for IIFT and WB for *Borrelia spp*. IgG were also tested for co-infection with *Bartonella quintana/henselae* IgM and IgG (2 cases positive IgM and 10 positive IgG) and *Babesia* spp. (no positive cases). Out of the 53 positive samples for IIFT *Borrelia* spp. – 18 (33.31%) were confirmed using WB: 5 cases IgM and 13 IgG positive; 35 showed negative WB. The patients were aged between 7-54 years; 24 females and 29 males. For the confirmed cases, the main symptoms were: edema, headache, asthenia, tiredness, memory lost, anxiety and depression, troubles of sensitivity and movement and arthralgia. Eighteen patients recognized the insect bite.
Conclusion: The indirect immunofluorescence is a good screening test but must be confirmed by Western blot. All the positive samples using IIFT for detection should be further tested by Western blot for confirmation and correlated to clinical signs and symptoms. If the patients are symptomatic and the serology is still negative they must be re-tested periodically.

SY14/3

VECTOR-BORNE INFECTIONS IN IMPORTED DOGS - A SEROLOGICAL AND MOLECULAR SURVEY

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Several thousand dogs are imported into Germany every year, but only a fraction is submitted for diagnostic screening for vector-borne infections. This is mostly based on haematological and serological techniques such as the examination of a blood smear, Knott´s test as well as the evaluation for *D. immitis*-antigen and antibodies against *Babesia*, *Ehrlichia* and *Leishmania*, while molecular-biological methods are applied to a very low extent. In this study, a total of 222 dogs imported from South and South East Europe were evaluated by a “classical” profile as well as PCR for vector-borne infections.
A total of 44.1% of the animals were positive for antibodies or DNA. DNA of eight different pathogens – *Babesia canis canis*, *B. c. vogeli*, *Hepatozoon canis*, *Leishmania* spp., *D. immitis*, *Anaplasma platys*, *A. phagocytophilum* and *Ehrlichia canis* – was detected. Coinfections were identified in 8 samples by direct methods. Fifteen samples were positive for DNA of *B. c. canis*. This has considerable implications in case of import of such animals into environments with an adequate vector population (e.g., *D. reticulatus*).

VECTOR-BORNE INFECTIONS IN DOGS FROM KIEV, UKRAINE

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Vector-borne infections are an important issue in small animal practice, but information on the occurrence of distribution is comparably limited for some parts of Eastern Europe. To add to available data, engorged ticks from infested dogs were collected in spring 2010 and canine EDTA-blood samples and blood smears were obtained in 2011 in a veterinary clinic in Kiev, Ukraine. A total of 52 ticks (33 *Dermacentor reticulatus,* 19 *Ixodes ricinus*) and 23 EDTA-blood samples were screened by PCR for vector-borne infections. DNA of eight pathogens was detected: *Babesia canis canis*, *Anaplasma phagocytophilum*, *Dirofilaria repens*, *Rickettsia helvetica*, *R. monacensis* and *R. raoultii* in ticks and *B. c. canis*, *Hepatozoon canis*, *D. immitis*, *D. repens* and *Mykoplasma haemocanis* in canine blood samples.
DNA of *B. c. canis* was identified in 6 canine blood samples and in two ticks, *Dirofilaria* spp.-DNA (6x *D. repens*, 1x *D. immitis*) in three canine blood samples and as xenodiagnosis in 10 ticks collected from four different dogs.
Additionally, DNA of *R. raoultii* was recorded for the first time in *D. reticulatus* ticks in the Ukraine. The spectrum of vector-borne pathogens is comparable to that of neighbouring countries.

IDENTIFICATION OF RELAPSING FEVER *BORRELIA* SPECIES BY DIAGNOSTIC SPECIES-SPECIFIC PCR BASED ON FLAGELLIN GENE

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Tick-borne relapsing fever is an endemic disease in Iran. *Borrelia persica* is the causing agent for most of cases in the country, however, the epidemiological evidence for *B. microti* relapsing fever in southern and central Iran is also strong,. This study describes identification of two abovementioned relapsing fever agents in blood samples using species-specific PCR based on Flagellin (*flaB*) gene. The spirochete *B. microti* was originated from *Ornithodoros erraticus* ticks collected from rodent burrows in Hesark, near Karaj city in Alborz Province, and *B. persica* was isolated from *O. tholozani* ticks that were obtained from rural areas in Ardebil Province. The pathogenicity test on laboratory animals confirmed the identity of both isolates; *B. persica* caused heavy infections in guinea pigs; while, *B. microti* caused occult infections in guinea pigs and heavy bacteremia in adult mice. Molecular typing of the both species based on partial sequencing of *flaB* gene over 718bp exhibited 88% homology (51 nucleotide difference and 33 gaps). Presence of 12% nucleotide difference between two DNA sequences enabled designing species-specific primers using Gene Runner software, which amplified 224 and 417 bp fragments for *B. persica* and *B. microti*, respectively. Also, the shared reverse primer worked successfully with species –specific forward primers for both species and amplicons of about 200bp and 376bp were obtained with DNA from *B. persica* and *B. microti* infected blood samples, respectively. The species-specific primers could detect the DNA equivalent to as low as 20- 40 spirochetes of *B. microti* and *B. persica* in 200µl of blood samples.

DIFFERENTIAL ALTITUDINAL DISTRIBUTION OF TICKS IN DOGS OF NOMADIC TRIBES FROM NORTHERN KENYA

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Studies regarding distribution and epidemiology on ticks in Nord-Est of Kenya are rare and incomplete.Our research on this topic is based on a 5 years screening project on ticks spread over Lake Turkana and Mt. Kulal areas. An amount of more than 7000 ticks were collected from over 1000 dogs of all ages and sex. Identification was performed using morphological keys and the data were analyzed by Repeated Measures ANOVA, post-hoc Scheffe test and F test relating independent variables as seasons and regions. Final results were transcript on maps using GIS. *Rhipicephalus pulchellus, Rhipicephalus sanguineus, Rhipicephalus armatus* and *Amblyomma gemma* were identified using binocular microscopic magnifier. Results showed statistical differences between tick community structure, seasonality and geo-climatic distribution. Regarding season, variations we can assume that parasitism with *R. armatus* and *R. pulchellus* is more intense in September-October compared with January. Parasitism by *R. sanguineus* is not influenced by season. Considering the variability of climatic areas is relevant to affirm that *R. armatus* infest exclusively dogs living in semi desert areas, while *R. sanguineus* represent the exclusive species present on the shore of Lake Turkana. We also showed the presence of *R. pulchellus* on the entire area of research with a higher prevalence (both on dogs and as percent of tick community) in the afro-alpine region of Mt. Kulal, characterized by elevated humidity. This geo-climatic distribution is typical also for *A. gemma* which is present exclusively in Mt. Kulal alpine area.

SY14/4

MOSQUITO (CULICIDAE) PREVALENCE IN THE KOŠICKÁ KOTLINA BASIN – EASTERN SLOVAKIA

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Mosquitoes in Central Europe are the most important hematophagous insects, that epidemiological role as vectors are increasing in ever changing climate.
Mosquitoes prevalence was studied in 7 localites in the Košická kotlina Basin, that is in sutiable area for mosquitoes reproduction. Mosquito larvae and adults were collected using valid standard methods (Kramář, 1958) and stored in 96% ethanol resp. ethyl acetate and by freezing (-18 ˚C). Larvae and adult mosquitoes were determined according to keys by Becker (2010) and Kramář (1958).
During the period of 2010 and 2011, 919 adult mosquitoes belonging to 10 species and 18 825 mosquito larvae belonging to 16 species were collected and identified. Most frequently were found larvae of *Culex pipiens /resp. torrentium/* (28 %), *Aedes vexans* (19.4 %), *Ochlerotatus cantans* /resp. *annulipes*/ (18.9 %), *Culiseta annulata* (6.2 %), *Oc. punctor* (6.1 %), *Ae. cinereus* (5,6 %) and *Oc. cataphylla* (5,7 %) species. Number of trapped larvae was the highest in April (42 %) and

July (19.5 %). Adult mosquitoes of *Culex pipiens* (40.2 %), *Aedes vexans* (19 %), *Culiseta annulata* (15.2 %), *Oc. sticticus* (10 %), *Ae. cinereus* (4.6 %) and *Oc. punctor* (4 %) species were the most prevalent. These species are relatively common and widely distributed and occur mainly in lowlands with warm climate, *Oc. punctor* and *Cs. annulata* also in uplands. The highest abundance of adult mosquitoes was recorded in June (21.2 %), July (27 %) and August (32.8 %). The ascertained prevalence of mosquitoes is closely related with the environmental situation that arose during the studied period in the subjected area.

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STOMOXYS SPP. (DIPTERA: MUSCIDAE), POTENTIAL VECTORS OF MANY DIFFERENT PATHOGENS

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Stomoxys flies are, like tabanids, potential mechanical vectors of a number of pathogens present in the blood of their hosts, animals, especially livestock, but occasionally humans. In livestock, their direct effect is mainly disturbance, skin lesions, reduction of food intake, stress and blood spoliation, however, they also induce a global immunosuppressive effect and the gathering of animals for mutual protection; meanwhile they favour development of pathogens in the hosts and their transmission. Their indirect effect is the mechanical transmission of pathogens. In case of interrupted feeding (due to host defence movements), *Stomoxys* can re-start their blood meal on another host; meanwhile, when injecting some saliva prior to blood-sucking, they can inoculate some infected blood remaining on their mouth parts. Beside this immediate transmission, it was observed that *Stomoxys* may keep some blood in their crop, which offers a friendly environment for pathogens that could be regurgitated during the next blood meal; thus a delayed transmission (12-72 hours) by *Stomoxys* seems possible. Such mechanism has potentially considerable epidemiological impact since it allows inter-herd transmission of pathogens. Viruses such as Equine infectious anaemia virus, African swine fever, West Nile and Rift Valley fevers are known to be transmitted by *Stomoxys*, while others are suspected such as Lumpy skin disease, Bovine leukaemia and Blue tongue viruses. Rickettsia (*Anaplasma*, *Coxiella*) and other bacteria such as *Bacillus anthracis*, *Enterobacter sakazakii*, *Staphylococcus* spp., *Pasteurella* sp., *Leptospira* sp. and *Escherichia coli* are also transmitted by *Stomoxys*. Parasites such as *Trypansoma evansi* and other *Trypanosoma* sp., but also *Leishmania sp.* are transmitted by *Stomoxys*, while their role in the transmission of *Besnoitia* is under study. Finally, *Stomoxys* was also found to act as an intermediate host of the helminth *Habronema microstoma* and may be involved in the transmission of some *Onchocerca* and *Dirofilaria* sp. being cosmopolite, *Stomoxys calcitrans* might have a worldwide and greater impact than thought on animal and human pathogens transmission.
Keywords: *Stomoxys* flies, mechanical vectors, viruses, bacteria, protozoa, helminths.

BARTONELLOSIS AND THE HUMAN DISEASES PRODUCED BY BARTONELLA SPECIES

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Bartonellosis is an antropozoonosis produced by bacteria of the genus *Bartonella*, that includes at least 23 bacteria species, some of which cause human diseases, including Cat-scratch disease (CSD), Carrión´s disease, Trench fever, bacillary angiomatosis, peliosis hepatis, endocarditis, chronic lymphadenopathy, and neurological disorders. Members of the genus *Bartonella* are facultative intracellular bacteria belonging to the alpha 2 subgroup of the class *Proteobacteria* and are phylogenetically closely related to *Brucella* species. Bacteria of the genus *Bartonella* are responsible for emerging and reemerging diseases worldwide and can present as illnesses ranging from benign and self-limited diseases to severe and life-threatening diseases.
In mammals, each *Bartonella* species is highly adapted to its reservoir host as the result of intracellular parasitism, and can persist in the bloodstream for a period of time. Intraerythrocytic parasitism is only observed in the acute phase of Carrión´s disease. *Oroya fever* is characterized by a red blood cell deficiency (hemolytic anemia) and fever, this acute form of bartonellosis being potentially fatal. *Bartonella* spp. have a tropism for endothelial cells, observed in the chronic phase of Carrión´s disease (*Verruga Peruana*) and bacillary angiomatosis.
The clinical course of human disease can vary with the immune status of the host. Trench fever, also known as 5-day fever or quintan fever, is a manifestation of initial infection with *B. quintana* and is characterized by infection of human red blood cells. Clinical manifestations range from asymptomatic infection to severe illness. Infection with *Bartonella henselae* can result in a focal suppurative reaction (CSD in immunocompetent patients), a multifocal angioproliferative response (bacillary angiomatosis in immunocompromised patients), endocarditis or meningitis.
While Cat-scratch Disease was recognized nearly 100 years ago, the bacteria that causes this and other *Bartonella*-related disease manifestations and the routes by which humans become infected needs much more research. *Bartonella henselae*, *B. clarridgeiae*, *B. koehlerae* can be transmitted by infected fleas and the inoculation of flea feces. The infected nail beds and saliva of cats are routes of transmission following a bite or scratch. Other mammals can be vectors. The transmission by sucking insects is possible. Some of the diseases due to *Bartonella* spp. can resolve spontaneously without treatment, but in other cases, the disease is fatal without antibiotic treatment and/or surgery. We studied in the last 5 years 34 cases of CSD, and 3 cases of endocarditis produced by *Bartonella henselae*, and in our oppinion there are many other human diseases, undiagnosed, due to *Bartonella* species.

BARCODING TICK SPECIES OF ROMANIA

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Ticks are of great medical and veterinary importance, and they cause direct damage to their host through attachment and feeding. They are regarded as the most relevant vectors of disease-causing pathogens in wild and domestic animals or in humans in non-tropical areas. Adults of the ticks are relatively easy to distinguish by morphological traits but identification of fully engorged nymphs or larvae is more difficult. An easy tool to identify a species is the DNA-barcode. For this purpose the most widely used is a sequence from the cytochrome oxidase subunit I gene (COI). In our study we aimed to characterize the partial COI sequence of certain Romanian tick species. The COI sequences can help us in the reconstruction of phylogenetic relatedness between species and also in developing a molecular key for rapid assignment of nymphs and larvae to a certain species. In our preliminary results we present species relatedness based on COI gene and possibilities to discern between species using PCR – RFLP methods when morphological analysis is dubious.

DETECTION AND TYPING OF *BORRELIA BURGDORFERI* SENSU LATO SPECIES IN *IXODES RICINUS* TICKS FROM ROMANIA

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A total of 464 *Ixodes ricinus* ticks (larvae 6%, nymphs 22%, adults 72%) were collected by flagging in 4 counties (Mureş, Braşov, Bacău, Bihor) of Romania and examined for the presence of *Borrelia burgdorferi* sensu lato by polymerase chain reaction (PCR) followed by RFLP targeting the *osp*A gene. The overall prevalence of infection was shown to be 6.7%, the infection was higher in adults (65%) than in nymphs (35%); no infection was found in larvae. The prevalence of *Borrelia burgdorferi* s.l. was higher in females (81%) than in males (19%). Genotyping was performed using nested-PCR-RFLP method. At last, two *Borrelia burgdorferi* s.l. species were identified: *B. afzelii* (68%) and *B. garinii* (32%), no mixed infection was detected. This is the first report of *Borrelia burgdorferi* in ticks in the northwestern part of Romania.

STRUCTURAL EVALUATION AND PARASITISM OF THE FEMALE REPRODUCTIVE ORGANS OF DOGS NATURALLY INFECTED BY *LEISHMANIA* (*LEISHMANIA*) *INFANTUM*

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Canine Visceral leishmaniasis (CanL) caused by *Leishmania infantum* is endemic disease in Brazil, where the dogs usually present a variable clinical, but sometimes asymptomatic animals are more prevalent than symptomatic. The infection results in clinical manifestations, ranging from unapparent sub-clinical infections to a systemic disease characterized by fever, anemia, progressive weight loss, hepatomegaly, splenomegaly, generalized lymphadenopathy, and cutaneous lesions. The most important microscopic lesion is accumulation of macrophage-containing amastigotes in the spleen, lymph nodes, liver, and skin. Genital lesions associated with Leishmania amastigotes have been more often described in male human patients, affecting the glans penis, prepuce and scrotum. However, an ulcerative lesion, containing macrophages parasitized with *Leishmania*, was reported in the vulva of a woman who contracted the infection venerally. Recent reports indicate the presence of *L. infantum* in the canine genital system. The goal of this study was to evaluate the structural changes and the level of parasitism in the genital system of bitches with natural infection with *L. infantum*. Fragments of vulva, vagina, cervix, uterine body, uterine horns and ovaries from nine bitches were collected, fixed in formalin and processed for histopathology and immunohistochemistry. Two groups of animals were used: the first was composed by dogs with positive ELISA test and bone narrow biopsy (Group I) and the second one only by positive ELISA test (Group II). Histological evaluation showed mild-to-moderate vulvar dermatitis, characterized by a histio-plasmo-lymphocytic infiltrated, particularly in the dermis layer in 100% of animals from Group I. No microscopic lesions were observed in cervix, uterus and ovaries in all animals. Amastigotes forms of *L. infantum* was detected by histological and immunohistochemistry methods in the vulva (4/5) and vagina (1/5) of animals from Group I. In conclusion vulvar and vaginal lesions were related with *L. infantum* infection.
Keywords: Canine Visceral Leishmaniasis, diagnosis, pathology, genital system.

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SY10/1

GENETIC VARIABILITY OF *ECHINOCOCCUS GRANULOSUS* SENSU STRICTO IN EUROPE

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The genetic diversity of *Echinococcus granulosus* sensu stricto (s.s.) metacestodes from four European countries was evaluated by the DNA sequence analysis of the cytochrome c oxidase subunit 1 (cox1) mitochondrial gene. Of the 312 organisms investigated, 132 were from Bulgaria, 35 from Hungary, 89 from Italy and 56 from Romania. Considerable intraspecific variation was observed in the mitochondrial cox1 sequences: 24 haplotypes were detected in the Eastern European population and seven in the Italian population. The Eastern European population parsimony network displayed a star-like features consisting of the most common haplotype EG1 (G1 genotype) and the three major haplotypes: EG2, EG3 and EG4. The EG1 was also the major haplotype in the Italian population network, though with a higher prevalence (73%) compared to the Eastern European network. The percentage of the population constituted by the G1 genotype was used as an indirect index to evaluate the genetic diversity within *E. granulosus* s.s. populations of Eurasia. A clinal correlation between the percentage of the G1 genotype and the geographical regions of Eurasia was observed: the G1 genotype is highly represented in the Mediterranean Basin; it decreases in Eastern Europe and South-West Asia and increases in China. This clinal correlation could reflect the spreading of livestock domestication from Southern-Western Asia during the Neolithic period, beginning around 12,000 BC.

ECHINOCOCCUS GRANULOSUS IN FRANCE: UPDATE OF THE DISTRIBUTION AND MOLECULAR CHARACTERIZATION

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Cystic echinococcosis is a parasitic and zoonotic disease of worldwide importance caused by *Echinococcus granulosus*. The *E. Granulosus* complex is composed as several species/genotypes described with different definitive and intermediate hosts. In Europe, *E. granulosus* is mainly present in the Mediterranean basin. Today France is commonly considered as endemic due to the results of the last survey occurring in 1989. Nevertheless, there is no obligation of report from slaughterhouses concerning *E. granulosus* spp. then no case is officially declared since the 90's and reported yearly to the EFSA.
In order to confirm the current presence of the parasite, a one year survey was organised in 12 departments in South of France and Corsica Island in 2009-2010. All hydatid cysts observed from liver and lungs during the routine meat inspection at the slaughterhouse were transferred to the lab for molecular characterization (cox1 and nad1). Genotypes G1 (n=9), G2 (n=10) and G3 (n=5) of *E. granulosus* were determined in 24 sheep, G1 (n=2) and G3 (n=2) in four cattle in the South. Genotype G6-7 was identified in 180 pigs in Corsica. The presence of genotype G2, G3 of *E. granulosus* senso stricto and G6-7 of *E. canadensis* were described for the first time in France.
Due to this confirmation of presence, a national scale survey has been organised by the French Agriculture ministry in 2012. All slaughterhouses in France must transfer all suspected hydatid cysts to the national reference laboratory for molecular diagnosis. After the early months, genotype G1, G2, G3 in sheep, G1 and G3 in cattle and G6-7 in pigs were confirmed. These results

confirmed previously identified endemic areas in South in sheep and pigs in Corsica. New areas were also observed notably cattle in the Pyrenees a historical highly endemic area. At the end of the survey, the first national molecular overview of the presence of *E. granulosus* in France will be available and can be compared to the European neighbouring countries. Breeders concerned by *E. granulosus* cases will be informed and advised to prevent future cases. Further studies will be realized on particular high endemic foci identified to determine the origine of the lifestock contamination.

LAPAROSCOPIC VS. OPEN SURGERY FOR HEPATIC HYDATID CYST

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Introduction: Considering the risk factors for the postoperative complications for patients operated for hepatic hydatid cysts described in the relevant literature, we wanted to make a comparison between the classical surgery and the laparoscopic one starting from these factors, and underline the advantage of laparoscopic surgery.
Method: We realized a retrospective case-control controlled study on a total of 52 cases, 26 of which undertook laparoscopic surgery (case), 26 classical surgery (control), operated between 2003 and 2009. The factors considered for the selection of the control group were: Gharbi type, preoperative biliary fistulae, gender, age interval (± 10 years), the intervention type. We used "minor" selection factors: co-morbidities, rural/urban provenience, the operating team, associated procedures. The factors that were compared between the two groups were: duration of the intervention, length of postoperative stay, intra- or post- surgery complications, drainage duration, reinterventions, recurrences, postoperative pain medication.
Results: The groups contained 40 women and 12 men, ages between 14 and 70 years (average 36.47); 29 cases come from rural areas and 23 from urban ones. Most of the cysts were Gharbi I (16 - 30.76%). Each group had one case with pre-operatory biliary fistula. The cyst sizes varied between 3 and 20cm (av. 7cm) in the case group and 4 to28cm (av. 9.34cm) in the control one.
The average duration of the laparoscopic intervention was 77.72 minutes (median 65, min. 30, max. 170). For the classical surgery, the average time was 75.45 minutes (median 70, min. 50, max. 110). No intra operatory complications appeared.
The drainage was maintained in average 5 days for the laparoscopic group (median 3, min. 1, max. 17 – for a post operatory biliary fistula). In the control group, the drainage duration averaged 5.72 days (median 4, min. 2, max. 13).
None of the groups received routine post operatory anti-parasitic medication, but both received routine antibiotic treatment. Fever appeared equally in both groups (30%). Major pain medication was necessary in 5 cases all in the classical surgery group. Biliary fistulae appeared in 2 cases (7.69%) in the case group, and 3 cases (11.53%) in the control group.
The average postoperative hospital stay was 6.23 days for laparoscopic interventions, (median 4.5, min. 3, max. 17). For classical surgery the average was 9.03 days (median 6.5, min. 4, max. 25).
There were no recurrences or reinterventions for the studied cases. There was one case of anaphylactic shock post operatory in the control group. There were no post operatory abscesses.
Conclusions: Maintaining a strict selection of cases as to reflect equally the risk factors established in the literature, we concluded that although it is technically more difficult and might take longer, the laparoscopic intervention is better, reducing the hospital stay, the impact on the abdominal wall, the postoperative pain. There are no indications of more post operatory complications after laparoscopic interventions. The small number of cases in the study requires a larger study for a definitive conclusion.
Keywords: hepatic hydatid cyst, surgery, laparoscopy, classic, comparison.

HYDATIDOSIS IN BOYER AHMAD DISTRICT, SOUTH OF IRAN

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Hydatidosis is an endemic zoonotic disease in Iran specially in the areas with nomadic life style with animal husbandry. In the most parts of Iran the disease is caused by *Echinococcus granulosus*. This study aimed to determine the last condition of hydatidosis in different hosts in Boyer Ahmad district in south of Iran.
Material and Methods: The number of new operated patientes was collected from hospitals and the number of infected livestock was collected from industrial slaughterhouse. Twenty four dogs necropsied and the worms were detected and diagnosed morphologically after staining. Molecular characterization of the parasitic agent in larval stage conducted by PCR-RFLP with Alu-I and Rsa-I enzymes on isolated hydatid cysts from infected livestock.
The number of new operated patients in last eleven years was 113 cases (67% female and 33% male). The number of new infected livestock was 18892 cases (4.97%) of total 380060 livestock. The most infection was seen in the lung of sheep (4.66%). From twenty four necropsied dogs, eight cases (33.3%) had *Echinococcus granulosus* in their intestine. Ninety three isolated hydatid cyst from livestock examined by PCR-RFLP and all of them were G1 genotype.
Different hosts were infected with *Echinococcus granulosus* in this area and the life cycle of the worm is established in the region. The main parasite that causes hydatidosis in this region was *Echinococcus granulosus* (G1) the same as some parts of Iran. Then the life cycle of parasite is established between dogs and domestic animals and human is an incidental host.
Keywords: Hydatidosis, *Echinococcus granulosus*, livestock.

SY10/2

SURGICAL APPROACH OF THE HYDATIC CYSTS DEVELOPPED IN THE RIGHT HEPATIC LOBE

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During 2000 – 2011, 268 patients with hydatic hepatic cysts were operated in "Colentina" General Surgical Clinic. 76 patients (28.3%) had hydatic cysts with large dimensions (over 10 cm), 164 patients had medium size cysts (5-10 cm) and 28 patients had cysts under 5 cm diameter. In 31 / 55 / 12 of these cases, multiple hydatic hepatic cysts were found (total of 97 cases, 37,3%). For the right hepatic lobe, the presence of the hepatic hydatic cysts was: 69 large / 158 medium/ 57 small cysts. The single hydatic cyst of the right hepatic lobe were found: 52 large / 84 medium / 15 small.
The surgical approach was performed as follows: 36 left hepatectomies (left hepatic lobe was found quite fully destroyed), 64 wedge resections/segmentectomies, 61 cystectomies with pericyst -digestive anasthomosis and 180 Lagrot operations (partial pericystectomy, cyst removal, drainage of the restant cavity). The external biliary drainage was used if the cysts had a massive biliary contamination (102 cases, 38.06%). Endoscopic sphincterotomy was required in postoperative care to decrease the bilious draining flow in almost 50% of cases.
The evolution was good in all cases. Hospitalisation was between 8 – 26 days, 16 days in average.
Conclusions:
- Lagrot operation remains a good option in the posterior hydatic cysts of the right hepatic lobe, no matter their size; therefore it was the most used procedure.

- Large hydatic cysts of the anterior and visceral surface of the liver allow a pericyst-digestive anasthomosis, which can provide a good management of the biliary leakage.
- Medium and small sized cysts of the right lobe allow resectional procedures, especially in favourable locations.
- There are many cases of multiple hydatic hepatic cysts which require complexe surgical procedures.
- For the multiple hepatic hydatic cysts, either for the large hydatic cysts, prezervation of the liver parenchima avoiding the resectional procedures as many times there is possible should be an important goal.
- Endoscopic sphincterotomy is a good solution for a persistent bilious drainage.
- Surgery for hydatic hepatic disease requires well trained teams which could perform various procedures.

Keywords: left hepatectomy, wedge resection, segmentectomy, cystectomy with pericyst-digestive anasthomosis, Lagrot operation, external biliary drainage, biliary contamination, conservative procedures, resectional procedures.

BILIARY FISTULAE - IMPORTANT ISSUE IN THE SURGICAL TREATMENT OF THE HYDATIC HEPATIC CYSTS

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During 2000 – 2011, 268 patients with hydatic hepatic cysts were operated in "Colentina" General Surgical Clinic. 76 patients (28.3%) had hydatic cysts with large dimensions (over 10 cm), 164 patients (61.2%) had medium size cysts (5-10 cm) and 28 patients (10.5%) had cysts under 5 cm diameter.
For the large and medium sized hydatic hepatic cysts, the most used surgical procedures were Lagrot operations, then pericysto-digestive anasthomosis and, only in those cases of hydatic cysts of the left hepatic lobe with almost total distruction of the parenchima, left lobectomies were performed.
The most often seen complication, intraoperative but mainly postoperative, for those conservative procedures which keep the pericyst laying over the liver surface, is the biliary fistula. According their flow, biliary fistulae could be healed using a conservative treatment, or they require endoscopic procedures (endoscopic sphincterotomy) in almost 50% of patients. Thus, the drainages of the restant cavities are obviously mandatory until the complete closure of the biliary fistulae.
Following this issue, an external biliary drainage performed during the surgical procedures, which decreases the pressure in the biliary ducts, could be useful, but in many cases the endoscopic sphincterotomy is needed. We performed this external biliary drainage in 102 patients (38.06%). The efficient alternatives to remove the danger of the biliary fistulae are the pericysto-digestive anasthomosis (conservative procedures) and the resectional procedures.
Well conducted and getting the important help of the endoscopic procedures, the biliary fistulae of the hydatic hepatic cysts have a benign course, but they need experience and alertness of the surgical team.

Keywords: biliary fistula, conservative procedures, resectional procedures, conservative treatment, endoscopic sphincterotomy, external biliary drainage.

GENETIC VARIATION OF *ECHINOCOCCUS GRANULOSUS* IN WILD BOARS FROM ROMANIA

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Introduction: In Romania, echinococcosis/hydatidosis is the most important zoonotic disease taking in consideration the high prevalence of the disease in animals and human population. In total 10 distinct genotypes of *Echinococcus granulosus* have been described using DNA sequence data: G1 (common sheep genotype), G2 (Tasmania sheep genotype), G3 (buffalo genotype), G4 (horse genotype), G5 (cattle genotype), G6 (camel genotype), G7 (pig genotype), G8 (cervid genotype), G9 (human genotype) and G10 (Fennoscandian cervid genotype).
The aim of this study was to determine the presence of *Echinococcus* sp. in wild boars living in a Romanian endemic area and to characterize the genetic variants of the parasite, correlated with the epidemiology of the disease in different species investigated in Romania.
Materials and method: Samples were obtained from 267 wild boars hunted in a private ground from Bihor County from western of Romania, in January 2012. PCR and PCR-RFLP analysis of ribosomal DNA was performed, using the pairs of primers BD1-4S, Eg9for.-Eg9rev. and Eg16for.-Eg16rev. All positive samples in PCR were digested with 5 restriction enzymes [MspI (BsisI), RsaI, CfoI (HhaI), AluI şi TaqI].
Results: From the total of 267 wild boars examined, 33 were found infected in necropsy. The ITS1-PCR with the pair of primers BD1-4S, Eg9-PCR revealed 28 positive. Eg16-PCR yielded for 13 (39.4%) samples two amplification products of 500 and 450 bp, characteristic for G7 (pig genotype), and for 15 (45.5%) samples two amplification products of 400 and 100 bp specific for G1 (common sheep genotype). The PCR-RFLP analysis of ITS1 region of rDNA and PCR-RFLP of the products obtained with pair of primers Eg9for.-Eg9rev. revealed the identity of samples obtained with pair of primers Eg16for.-Eg16rev.
Conclusions: *Echinococcus granulosus*, which is liable to cause severe disease in human population, is very spread in Romania and most in the North-West of the country. The DNA nalyses demonstrate that the common sheep genotype (G1) and the pig genotype (G7) are the most important in wild boars in Romania. These two genotypes being infectious for human population, we conclude that wildlife reservoirs should be taken in consideration for the management of the disease.
Keywords: *Echinococcus granulosus*, PCR, PCR-RFLP, genotype, wild boars.

SY10/3

**HEPATIC HYDATID CYST IN PEDIATRICS –
A CONTINUOUS CHALLENGE**

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Hydatid disease is a severe and common parasitic disease caused by larval stage of *Echinococcus granulosus*. Ultrasonography and computed tomography, the most important diagnostic tools, are essential for staging the disease, establishing the complications and planning the treatment. Ultrasonography is extremely helpful in diagnosis, staging and follow-up of hepatic hydatid cysts in children. Patients with abdominal pain, hepatomegaly, decreased appetite, fatigue were examined clinically, biologically and by imagistic methods. Ultrasonography offered the diagnosis of giant multiple hepatic hydatid cysts in the first case of a 15 years girl (one of the cyst was infected); the

second girl of 8 years of age presented on ultrasonography two large hepatic cystic mass in the right lobe of the liver containing multiple daughter cysts separated by a matrix of mixed echogenicity. Surgery was the treatment of choice in both cases, under medical treatment with Albendazole. In conclusion, ultrasonography is the cornerstone in the diagnosis, staging and follow-up of children with hepatic cysts.
Keywords: hepatomegaly, hepatic hydatid disease, ultrasonography, child.

DIAGNOSIS OF HYDATID CYST: IMAGING, SEROLOGY AND MOLECULAR

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Introduction: Hydatid cyst is one of the most common parasitic diseases in Romania, determined by the small cestoda of the dog, *Echinococcus granulosus*. The human is an accidental intermediate host, which allows the development of the larval stage of the parasite.
Aim and methods: to report a very close correlation between the three methods - imaging, serology and molecular diagnosis. A number of 126 patients admitted in Colentina Hospital and Eco-Para-Diagnostic Medical Center were evaluated between 2007-2011. The laboratory tested protoscolices viability and preserved samples for genotyping. All the patients were operated in Colentina Hospital and "Marius Nasta" Institute, Bucharest and received medical treatment with albendazole before and after, or only after surgery in Eco-Para-Diagnostic Medical Center, or in Colentina Hospital – Parasitology Department. Out of the 126 cases, 115 cases underwent conservative surgery and 11 cases underwent radical methods. Location of the cysts: brain (2), kidney (1), liver (94), lung (24), peritoneum (1), lung and liver (4). The size of the cysts: large > 10 cm (48), medium 5-10 cm (40) and small < 5cm (38). Imaging methods: ultrasound, according to WHO classification: CE1 (40), CE2 (44), CE3a (9), CE3b (7). Chest X-Ray performed in all the cases, reveled lung cysts in 28. CT has been performed in 24 patients and MRI in 1. The viability of the protoscolices tested in 125 cases, showed positive results in 112 cases. Seventy patients received Albendazol therapy before and 83 after the surgery, 42 did not receive any specific medication and for 1 it is unknown. ELISA test was positive in 92 patients, negative in 32 and was doubtful in 2 cases. Western blot was positive in 116 and negative in 10 patients. Out of the patients with ELISA negative, 24 had a positive result using Western blot technique. The specific patterns detected were P1, P2, P4 and P5, all specific for *E. granulosus.* Molecular diagnosis for genotyping was performed for 82 samples in University of Pavia and University of Milan, Italy. The following genotypes were detected: G1-G3 in 59 patients, G6-G10 in one patient and remained unclear in 22 patients.
Conclusion: Cystic echinococcosis is a very common and important disease in Romania. In order to get a real improvement of patients' condition, it is very important to properly manage the diagnosis, using serology and imaging methods. Molecular diagnosis in human CE will be continued after these preliminary results on all the samples.

SURGICAL DRAINAGE MODALITIES IN HEPATIC HYDATIC CYST – THE EXPERIENCE OF COLENTINA GENERAL SURGERY CLINIC

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In the last 5 years (2007-2011),in the General Surgery Clinic of Colentina Hospital, there were 146 patients operated for abdominal hydatidosis, most cysts being with hepatic localisation (124 cases). Hepatic cysts benefited from radical surgery in 27 cases (pericystectomy - 18 cases, hepatic resection - 9 cases), in 68 cases of cysts with diafragmatic hepatic topography were performed cystectomy with content evacuation, partial pericystectomy and external drainage of residual cavity, the remaining 29 cases, located on the liver visceral face, receiving cystectomy with partial pericystectomy and pericysto-digestive diversion, the diversion being executed using the stomach in 5 cases and the jejunum in 24 cases, for the last ones using an "omega-like" anastomosis in 13 cases and "Y-loop" a la Roux anastomosis in 11 cases. The pericysto-jejunal anastomosis was protected, in 10 of the cases, by a transanastomotic drainage of the residual cavity exteriorized a la Witzel through the jejunal partner, the drainage being kept for 3 weeks. For the cases that we used the external drainage of the residual cystic cavity, the drainage tubes were kept, on average, for a period of 6 weeks. The medical treatment with Albendazol that was initiated in preoperative was continued in postoperative, under the control of laboratory analysis. In 59 cases of those who have benefited from external drainage of residual cavity was need for postoperative endoscopic sphincterotomy 10-14 days after intervention. Evolution of cases was good, except for 6 cases that have developed abscess of the remaining cavity and undergone for reintervention 3-6 months postoperatively. Mortality was null.
Keywords: hepatic hydatic cyst, external drainage, pericysto-digestive diversion, endoscopic sfincterotomy.

THE ALBENDAZOL TREATMENT'S EFFICACY IN HYDATIC CYSTS WITH DIFFERENT LOCATIONS

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Evaluation of the albendazol treatment's efficacy in hydatic cysts with different locations in comparison with other types of therapies.
Evaluation of treatment's efficacy according to: cysts' location, size, type and immunological status of patient.
We performed a prospective study on 320 patients, during 5 years. We performed the drug-based treatment in cases of small and medium cysts sizes (< 7 cm).
In the case of exclusive drug-based therapy, the number of cures with albendazol was significantly bigger in comparison with the number of cures followed in the combined therapy. Therapeutic failure is associated with the usage of the exclusive albendazol-based treatment, thus surgical intervention combined with the drug-based treatment probably being much safer and with a higher success rate. $p<0.05$), p=0.0145 test $\chi 2$. Statistics do not show a significant difference between the hepatic and pulmonary hydatic cysts' healing rates, $p>0.05$ ($p = 0.5987$). Albendazol treatment's efficacy is higher in the case of hepatic localization than in other localizations. The efficacy of the albendazol therapy is significantly higher in the case of CH < 7cm in comparison with CH > 7cm, which requests a combined treatment. The efficacy of the albendazol treatment is higher in the case of the mono-located CH than in the case of multi-located CH. Patients with CD4 > 1000 cells/mm3 responded better to treatment in comparison with the patients with CD4 < 1000 cells/mm3. The efficiency of the albendazol treatment is smaller for the patients presenting co-morbidities.
Treating hidatid diseases with albendazol 800mg/day for 4 weeks, in repeated cures separated by free intervals of 2 weeks, had as a result the healing of 91.25% of the cases. The treatment has a

similar efficacy in the hepatic and pulmonary hidatic disease (91.63%, respectively 88.88%). The efficacy of the albendazol treatment is higher in the case of hepatic and pulmonary hidatic disease than in other localizations.
The positive predictive factors of the albendazol treatment response were: hepatic or pulmonary localization of the hydatic cysts, small dimensions, below 7cm in diameter, presence of the type1 hydatic cists, absence of co-morbidities and unaltered immunological status (CD4 > 1000 cells/mm3). The therapy is well tolerated, the percentage of the severe adverse responses which impose interrupting the treatment being 0.93%.

SY10/4

CURRENT STATUS OF ECHINOCOCCOSIS IN SERBIA

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Echinococcosis (hydatidosis) is traditionally endemic in South-East Europe, Serbia included. In Serbia, echinoccosis is mandatory reportable, and here we review the officially reported data as well as the research data published between 1998 and 2010. Official data on human and animal infections were obtained from the Reports on Infectious Diseases in Serbia, and from the Ministry of Agriculture, Trade, Forestry and Water Management and the Statistical Office, respectively. Published data were obtained by searching the Medline, Scopus and Google databases using “echinococcosis”, “hydatidosis” and “Serbia” as keywords. In addition, the search included national journals and doctoral theses, as well as conference proceedings. Only *Echinococcus granulosus* has been reported in Serbia, with a total of 409 cases of human infection officially reported during the observed period as opposed to 820 cases described in clinical studies. No trend in the incidence of infection was shown among adults, but the number of cases in children continuously decreased over the period. Patients were more frequently female (55-69%) and from rural areas (54-60%). Differences in the geographic distribution of cases were noted, with a lower incidence in the central part of country. Liver disease was by far the most common presentation, but cases of unusual cyst localizations have been described. Among domestic animals, a gradual but strong and sustainable decrease in the prevalence of CE during the last decade was evident in all analyzed species (from 14 to 1% in sheep, from 13 to 2% in cattle and from 9 to 4% in swine). This continues the decreasing prevalence of CE in animals that has been noted since the seventies. Echinococcosis remains endemic in Serbia in the 21st century, but despite predictions to the contrary, neither official data nor those from clinical studies indicate its re-emergence. However, there is gross underreporting. Public health authorities should actively work to increase reporting as only valid reported data provide an accurate basis for future control plans.

ECHINOCOCCUS MULTILOCULARIS DETECTION WITH REAL TIME PCR IN CONTRASTED ENDEMIC REGIONS

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Echinococcus multilocularis (Em) eggs are released into the environment with carnivore faeces. After ingestion by rodents or human the parasite can lead to the chronic Alveolar Echinococcosis.

The follow up of animal contamination is a key point in Alveolar Echinococcosis surveillance program.
The recent development of a real time PCR based on the mitochondrial target *rrn* amplification was done on a collection of fox colon contents (CC), sampled after necropsy (29 positive for Em adult worms detection (scratching technique SSCT), 1 to 6145 worms present (CC+) and 28 negative (CC-). The specificity was tested on a European panel of *Echinococcus* and *Taenia* from canines. Three areas were thus tested for the presence of the parasite in field faeces: a highly endemic area (Em prevalence around 70%; 50 copro-samples) and low endemic area (Em prevalence around 5%; 50 copro-samples) in the East of France and in an Em-free area (14 samples from Greenland). Total DNA was extracted from CC and field faeces (QIAamp DNA stool kit, Qiagen). The presence of inhibitors was assessed by the detection of an inner control in each extract. In case of inhibitors present in the sample, 1:10 and 1:100 dilutions were performed before a new *rrn* PCR. The positive samples were sequenced.
The European *Echinococcus* spp. and 2 *Taenia* not present in fox were amplified but Ct were very high. In total 25/29 CC+ were positive in qPCR (Cq 23.8 to 39.2; 1 sample inhibited) and 2/28 CC- were found positive (Cq 36.9 to 41.2; 1 sample inhibited). In the highly endemic French region, 20/50 samples (40 %) were positive (Cq 31.3 to 41.2) and in the low endemic area 5/50 were positive (10%) (Cq 29.3 to 38.7; 2 samples inhibited). For the Em-free area, no *rrn* amplification was observed. The *rrn* qPCR appears as a more sensitive technique than the SSCT technique. However the presence of inhibitors is an important obstacle in PCR diagnosis. The combination of different targets could increase the sensitivity of the qPCR diagnosis. The infectivity of copro-samples in the environment can be assessed by the present qPCR technique and could complement the copro-ELISA and copro-PCR technique and take place on necropsy.

EMSB MICROSATELLITE APPROACH ON THE EXPANSION OF *ECHINOCOCCUS MULTILOCULARIS* ENDEMIC AREAS IN FRANCE

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Alveolar echinococcosis is a severe parasitic disease caused by the fox tapeworm *Echinococcus multilocularis*. In the last twenty years, an expansion of the European endemic areas has been observed. Between 2005 and 2010, a large scale survey of *E. multilocularis* in fox was realized in north-eastern half of France covering 42 departments (French administrative unit). New endemic areas were identified. The emerging or re-emerging status of the parasite at the West of the French historical focus (the Alpine Arch) was assessed by the EmsB tandemly repeated microsatellite. Genotyping was realized on 303 *E. multilocularis* worms isolated from 128 intestines of positive foxes originating from 15 departments grouped in three areas: Eastern (7 departments), the historical endemic area, part of the European global focus (prevalence in fox of 33.0 % ± 3.6 %); Northern (5 departments), newly recognized as endemic areas, between Paris and Belgium (prevalence in fox of 16.7 % ± 3.4 %); Western (3 departments), newly recognized endemic areas, at the West of Paris (prevalence in fox of 10.3 % ± 3.7 %). The *E. multilocularis* panel presented three mains EmsB assemblages corresponding to three of the five principal European assemblages previously described. These assemblages are constituted of 22 genetic clusters with three numerically dominant clusters (48.5 % of the sample collection). One cluster is constituted of worms originating from only one department of the eastern area. The two other clusters are present in the three areas. The eastern area exhibited a higher genetic diversity than the northern and western areas, with the presence of 18, 8 and 5 clusters, respectively. Some clusters are highly represented in the two new endemic areas (northern and western) but poorly in the eastern area. Genetic distribution of worms is more homogenous in clusters from the northern area than in those from the western area, where almost two thirds of the worms owned to the same cluster. These results on the genetic distribution of *E. multilocularis* in France show evidence that genetic clusters from newly recognized endemic areas have been supplied by the historical area, due to the fox dispersion and leading to funder events. Thus we confirm the mainland-island parasite

system already described for the global European focus of *E. multilocularis*. The genetic diversity gradient observed here support the hypothesis of a progressive spread of the parasite from the Eastern to the Western part of France via the North.

SY11

ACANTHOCEPHALANS OF GENUS ECHINORHYNCHUS (SENSU LATO) IN THE BAIKAL RIFT ZONE

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In the Lake Baikal recorded 10 species and subspecies of the phylum Acanthocephala (Zaika, 1965; Baldanova, Pronin, 2001; Rusinek, 2008). Three species belong to the family Echinorhynchidae.
Taxonomy of genus Echinorhynchus Zoega in Muller, 1776 still remains controversial. V.I. Petrochenko (1956) divided the genus into three genera: Echinorhynchus Muller, 1776; Pseudoechinorhynchus Petrochenko, 1956; Metechinorhynchus Petrochenko, 1956. However, many zoologists did not accept this division (Yamaguti, 1963; Hoffman, 1967). O. Amin and M.J. Redlin (1980) concluded that the division conducted on the basis of the structure of the cement glands unreliable, and considered Pseudoechinorhynchus and Metechinorhynchus as synonymous of Echinorhynchus. Vainola R. et al. (1994), researched the genetic divergence of Echinorhynchidae, discovered a clear differentiation between marin *Eshinorhynchus gadi* Muller, 1776, and freshwater and brackish-water *E. salmonis*. The genetic similarity was close to the generic, though not entirely consistent with the division on the Echinorhynchus (sensu stricto) and Metechinorhynchus. O.N. Bauer and E.S. Scryabina (1987) retained the classification of V.I. Petrochenko. *Pseudoechinorhynchus borealis*, *Metechinorhynchus salmonis* and *M. truttae* are the widespread species with large host range and high morphological variability, which depends on the parasite age, host species and geographical location, therefore possible an inaccurate identification of parasites. To solve the problems with the systematics of Lake Baikal Echinorhynchidae we studied parts of the nucleotide sequences of the nuclear small subunit of ribosomal DNA and the internal transcribed region ITS-1 of P. borealis, *M. salmonis*, and M. truttae. For the first two species nucleotide sequences of these parts of DNA were held for the first time. Sequencing of small subunit ribosomal DNA showed that the genetic differences between the studied species are low, they are clustered into one group. At the same time clear separation of the group, bringing together these species, on the high (generic) level of *E. gadi* (AY218123.1, U88335.1) from the Genbank and E. truttae (AY830156.1, AF469412.1) with an external group *Pomphorhynchus tereticollis* (AY423347.1) is revealed. It is need revision of morphological characters of Echinorhynchidae of the Lake Baikal and the identification of systematic characters for the taxon, uniting these species.

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THE DISCOVERY OF TWO LIGAMENT SACS IN A MEMBER OF THE CLASS PALAEACANTHOCEPHALA (ACANTHOCEPHALA)

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The number of ligament sacs (one or two) is considered to be one of the primary diagnostic character for the classes within Acanthocephala (Morris, Crompton, 1982; Amin, 1985, 1987). Members of the Classes Eoacanthocephala, Polyacanthocephala and Archiacanthocephala possess two (ventral and dorsal) ligament sacs. In females of Eoacanthocephala, posterior end of

the ventral ligament sac is attached to the uterine bell, and in females of Archiacanthocephala attached to the uterine bell is the posterior end of the dorsal ligament sac. It was considered so far that all members of the Class Palaeacanthocephala possess only one ligament sac; in females its posterior end is attached to the inner side of the uterine bell. Morris and Crompton (1982) considered this morphological feature as evidence of the primitiveness of Palaeacanthocephala. In the present study, the cystacanths of *Southwellina hispida* (Van Cleave, 1925) (Polymorphidae) from the intermediate host *Orchestia* sp. (Amphipoda: Talitridae) were examined. Two ligament sacs, dorsal and ventral, were observed both in males and females of the species. In females, anterior end of the dorsal ligament sac is attached to the lateral wall of proboscis receptacle, and posterior end is attached inside the uterine bell. Anterior end of the ventral ligament sac is attached to the bottom of proboscis receptacle, and the posterior end is attached to lateral body wall close to posterior end of the metasoma. A single mass of ovarian tissues is located inside the ventral ligament sac. In males, two ligament sacs are attached anteriorly to the bottom of the proboscis receptacle. Posterior ends of both ligament sacs of males are attached to the genital pore. Each ligament sac contains a testis. Two ligament sacs of males and females were clearly observed in cystacanths from the intermediate hosts and in juveniles from the definitive hosts. In gravid specimens from the definitive hosts the ligaments become destroyed and inconspicuous. Presence of two ligament sacs has been never reported in members of Palaeacanthocephala. *Southwellina hispida*, a common parasite of ciconiiform birds, is widespread from Japan to North America (Schmidt, 1973; Khokhlova, 1986; Lisitsyna, 2008). It is one of the two known species of the genus *Southwellina* Witenberg, 1932 from the family Polymorphidae Meyer, 1931. Presumably, the discovery of two ligament sacs in *S. hispida* will stimulate detailed morphological studies of *Southwellina* species and the revision of the systematic position of the genus.

THE DESCRIPTION AND HOST-PARASITE RELATIONSHIPS OF A NEW SPECIES OF ACANTHOSENTIS (ACANTHOCEPHALA: QUADRIGYRIDAE) FROM THE PERSIAN TOOTHCARP, APHANIUS FARSICUS (ACTINOPTERYGII: CYPRINODONTIADE) IN IRAN

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A new species of Acanthosentis (Quadrigyridae) is described from the Persian tooth carp *Aphanius farsicus* (Teimori, Esmaeili and Reichenbacher, 2011) (Cyprinodontidae) in the Maharlu Lake Basin, southern Iran. *Aphanius farsicus* is an endemic freshwater fish found in streams and springs that drain into Maharlu Lake, Shiraz, Iran. The new species is the smallest of all the 44 known species of the subgenus *Acanthosentis* Verma and Datta, 1929, measuring between 0.26 and 1.68 mm in length. It is further distinguished by having a short cylindrical proboscis with very long anterior hooks widely separated from very small hooks in two very close circles posteriorly (hook length ratio about 4:1). It is separated from 4 other species of *Acanthosentis* with similar proboscis armature but with less extreme diversification of hook length. The new species is also distinguished by having anterior and posterior para-receptacle structures (PRS) that have been reported in only one other species of *Acanthosentis* from Japan. Proboscis receptacle single walled with large triangular cephalic ganglion. Testes are large, pre-equatorial, and Saefftigen's pouch is prominent. Fourteen to 25 circles of rose-thorn-shaped spines cover the anterior 50-70% of the trunk. This is the first species of *Acanthosentis* where SEM images, showing external morphological details, are provided. Of a total of 357 fish specimens examined between July, 2006 and June, 2007, 173 specimens (48.5 %) were infected with the new species. The prevalence of infection decreased with increasing fish size. The parasite was observed all year with the highest abundance and intensity in May while the prevalence was highest in February. The host-parasite relationships between the fish length, body weight and seasonality and infection were also investigated.

ACANTHOCEPHALA OF FRESHWATER FISHES OF MEXICO

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The helminth parasite fauna of freshwater fish of Mexico ranks amongst the best studied in Latin America. During past decade our knowledge of helminth parasites of freshwater fishes of Mexico has increased dramatically, more than 260 helminth species (both larvae and adults) have been recorded, however, acanthocephalan parasites (as well as cestodes), are the groups with the least species reported: only 10 species of adult acanthocephalans have been recorded from freshwater fishes of Mexico. We compiled a complete data base including all records of acanthocephalans from Mexico, and unpublished data from our own research. In this work we analyse the record of acanthocephalan parasites of freshwater fishes of Mexico and Central America, comment about the geographical distribution and the origin of the species, and explore answers to the question about the comparatively few number of species.

TAXONOMY AND HOST PARASITE RELATIONSHIPS OF POLYMORPHUS SPINDLATUS (ACANTHOCEPHALA) IN ITS VERTEBRATE HOSTS IN PERU

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Polymorphus spindlateus Amin and Heckmann, 1991 was described from the black-crowned night heron, *Nycticorax nycticorax* in Lake Titicaca, Peru. Its taxonomic position among the 27 known species of the subgenus *Polymorphus* is discussed. It is characterized by its spindle shaped proboscis and trunk, among other features. The cystacanths of the same species were described from the body cavity of 4 species of killifish of the genus *Orestias* also in Lake Titicaca, by Amin, Heckmann, Mesa, and Mesa in 1995. A morphological comparison between adults and cystacanths is made; attachment structures in both stages were found to be similar. Histopathological sections of attachment sites of adults and cystacanths show the damage caused by these parasite to their respective hosts. Damage involved villi compression, inflammation, rupturing, fibrous capsule formation, and production of numerous macrophages. A more recent study of the early developmental stages of *P. spindlatus* was also conducted and reported.

THE PARASITE SYSTEMS OF ACANTHOCEPHALANS IN THE LAKE BAIKAL

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The parasite system, by definition, consists of interacting populations of a parasite and its hosts (Roitman, Beer, 2004; Granovich, 2009). The structure of the system is determined by life cycle of the parasite species. Here, we provide information on acantocephalans life cycles and parasite systems in a complex ecosystem with a wide range of potential intermediate and definitive hosts.
A total of 3,000 fish and 26,000 amphipods were collected in Lake Baikal and analyzed with standard parasitological methods (fish – by Bykhovskaya-Pavlovskaya, 1985; amphipods – by Petrochenko, 1956). It is found that intermediate hosts of acantocephalans' parasites in Baikal are presented by 10 species from 4 genera of suborder Gammaridea.
As the definitive hosts of acantocephalans in Lake Baikal there are recorded 23 species of fish. The parasites of Lake Baikal are characterized by broad specificity to the definitive hosts. The host range of *Neoechinorhynchus salmonis* comprises 3 species from 2 genera; *Pseudoechinorhynchus borealis* - 9 from 8, *Metechinorhynchus salmonis salmonis* - 9 species from 8 genera, *M. s. baicalensis* - 15 from 10, M. truttae - 3 species from 3 genera. It was shown in some helminths that

invasive elements in the system are often provided only by one of the parasite hosts (Kontrimavichus, Atrashkevich, 1982). This is true in the parasite systems of *P. borealis*, *M. s. salmonis* and *M. s. baicalensis* in Lake Baikal. However, the invasion flow in the system of M. truttae is carried out through two host species.
We consider the parasite systems formed by acantocephalans in Lake Baikal as two-level metaxenic systems in terms of parasite system classification of V. N. Beklemishev (1956, 1960). Herewith, each level of the parasite system is polyhostal (i.e., there are few paraxenic hosts at each stage of parasite life cycle). In Baikalian acantocephalans the number of hosts at the larval stage increases due to acquisition by parasites of Baikalian endemic fauna of amphipods, at the imaginal phase – of endemic sculpin fish species and, in the case of P. borealis, due to inclusion of Baikal seal.

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SY02

EXPRESSION OF THE MUCIN-ASSOCIATED SURFACE PROTEINS (MASP) MULTIGENE FAMILY DURING THE LIFE CYCLE OF THE *TRYPANOSOMA CRUZI*

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MASPs are the second largest gene family in the *T. cruzi* genome. Due to the big number of genes that is composed, this multigene family must be essential in the complex life cycle of *T. cruzi*. In the present work we found that IgG antibodies specific to the catalytic region of MASP52 protein located at the surface and cytosol significantly reduce the parasite´s capacity to infect the host cells. Using the conserved signal peptide of this family, we found considerable changes in expression during the parasite's intracellular cycle, particularly when *T. cruzi* left the parasitophorous vacuole 24h after infection. Also, we clone 15 transcripts from cDNA of metacyclic trypomastigote forms of three different strains of *T.cruzi.* As a result of a comparative analysis of these sequences, we obtained 4 groups of paralogy, and three expressed (pseudo)genes as "strain specific". These data support the importance of this family during the invasion of the host cells and the contention that there are strain-specific MASP (pseudo)genes with divergent evolution and other (pseudo)genes groups subjected to a strong positive selection and concerted evolution in their sequences, over the genetic background that represents the MASP family, giving rise to groups of paralogy among them, according to the "birth-and-death" model of evolution.

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SY04/1

MALARIA SITUATION NOWADAYS IN HUNGARY

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Malaria was an endemic disease in Hungary for many centuries. The highest number of reported cases was several thousand per year (1933-1943), but the actual number of malaria cases was estimated as high as 10-100 000. The major breakthrough came in 1949 by the organized antimalarial campaign applying DDT for mosquito killing. The drastic reduction of the vectors resulted in the rapid decline of malaria cases. Since 1956, there have not been reported any

indigenous case in Hungary. In 1963, Hungary entered on the Official Register of the WHO to the areas where malaria eradication has been achieved. Aim of this work is to present the malaria situation nowadays in Hungary. During the period of 1963-2011, there were 215 Hungarians and 293 foreigners who imported malaria to Hungary. Majority of cases (266) were caused by *Plasmodium* (*P.*) *falciparum*. Further 242 cases were caused by *P. vivax* and other *P.* species. During that period, 7 fatal cases were reported (*P. falciparum*). Diagnostic tools: microscopic examination of Giemsa stained thin and thick blood film, antigen detection and DNA detection by LC and multiplex semi nested PCR. The expansion of migration (both the increase of the number of foreigners travelling into Hungary and of Hungarians travelling to abroad) favours to the appearance of imported cases. In order to avoid the importation of malaria to Hungary, attention is called of all the persons travelling to malaria endemic countries to the importance of malaria prevention by the International Vaccination Stations located in the National Centre for Epidemiology and in the Public Health Institutes of 19 counties and of Budapest.

DECREASE OF IMPORTED MALARIA IN FRANCE DURING THE 2006-2011: EPIDEMIOLOGY AND CLINICAL DATA THROUGH THE FRENCH NATIONAL REFERENCE CENTRE FOR MALARIA "CNR DU PALUDISME"

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The CNR du paludisme (national reference centre) was supported by the French National Institute of Disease Surveillance (INVS) to provide health public authorities with information on epidemiology and antimalarial resistance of imported malaria in France. Data were recorded from a network of about 80 hospitals all around the country. Each confirmed malaria case is reported to the CNR on an electronic uniform case-report form (epidemiological & clinical & biological data). Species confirmation and genotype and phenotype drug resistance analyzing were performed by the CNR laboratories. Between 2006 and 2010, about 4,400 (3970-4868) annual cases of imported malaria were estimated: in 2011, there is a decrease in the number of cases by 20% (3650 cases) compared to 2010. The median age was 33 years and about 16% of patients were children (< 15 year-old), the sex-ratio was 1.8. During the studied period, countries of contamination were mainly located in sub-Saharan Africa (more than 90%) and eighty three per cent of cases were due to *P. falciparum*. From 2006 to 2011, the proportion of severe cases increased, from 5.2% to 8.4% without impacting the lethality of all reported cases (0.33%).
In 2008, based on the data on the evolution of drug resistance to antimalarial drugs, French health authorities finally considered all African counties except Madagascar in group III for the resistance (multi-drug resistance). None failure of the atovaquone-proguanil chemoprophylaxis has been associated with a *falciparum* resistance. However, travelers didn't respect prevention recommendations: the proportion of patients reporting taking chemoprophylaxis was less than 40%. Atovaquone-proguanil (Malarone®) has become the first line therapy followed by quinine. The prescription of artemether-lumefantrine (Riamet®), the only ACT available in France, was still limited. Curative treatment failures due to a *falciparum* resistance to Malarone® were less than 3% of the isolates tested during the 2006-2011 period.
These data provide information on the malaria burden in France showing a decrease in the number of imported malaria cases which tends to be confirmed for the coming years. Consistently more than 70% of cases were observed in migrants and since the early 2000s, among both the total cases of imported malaria and the severe malaria cases, the proportion of tourists decreased while the proportion of visiting friends and relatives increased. They demonstrated the large disparity in the suffered population with an ever increasing proportion of migrants, misinformed or not adherent to risk prevention messages.

HAZARD AND RISK MAPPING OF MALARIA DISEASE IN IRAN: AN 11 YEAR TREND

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Malaria as a major public health problem in the tropical and subtropical regions of the world is an endemic disease in the south and southeastern of the Iran. There is a few evidence about the vulnerability, hazard and risk of this disease in Iran and its awareness can be an essential issue. The aim of this survey is the assessment of the 11 year trend and determination of the hazard and risk of this disease in Iran. Materials and Methods: Data was obtained in Iran's Centers for Disease Control and Prevention (CDC). Data regarding to frequency of Malaria which reported to CDC, classified according to provinces and the year of occurrence. The linear trend of Malaria was plotted in the EXCEL (2007) software. The hazard and risk maps were drawn using ARC GIS software. Results: The results showed that the cases of malaria decreased during the first 5 years of the study (1998 to 2002) despite of a slight increase in 2003. The most cases were reported from provinces of the Sistan and Blochestan, Hormozgan and Kerman with frequency of 108750, 44650 and 16759 respectively. The most hazard rates belonged to Sistan and Blochestan and Hormozgan Provinces, and the most risk maps were related to these three provinces. Conclusion: This survey showed that although in the high risk provinces the numbers of the reported cases are still high but in recent 11 years the trend of the Malaria decreased, that can be due to some effective interventions and also decreasing raining rate in recent years that affected the generation of Anopheles mosquitoes.
Keywords: Malaria, Risk, Hazard, Trend, Iran.

SY04/2

A BAYESIAN-BASED APPROACH FOR SPATIO-TEMPORAL MODELLING OF COUNTY LEVEL PREVALENCE OF MALARIA IN JIANGSU PROVINCE, CHINA

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Spatio-temporal variations of malaria in Jiangsu province were examined and the relationships between the key climatic factors and malaria prevalence at the county level were determined. The parasitological data were collected annually by means of cross-sectional surveys carried out in 47 counties from 1973 to 1999. Climatic factors, like average temperature, relative humidity and rainfall from 1973 to 1999 were obtained from China Meteorological Data Sharing Service System. Bayesian spatio-temporal models were employed to analyze the data. The best fitting model showed that spatial autocorrelation in Jiangsu province decreased dramatically from 1975 to 1981. A likely explanation of this finding arises from the large-scale administration of anti-malaria measures for malaria cases. Our analysis suggested a positive association between rainfall and risk of malaria incidence. On the other hand, an increase in relative humidity contributed to a significant negative effect on malaria prevalence. We conclude that combining geographic information system and Bayesian-based statistical approaches facilitate integrated risk modeling of malaria, which in turn is of relevance for allocation of scarce resources for monitoring of malaria during malaria elimination in Jiangsu province and elsewhere in China, where the disease remains of public health and economic significance.

POST-ARRIVAL SCREENING FOR MALARIA IN ASYMPTOMATIC REFUGEES USING REAL-TIME PCR

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Malaria is a significant health risk to refugee populations originating from endemic areas yet there is little consensus on screening and/or treatment approaches for this population. Furthermore, detection of malaria in semi-immune asymptomatic refugees is limited by the sensitivity of diagnostic tests used for screening. We determined the prevalence of malaria by microscopy and real-time PCR in asymptomatic refugees upon arrival in Edmonton, Canada over a one-year period.
A total of 324 refugees who underwent a medical exam at the New Canadians Clinic in Edmonton between March 2009 and 2010 were included in the study. All were asymptomatic for malaria. Patient demographics, clinical and laboratory data were obtained through review of medical charts and results from electronic laboratory information systems. Blood samples were processed by microscopy immediately following medical screening and served as the standard for patient care. Real-time PCR was performed retrospectively on de-identified samples at the end of the collection year.
The majority of refugees (97%) originated in countries that are endemic for malaria. While all thick and thin blood smear results were negative, ten subjects (3.1%) tested positive for *Plasmodium* DNA by PCR. Speciation by real-time PCR identified 3 cases of *P. falciparum*, 5 cases of *P. vivax*, and 1 case of *P. ovale*. One sample could not be speciated. Infections were identified in subjects originating from 3 countries: Democratic Republic of Congo (2 *P. falciparum* and 1 *P. ovale*), Burma (5 *P. vivax*) and Liberia (1 *P. falciparum*). Pre-departure treatment was self-reported in only 11/324 subjects, including three subjects infected with *P. falciparum*.
These results highlight the potential usefulness of real-time PCR in the diagnosis of asymptomatic malaria. Furthermore, our findings suggest that appropriate guidelines for malaria screening should consider the risk of relapsing infections, particularly in refugees arriving from Southeast Asia.

A MULTIPLEX REAL-TIME PCR ASSAY FOR DETECTION AND QUANTIFICATION OF *PLASMODIUM* SPP. INFECTION IN MALARIA VECTORS

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The enzyme linked immunosorbent assay specific for circumsporozoite protein (CSP-ELISA) is the gold standard method for the detection of malaria parasites in the vector despite several limitations. Here, we developed a new multiplex PCR-based method to detect and quantify the mixed infection rates of *Plasmodium* species in the African malaria vectors to better estimate the level of parasite infection in field populations and to ensure more accurate evaluation of the level of transmission following the implementation of vector control interventions. Methods: TaqMan duplex real-time PCR was first evaluated using different ranges of plasmids. The efficiency of real-time PCR was compared with the CSP ELISA using field caught *Anopheles gambiae* and *An. funestus* mosquitoes collected from two localities in southern Benin. Finaly, quantification of DNA of *Plasmodium* spp was performed and normalized using a housekeeping gene RS7. Results: A total of 200 mosquito samples (100 *An. gambiae* and 100 *An. funestus*) were used to develop and validate the RT-PCR method. The validation of these oligonucleotides this technique on the mosquito homogenates showed that RT-qPCR was more sensitive than the ELISA-CSP for the detection of *P. falciparum* (RT-PCR= 97% and CSP-Elisa=87%). These results indicated high

specificity of the multiplex real-time PCR to detect the other *Plasmodium* species (notably *P*. *malariae* and *P*. *ovale*) in anophelinae mosquitoes. The relative quantification shows that the amount of DNA varies between 3 and 90 copy number/ng per samples in *An*. *gambiae* and 4 and 20 in *An*. *funestus*. The average number of copies / ng in *An*. *gambiae* is (28.35767) and (7.16700) in *An*. *funestus* (*p*-value = 0.1045). Conclusion: This study describes a new method for the detection and quantification of the four Plasmodium species in the African malaria vectors. This will ensure a better diagnostic of malaria parasite's infection in field populations and allow for new basic research on the fitness cost associated with malaria infection during the life of the mosquito.
Keywords: *Plasmodium* spp., *An*. *gambiae*, *An*. *funestus*, Real-time PCR, Infections, Quantification.

THE ROLE OF LECTIN ON INTERACTION BETWEEN OOKINETE OF *PLASMODIUM BERGHEI* AND MIDGUT EPITHELIAL CELLS OF *ANOPHELES STEPHENSI* BY USING NANO PARTICLE QUANTUM DOT

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Malaria transmission needs development of the *Plasmodium* parasite in mosquito vector. *Plasmodium* invasion of the mosquito midgut is the critical primary step and involves adhesion to host epithelial cell ligands. There is some evidence suggesting that midgut lectins are important ligands for parasite adhesion. However, the identity of these glycans remains unknown.
We used fluorescent core shell quantum dots (QDs) as fluoresent markers to studies on *Plasmodium berghei* and *Anopheles stephensi* interaction. Two lectins, WGA and Con A were labeled with cadmium telluride quantum dot (CdTe QDs) and cadmium selenide quantum dot (CdSe QDs) separately. The mosquitoes were allowed to fed on mice blood contain *P. berghei* and labeled WGA or Con A. The ookinete invasion midgut was followed 20 hours after blood feeding by dissecting the insects and using conventional optical microscope.
The results show that Con A blocked the ookinetes invasion onto the midgut cells of the mosquito while WGA did not preserve ability of ookinetes to attack the mosquito midgut wall cell. There was no significant difference between the results of conjugated and non-conjugated lectins with respect to ookinete activities.
Bioconjugation of QDs with the lectins did not significantly effect on the vitality of parasite and/or vector comparing with controls. Therefore, our results show that QDs can be used as physiological fluorescent markers capable to label living parasites and insect vector cells. In addition, conjugating QDs with two lectins assist us to visualized and understood interaction of surface carbohydrates on the perimicrovillar membrane of mosquito midugt cells with ookinate of the plasmodium.
Keywords: Nano particle Quantum Dot, lectin, malaria parasite, anopheles mosquito.

STRAIN AND SPECIES-TRANSCENDING IMMUNITY INDUCED BY EXPOSURE TO LOW DOSES OF BLOOD STAGE MALARIA PARASITES - A NEW PARADIGM FOR VACCINE DEVELOPMENT

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There has been limited progress in the quest to develop a subunit blood stage vaccine since malaria antigens were first cloned in 1983. Efforts have almost entirely focused on the identification of suitable merozoite surface proteins as vaccine targets. There has been no progress beyond Phase II trials where the results have been largely negative because of poor immunogenicity and/or antigenic diversity within target B cell epitopes. In recent times, there has been a re-

appraisal of the role of cell-mediated immunity in protection against malaria. We have observed that exposure to low, but not high doses of blood stage parasites induces cell-mediated immunity and apparent protection in humans and mice with no or minimal requirement for antibody (1-4). These experiments set the stage for new approaches to vaccine development and delivery. Thus, there is growing interest in GM parasites as malaria vaccines but our recent focus has been on chemical attenuation using a class of chemicals typified by gentanamycin which binds in AT-rich regions of the parasite genome. Chemically attenuated parasites (CAP) do not grow in immunodeficient mice. We observe that low doses of *Plasmodium chabaudi* ring stage CAPs induce profound protection against different strains and species of *Plasmodium*. CAP prepared using other species of *Plasmodium* are also effective. A single immunization is sufficient. Vaccination does not induce detectable antibodies and protection is T-cell dependent. CAP administration results in parasite DNA being detectable in the blood for several weeks post vaccination, which may be critical to immune induction. This approach is now being applied to P. falciparum where we plan to vaccinate humans with low doses of *P. falciparum* CAP, determine their immune responses and then challenge vaccines with viable *P. falciparum*-infected human red cells to determine vaccine efficacy.

SY25/2

CONTROL OF CRYPTOSPORIDIOSIS OF RUMINANTS IN GREECE

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Cryptosporidiosis is a very common gastrointestinal disorder due to the protozoan parasite *Cryptosporidium* spp., located in the intestine of various animals, particularly ruminants. In Greece, it is found in high prevalence in dairy farms of ruminants, especially in very young animals and it is considered a main (primary or component) pathogen for neonatal losses due to diarrhoea. The aim of this extended set of studies was to investigate the prevalence and involvement of *Cryptosporidium* spp. in neonatal diarrhoea and other disorders, the main risk factors, compare diagnostic techniques and control. Towards this end, 475 cattle from 18 farms and 523 sheep from 31 flocks, both with a history of diarrhoea, were examined. Cattle and sheep were 25.05% and 29.06, respectively, infected, with highest percentages 48.62% and 55.07% for calves and lambs, respectively, younger than 2 weeks. It was found that the main risk factors were the season of lambing, in late lambing flocks the infection reached 80-89.3%, the number of head per herd was important risk factor for goats (>200head), the age, the history of diarrhoea in the farm in the past etc. *Cryptosporidium* spp. was a possible implication factor for umbilical infections and illthrift of lambs. Field trials were carried out to investigate the efficacy of halofuginone lactate to treat cryptosporidiosis using 230 lambs (5 flocks) and 250 kids (11 herds) suffering from diarrhoea due to cryptosporidiosis. Animals were treated with halofuginone lactate at 100μg/kg b.w. for 7 consecutive days. Treatment was 100% effective. Furthermore, 960 lambs (10 flocks) and 1980 lambs (12 herds) healthy, but living in farms with confirmed cases of cryptosporidial diarrhoea in the past, were treated with halofuginone lactate at 100μg/kg b.w. for 7 consecutive days in order to investigate the efficacy of this drug to prevent the disease. It was found that halofuginone L was highly effective to prevent the disease. It is concluded that halofuginone lactate at the dose rate of 100μg/kg b.w. for 7 consecutive days is highly effective to treat and prevent cryptosporidiosis of ruminants.

MOLECULAR CHARACTERIZATION OF *CRYPTOSPORIDIUM* ISOLATES FROM PRE-WEANED CALVES IN WESTERN FRANCE

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Cryptosporidium parasites are considered as a major cause of neonatal diarrhea in calves. *Cryptosporidium parvum* has been frequently recorded as the dominant species in diarrheic calves but others species could occur particularly in subclinical situation. Several studies have shown a sequence of *Cryptosporidium* species according to the age of calves but very few of them have taken into account the possible co-infections using longitudinal study.
Eighteen pre-weaned female calves (Parthenaise breed) from a single beef cattle herd were sampled weekly from birth to 2 months of age in order to characterize oocyst output, parasite species and clinical features associated with infection. Fecal samples were screened for the presence of oocysts after ethyl acetate concentration using immunofluorescence analysis. DNA was extracted from positive samples and then, nested PCR was performed to amplify the partial SSU rRNA gene of *Cryptosporidium*. For the identification of species, RFLP analysis with restriction enzymes SspI and MboII was performed. Finally, for the genotyping of *C. parvum,* a GP60 PCR will be carried out.
Eighteen (100%) animals excreted oocysts at least once. The highest percentage of excretion was observed in calves at the age of 17-31 days. The mean number of oocysts at peak of excretion (10-16 days) was $5x10^5$ oocysts per gram of faeces.
During the whole study a mild diarrhea was observed in 80% of these calves. No mortality was recorded. To date, PCR-RFLP analysis was successful for 53 of 62 positives samples for *Cryptosporidium*. Molecular characterization of the other positive samples is being done. To date, fourteen samples were identified as *C. parvum*, 13 samples as *C. bovis* and 16 samples as *C. ryanae*. Ten co-infections were identified: 4 *C .bovis- C. parvum*, 5 *C. bovis-C. ryanae* and 1 *C. parvum-C. ryanae.*
Calves excreted the following *Cryptosporidium* species: *C. parvum* between 7 and 24 days of age, *C. bovis* between 11 and 38 days of age and *C. ryanae* which infects older animals aged between 19 and 45 days.
This study showed that the species *C. parvum* and *C. bovis* can infect very young animals with an equivalent level of excretion with or without relationship with clinical signs.

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SEROPREVALENCE OF *TOXOPLASMA GONDII* IN DOGS IN UKRAINE

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Toxoplasma gondii is an important zoonotic intracellular protozoan parasite, which can affect all warm-blooded mammals and birds throughout the world, including humans. In recent years, surveys of *Toxoplasma gondii* infection in dogs have been reported worldwide. However, little is known about the prevalence of *T. gondii* in dogs in Ukraine. 1167 serums samples of dogs originating from different cities of Ukraine have been collected between 2007 and 2010: Kiev − 1003 samples, Odessa − 63 samples, Lviv − 49 samples and other cities − 52 samples. The serum samples were collected from an urban population of dogs, with owners, having different dietary habits (industrial, processed or raw meat, etc). The samples were tested by ELISA, a solid-phase

enzyme immunoassay for the qualitative determination of antibodies to *Toxoplasma gondii* in feline or canine serum or plasma "*Toxoplasma* IgG-CF EIA" (Xema-Medica, Moskow, Russia).
Overall, 51.4% (600/1167) serums samples of the dog population were identified as positive to *T.gondii* infection by means of ELISA, according to manufacturer's instructions. The seroprevalence varies from 49% (491/1003) in Kiev to 65.1% (41/63) in Odessa and to reach 83.7% (41/49) in Lviv, underlying the widespread of the zoonotic parasite in Ukraine, with a special emphasis on its presence in the western part of Ukraine. Extensive field investigations in the dog population of Ukraine (both rural and urban) are requested in order to confirm the present results, as well as to investigate the presence of the parasite in other animal populations.

DEVELOPMENT OF TRANSFECTION TOOLS IN *NEOSPORA CANINUM:* A PYRIMETHAMINE CASSETTE OF RESISTANCE WITH LAC-Z AS THE REPORTER GENE

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N. caninum is an obligate intracellular Apicomplexa frequently related to abortion in cattle. The genetic manipulation is one of the methods to describe the key pathways related to invasion and replication. Among the tools developed in *Toxoplasma gondii*, the insertion of genes by the pyrimethamine resistance cassette is one of most applied because it is well tolerated by the parasite and allows multiple insertions in the genome. As constructed for *T. gondii*, the NcDHFR-TS (Dihydrofolate reductase- Thymidylate synthetase) gene was point mutated in two aminoacids, the change of serine 36 to arginine (M2) and tyrosine 83 to aspartic acid (M3), generating DHFRM2M3. The DHFRM2M3 flanked by the promoter and 3' UTR Ncdhfr (NcDHFR-DHFRM2M3) conferred resistance against pyrimethamine after transfection. The cassette DHFR-DHFRM2M3 was ligated to the reporter gene Lac-Z (beta-galactosidase enzyme) controlled by the *N. caninum* tubulin promoter (NcTub-tetO/Lac-Z) and was transfected in *N. caninum*. After selection of resistant parasites, the expression of Lac-Z was evaluated. The tachyzoites were diluted ($1x10^6$, $5x10^5$, $1x10^5$, $5x10^4$, $1x10^4$, $5x10^3$ and 1 $x10^3$) and invaded vero cells in a 24 well plate for 2 hours. The activity of beta-galactosidase of each dilution was plotted in a graphic with a R^2 of 0,988curve. A growth assay was performed by the incubation of tachyzoites (1×10^4 and 5×10^4) in vero cells for 24 and 72 hrs. The activity of beta-galactosidase was proportional to the development of tachyzoites and the difference between 72 and 24 hrs resulted in a curve with R^2 of 0,968. The Lac-Z tachyzoites were also assessed with X-gal precipitation, where isolated tachyzoites were visualized with several dilutions ($1x10^6$, $5x10^5$, $1x10^5$, $5x10^4$, $1x10^4$). The insertion and detection of reporter genes as Lac-Z in *N. caninum* is an unprecedented fact and will allow the evaluation of drug effects and functional gene assays on *N. caninum*.

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SY25/3

COMPARISON OF DIFFERENT MOLECULAR TESTS FOR DETECTION OF CRYPTOSPORIDIOSIS IN ANIMALS

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Cryptosporidiosis is a self-limiting infection but can lead to severe disease in young or immunocompromised animals. There are numerous methods available for the detection of cryptosporidiosis such as traditional microscopic, immunologic and molecular methods.

Cryptosporidium species generally lack distinguishing morphological traits, and consequently, molecular methods are commonly used for parasite identification which is very important in epidemiological studies. Various methods for *Cryptosporidium* identification have been proposed, each with their advantages and disadvantages. In this study different molecular techniques were compared for the detection and identification of Cryptosporidium spp. in animal faecal samples. For the purpose of comparison of 6 molecular tests, 49 (Sheep n= 10, horse, n= 17 and cattle, n=22) samples were collected in 2010. Oocysts purification from faecal samples using Sheather's sugar solution was followed by isolation of DNA. Three nested PCR (nPCR) protocols all based on the amplification of the 18S rRNA, two nPCR targeting the actin gene and a real time PCR for amplification of tubulin gene of *C. parvum*, were performed on all samples. PCR products were sequenced for further identification of infected species. An animal sample was identified as infected when one of the tests was positive. The preliminary finding of this study shows that cryptosporidial DNA was detected in 29%, 79%, and 26% of the infected animals by amplification of 18S rRNA based on Xiao, Ryan and Nichols protocols, respectively. *C. parvum* specific real-time PCR assay resulted in detection of 21% of infected samples. Two actin nPCR assays were used in this study. One specifically amplifies actin gene of *C. parvum* while the other is only *Cryptosporidium* genus specific. Species specific and genus specific actin PCR detected the presence of *Cryptosporidium* in 13% and 31% of the samples, respectively. Sequencing of all PCR products revealed the presence of *C. ryanea*, *C. bovis*, *C. xiaoi*, *C. parvum*, and *C. ubiquitum* in the samples. In most instances, applying different PCR assays resulted in amplification of different Cryptosporidium species. The Ryan PCR protocol detected more samples with Cryptosporidium than any of the other tests and was also able to identify a wider range of *Cryptosporidium* species. Since different species were identified using different PCR methods, application of a combination of different nested PCR assays is recommended for epidemiological investigations and also identification of mixed infections.

PREVALENCE OF *BALANTIDIUM COLI* IN CATTLE FROM ROODSAR ABATTOIR, EAST OF GUILAN, NORTHERN IRAN

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Balantidium coli is a common protozoan disease of animals and it is a large ciliated intestinal protozoan parasite. *Balantidium coli* has been known about for over a century, but the process of infection has yet to be discovered. It is the only known ciliated parasite to infect humans and responsible for the Balantidiasis. *Balantidium coli* is found worldwide but predominately found in the areas where pigs are raised. Other potential reservoirs include ones that hold rodents and nonhuman primates. Less than 1% of the human population is infected with *B. coli*.
Prevalence of *Balantidium coli* in cattle from Roodsar abattoir was examined. A total of 100 fecal samples were collected from cattle and examined microscopically; 6 (6%) were positive for *B. coli* cysts and trophozoites. Prevalence of *B. coli* infection was relatively higher in adult cattle aged > 5 years than young aged > 2- ≤ 5 years and calves aged ≤ 2 years. Higher prevalence of *B. coli* was observed in female than that of male cattle which are not statistically significant. Prevalence of *B. coli* infection was significantly (p<0.05) higher in poor health cattle than healthy cattle and cattle reared in normal floor/muddy floor than that reared in concrete floor, respectively.
Keywords: *Balantidium coli*, Prevalence, cattle, Iran.

PRELIMINARY STUDY OF THE PRESENCE OF DOG HELMINTH PARASITES IN SOIL FROM TIMIS COUNTY: IMPORTANCE FOR HUMAN HEALTH

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Dog feces are an important source of soil contamination with helminths. Contaminated soil is an important route for transmission of numerous parasites which are capable of causing zoonotic disease in humans. Contact with soil or sand contaminated of dogs infected with some zoonotic parasites can lead to the development of larva migrans in people.
There is no current information regarding the presence of helminth parasites in soil in Timis County.
The aim of the present study was to analyze the environmental infestation with parasites of the dog in spring season. A total of 30 soil sample were collected from public places and children's playgrounds, backyards and gardens. Five sample of approximately 250g each were collected from different points of each square. A trowel was used to scrape the soil from the superficial layer 0-3 cm, and the deeper 3-10 cm layer and put into different sets of labelled polythene bags. The technique described by Laborde *et al.* and modified by Ruiz de Ybanez *et al.* method were used to recover parasites in soil and sand.
Thirteen square (43.3%) were found to be contaminated, and the most prevalent parasites were *Toxocara canis* (36.6%) which was identified in eleven squares, followed by *Trichocephalus vulpis*. (26.6%), *Ancylostoma* spp. (13.3%), *Strongyloides stercoralis* larvae (6.6%) and *Dipylidium caninum* (3.3%). In places where dogs were present, but even where dogs were not seen, soil and sand samples were positive for helminth parasites. Three types of parasites were found in sand samples. The results of this study suggest that dogs may contribute significantly to the spread of human diseases in these areas. Detection of zoonotic parasites in soil reveals the existence of real risk for human infection especially in children.

OCCURRENCE OF GASTROINTESTINAL NEMATODE PARASITISM AND SUBCLINICAL MASTITIS IN EWES REARED UNDER LOW INPUT MANAGEMENT SYSTEMS

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Gastrointestinal nematodes (GIN) and subclinical mastitis (SM) can cause important health problems in dairy sheep, leading to an increased use of chemical treatments. Abiotic stress factors caused by flock husbandry practices are known to affect the susceptibility of ewes to GIN and SM. The aim of this study was to investigate the effect of different management systems and lambing periods on GIN and SM and also to detect potential interactions between the two pathological conditions. This study included 10 extensive (diet based on free grazing of mountain pastures throughout the year, concentrated feed provided during wintertime, hand milking applied) and 10 semi-intensive (diet based on grazing in high quality private pastures, concentrated feed provided constantly, machine milking applied) sheep flocks in Crete, Greece. From each of those flocks 20 ewes were enrolled and monthly faecal and milk samples were taken, for 12 consecutive visits. GIN Eggs per Gram (EPG) numbers were determined using the modified McMaster technique, while milk samples were analyzed for CFU (Colony Forming Units) and Somatic Cell Count (SCC). Microbiological examinations were conducted when SCC exceeded 500,000 somatic cells/ml. FAMACHA and Body Condition Score (BCS) were also recorded for each sheep. The results showed that low parasitic egg output was encountered in both systems (i.e. mean EPG values of 43.6 ±2.3 and 31.3 ±3.6 for extensive and semi-intensive flocks, respectively). Extensive reared and early lambing ewes showed significantly lower EPG numbers compared to the 'semi-intensive' and late lambing ewes, respectively. Ewes bred in semi-intensive systems showed an EPG peak

during summer. GIN counts were weakly related to FAMACHA records (r_s=-.037, p<.05), while a moderate correlation was detected between EPGs and BCS values (r_s=-.179, p<.01). Management system (p<.01), lambing period (p<.01) and their interactions (p<.05) significantly affected EPG numbers. In average, 8.5% of udder teats, examined microbiologically, had subclinical mastitis. Higher numbers of microbiologically positive milk samples were observed in semi-intensively bred ewes. *Staphylococcus* spp was the most commonly identified pathogen (70%). Management system had a significant effect on SCC (p<.001). EPGs showed weak/no correlation with SCC (r_s=-.058, p<.01) and CFU (r_s=-.035, p>.05) values. Our results suggest that management system and breeding practices can influence the dynamics of gastrointestinal nematodes infection and subclinical mastitis. No correlation could be established for the two pathological conditions, possibly due to the low parasitic burden.

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A COMPARATIVE STUDY BETWEEN NECROPSY AND BIOCHEMISTRY OF HYDATIDOSIS IN CAMELS (*CAMELUS DROMEDARIUS*) IN THE REGION OF TOUGGOURT IN ALGERIA

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In order to compare the findings of the postmortem examination concerning hydatic cyst and the usual biochemical values corresponding. A study of 20 blood samples camels made at the jugular vein followed by an inspection of the organs which has tropism for the hydatid cyst in the slaughterhouse of Touggourt. Animal experimentation is composed of 10 males and 10 females, aged between 4 and 15 years. Our results showed that 11 camels had liver and lung cysts and biochemical analysis showed significant variations in ALT (alanine aminotransferase), AST (Aspartate aminotransferase) and GGT (gamma glutamyl transpeptidase), total bilirubin and total protein. The results are consistent with many authors in the literature,however, the interpretation of these results is very difficult because the camel is very susceptible to factors of change in diet, climate, and its physiological condition. We noted in animals infected with hydatid disease an increase in AST, ALT, and almost the majority showed an increase of GGT and relative stability of total bilirubin. These variations of AST and ALT are due to severe liver disease, which explains the destruction of liver cells are the result of hepatic necrosis. An increased plasma protein concentration is related particularly to food, and finally the total bilirubin is increased only when the bile ducts are compressed. For this purpose the study of biochemistry parameters in the dromedary can be a means of diagnosis of hydatid disease.
Keywords: Biochemistry, hydatid cyst, blood Serum, Post mortem, necropsy.

SY25/4

PREVALENCE OF SOME VECTOR BORNE DISEASES IN DOGS IN R. MACEDONIA

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Data related to canine vector-borne diseases in R. Macedonia are limited, despite the favourable climatic conditions for parasites and vectors, and existence of a large population of stray dogs. A serological survey for *Ehrlichia canis*, *Anaplasma phagocytophilum*, *Borrelia burgdorferi*, *Dirofilaria immitis* and *Leishmania infantum* infections in rural and urban dogs from different regions was

conducted. Blood samples were tested using a commercial ELISA assay kit (SNAP® 4Dx®; IDEXX Laboratories, Inc. U.S.A.) and in-house indirect fluorescent antibody test (IFAT) for detection of anti-*Leishmania* antibodies. From a total of 144 examined dogs' serums, the number of the serologically positive dogs for any of the five pathogens was 81 (56.2%). *L*. *infantum* was the most prevalent pathogen, being present in 50 (34.7%) of the examined dogs, followed by *E*. *canis* 20 (18.7%), *D*. *immitis* 23 (15.9%) and *A*. *phagocytophilum* 12 (8.3%). *B*. *burgdorferi* antibodies were not present in any of the examined dogs. The number of dogs with single, dual, triple and quadruple seropositivity was 47 (32.6%), 22 (15.3%), 5 (3.4%) and 1 (0.7%) respectively. *L*. *infatum*, *E*. *canis* and *D*. *immitis* were more prevalent in urban dogs, while *A*. *phagocytophilum* was more prevalent among rural dogs. There was significant difference in prevalence of *E*. *canis* and *D*. *immitis* (p<0.01) among urban dogs, while there was no significant difference in the prevalence of *L*. *infatum* and *A*. *phagocytophilum* among urban and rural dogs. This study indicates that urban dogs in R. Macedonia are at the higher risk of exposure to *E. canis* and *D*. *immitis*, while both urban and rural dogs are at the same risk of exposure to *L*. *infatum* and *A*. *phagocytophilum*.

BABESIOSIS OF DOGS IN BELGRADE AREA BETWEEN 2009-2011

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Babesiosis is a tick-borne disease of dogs caused by protozoan parasite *Babesia canis* and *B. gibsoni.* Babesios were became a significant health problem of dogs in Belgrade area since 1997 and since that day we performed continuous examination of its presence.
Examinations were performed usually at suspected dogs with common clinical manifestation of disease (anemia, haemoglobinuria, fever, pale of mucous membranes etc.) or infested with ticks. We used capillary blood to examination, this blood films were air-dried, fixed in absolute methanol for 1 minute and stained in 10% Giemsa stain for 20-30 minutes. The species of *Babesia* were identified using the schemes of Riek (1968) and Soulsby (1977).
In our paper we performed results from period 2009-2011. During that period average percent of infected dogs were 63.91% (375/593). We had next results: in 2009. Babesiosis was established in 66.66% (132/198) of examined dogs; in 2010 in 67.02% (126/188) and during 2011 in 58.54% (121/207) of suspected animals. Dominant *Babesia* species occurred during our examination was *B.canis* established in more than 95% of positive cases.
The dynamics of incidence of dog babesiosis was monitored from January to December. It was noted that the increase in incidence of dog babesiosis commenced in the interval March-April. May was the month of infection maximum, decreasing gradually until July. The autumn infection peak occurred in September, disappearing completely in December.
At same time we collected ticks from dogs. Ticks were found on 32.39% of examined dogs. Collected ticks of suspected genus (*Rhipicephalus* and *Dermacentor* species) were examined to presence of *Babesia*. Tick films were made by placing a small drop of blood onto a clean glass slide, air dried, fixed in absolute acetone for 5 minutes and stained by 5% Giemsa for 20-30 minutes. Each blood sample was examined under oil immersion. *B.canis* was detected in *R. sanguieneus* (66.10%), *D. reticulatus* (46.40%) and *D. marginatus* (18.70%).

PREVALENCE OF ERLICHIOSIS, ANAPLASMOSIS AND BORELIOSIS IN DOGS IN SERBIA

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Prevalence of erlichiosis, anaplasmosis and boreliosis of dogs in Serbia were investigated sporadically, at clinically suspicious dogs, using blood smears examination to erlichiosis and anaplasmosis and ELISA test to boreliosis.
During 2011 we have examined 387 blood samples of dogs from 10 cities from all parts of Serbia: Belgrade area, Novi Sad, Subotica, Smederevo, Niš, Kragujevac, Kraljevo, Sabac, Sremska Mitrovica and Čačak. To detection of erlichiosis, anaplasmosis and boreliosis we have used IDEXX 4Dx tests. Dogs that was presented for routine medical procedures, and exposed at least to one tick season and without history of treatment with doxiciclin, penicillin, cephalosporines and chloramphenicol was randomly included in study.
We obtained next results:
1. Erlichiosis:
In Belgrade area we examined Old housing district where prevalence are 3.12% (2/64), in north part of Belgrade nearby Danube River (Ovča & Borča) prevalence was 1.56% (1/37) and at west part of Belgrade nearby Sava and Danube River (New Belgrade & Zemun) we not established erlichiosis (0/60); In Smederevo and Subotica erlichiosis was established in 3.33% (1/30) and in Sremska Mitrovica (0/10), Šabac (0/15), Novi Sad (0/60), Niš (0/30), Kragujevac (0/20), Kraljevo (0/11) and Čačak (15/11) not found.
2. Anaplasmosis:
In Belgrade area in Old housing district anaplasmosis where prevalence of 6.25% (4/64), in north part of Belgrade nearby Danube River (Ovča & Borča) prevalence was 6.66% (2/37) and at west part of Belgrade nearby Sava and Danube River (New Belgrade & Zemun) was established in 5.40% (2/60) examined dogs. Anaplasmosis were established in Smederevo in 6.66% (2/30) and in Čačak in 20.00% (3/15), but not established in other cities.
3. Boreliosis:
Boreliosis not established in dogs during our examinations.
In total during our examinations erlichiosis was occurred in 1.33% (5/382), anaplasmosis in 3.92% (15/382) and boreliosis was not occurred (0/382).

EPIDEMIOLOGY AND ASSOCIATED RISK FACTORS OF ECTOPARASITES INFESTING GOAT POPULATION OF DISTRICT TOBA TEK SINGH, PAKISTAN

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Goats (*Capra hircus*) constitute an important source of essential proteins in many poor regions through meat and milk. In some parts of the world these are kept as a means of obtaining quick cash and for cultural functions, contributing to income generation and food security. Ectoparasites belong to the largest Phylum cause a serious nuisance to the livestock and dairy industries of developing countries like Pakistan. The current cross sectional epidemiological investigation reveals the possible risk factors which are associated with the prevalence of ectoparasites in domestic goats (*Capra hircus*) of district Toba Tek Singh, Punjab, Pakistan. Of the total 4020 goats screened during the study period (April 2010 – 2011), the overall prevalence of ectoparasites in domestic goats of study area was 11.14%. Among various ectoparasites, ticks

were found predominant (33.58%; 270/804; $p<0.05$) followed in order by lice (9.58%; 77/804), fleas (6.84%; 55/804), mites (3.23%; 26/804) and flies (2.49%; 20/804). The prevalent species of arthropods were identified. Among various subjected determinants, prevalence of ectoparasites was statistically equally distributed in hosts of different age, sex and breeds. However, grazing animals and those housed on uncemented floors were found more prone to ectoparasites. Seasonal abundance of fleas, lice and mites was highest during the winter while that of ticks and flies was highest in summer and spring seasons, respectively. Comparative hematological profile of infested and healthy goats revealed remarkable differences being lower cellular profile in former than latter. The results of the present study may implicit significant data for the farmers and local veterinarians for planning ectoparasitic control program in the study area.
Keywords: Prevalence, Ectoparasites, *Capra hircus*, Toba Tek Singh; Punjab, Pakistan.

Financial assistance for this study was provided by the Endowment Fund Secretariat, University of Agriculture, Faisalabad, Pakistan.

DETECTION AND MONITORING OF INCREASING HEALTH RISKS DUE TO PARASITE INFECTIONS IN CATTLE AS A RESULT OF GLOBAL CLIMATE CHANGE

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Pasture borne helminth infections have a considerable influence on health and productivity of cattle. Nearly every animal on pasture will be infected with some of these parasites at least once/one time during its life. Especially infections with *Fasciola hepatica* (*F. h*.), gastro-intestinal nematodes (GIN) and *Dictyocaulus viviparus* (*D. v*.) lead to severe production losses in ruminants. The life cycle of the parasites mentioned above includes intermediate hosts and/or free living stages respectively, which are affected by environmental conditions.
In the network KLIFF (climate impact and adaptation research in Lower Saxony) three regions with different climatic conditions were identified: (i) the coastal area in the North, (ii) the heathland (Mid-East) and (iii) a more hilly/mountainous area (South).
To determine the prevalence of the parasites of interest in dairy herds in the above mentioned regions, bulk milk samples were collected three times a year (before, in the middle and after the grazing season) from several dairy farms (100-130 per region) and were examined using ELISA techniques for the detection of these three parasites. Furthermore, during the grazing season faecal samples were collected monthly (coproscopical analysis) and blood samples (serum ELISA) three times from first season grazing (FSG) calves on selected tracer farms. Additionally the Body Condition Score (BCS) was determined and the body weight was recorded in monthly intervals.
On the tracer farms, the typical annual pattern for GIN-infections was observed in all FSG calves. The worm burden increased until late summer and declined around September although some slight differences in infections intensity and exact timing were noted between the farms/regions. Infections with lungworms and liver fluke were only detected in the North.
We have analysed a preliminary data set consisting of results obtained in the bulk milk ELISA for all three parasites in autumn of three consecutive years (2009 - 2011). The results revealed high prevalences of GIN (up to 90%) in comparison to *F. h.* and *D. v*. but with regional differences (higher in the North). We also observed slight annual variability with increasing results over the three years. The lowest prevalence was detected for *D. v*. (1.7%).
At the same time we want to represent the results in maps, combining GIS-data of the dairy farms with weather data and local climate projections to predict future high risk areas.

DETERMINING THE BEST SHEEP PROTECTIVE STRATEGY TO COMBAT INFECTION BY THE NEMATODE *HAEMONCHUS CONTORTUS*: IS RESISTANCE FUTILE?

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The gastrointestinal nematode *Haemonchus contortus* represents one of the most significant threat to ruminant production systems worldwide. Control is now looking towards selectively breeding sheep to meet production targets in the presence of such a threat. Breeding sheep which are resistant to the infection has been presented as a mean to reduce parasite-induced production losses. Yet there is mounting evidence to suggest that the protection mechanisms responsible for host resistance come at a cost and that immune-mediated pathology may render the breeding strategy counter-productive. Breeding sheep for resilience to the effects of *H. contortus* infection has instead been proposed as an alternative breeding strategy in which to minimize potential immunopathological negations on production. This study investigates the impacts a primary H. contortus infection confers on the body condition of sheep hosts under three different host-resistance strategies; where *H. contortus* has: no success (Martinik Black Belly ewes, n=25), low success (Martinik Black Belly rams, n=25) and high success (Romane rams, n=16). The sheep infection burden was evaluated by means of faecal egg counts (FEC). Their condition was evaluated using haematocrit as an indicator of anaemia, temperature as indicator of pain and relative weight gains. It then goes on to disentangle the worsening of condition and pain attributed to the parasite and to the cost of protection against the parasite. The study goes on to disentangle the relative parasite-induced and host-induced effects on the worsening of host condition. . The results suggest that resistance is not a costly strategy as long is it is complete as observed in Black Belly ewes: no infection, minimal impacts on anaemia or weight. Being susceptible to infection came at greater cost to the host condition as shown in the Romane rams: high FEC and lowest haematocrit levels by the end of the study. The most costly strategy was that of Black Belly rams in maintaining low levels of infection: low FEC, greatest decreases in body temperature (thereby possibly pain), and low haematocrit values These results carry significant implications for the selection criteria used in selective breeding and also provide further insight into the sheep costs of fighting a *H. contortus* infection.

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SY25/5

PREVALENCE AND RISK FACTORS OF TRICHOMONADS INFECTION IN PUPPIES FROM FRENCH BREEDING KENNELS

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Recently, *Pentatrichomonas hominis* (PH) and *Tritrichomonas foetus* (TF) have been identified in diarrheal stool of puppies. However prevalence of Trichomonads in this population of young dogs has never been described. The aim of this study was to estimate the prevalence and risk factors of Trichomonads infection in puppies from French breeding kennels.
215 puppies from 25 breeding kennels were included in the study. For each puppy a rectal swab was performed and inoculated in a commercially available system "In PouchTM TF test" (BioMed Diagnostics, Oregon USA). The pouches were incubated at room temperature. Cultured samples were evaluated by microscopic examination 2 days after incubation for the presence of motile

trophozoïtes. Negative cultures were maintained for 15 days, and reevaluated every 2 days. Positive culture systems were frozen and single-tube PCR assays were performed in order to sequence and identify the Trichomonads observed. For each puppy faecal quality was scored using a 13-point numerical scale. According to the expected mean adult body weight, puppies were divided into two groups: small and large breed dogs (breed with a mean adult body weight < 25 kg or > 25 kg). Kennels were also divided in two groups based on their size: small and large size kennels (i.e., kennels producing less than 30 puppies per year or more than 30 puppies per year).
A mean number of 9 puppies were sampled per kennel (range: 4–18). Trichomonads were isolated in 15.8 % of puppies (34/215) and 20 % of kennels (5/25). Their prevalence was influenced by kennel size (p<0.001) and breed size (p<0.001). A significantly higher prevalence was observed in large breeding kennels compared to small breeding kennels (34% vs 2%). None of small breed puppies were infected by trichomonads. Dogs with abnormal feces presented a significantly higher prevalence of Trichomonads than dogs with normal feces (27% vs 12%; p=0.007). PCR assays performed on DNA extracted from the 34 positive cultures identified 23 dogs as infected with PH. Extracts of fecal DNA from the remaining 11 dogs were PCR negative for TF and PH.
These results show the presence of trichomonads is associated with large breed dogs, large size kennels, and diarrhoea. The high prevalence of dogs infected could be explained the age and origin of dogs (young puppies from kennels). The poor specificity of the medium to distinguish TF from PH requires PCR for precise identification of species of Trichomonads.

RISK FACTORS OF *PENTATRICHOMONAS HOMINIS* INFECTION IN PUPPIES FROM A LARGE BREEDING KENNEL AND IMPACT ON FECES QUALITY

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Recently, *Pentatrichomonas hominis* (PH) and *Tritrichomonas foetus* (TF) have been identified in diarrheal stool of puppies. Trichomonads were isolated in 15.8 % of puppies living in French breeding kennels with an influence of breed and kennel size [unpublished data]. The aim of this study was to evaluate effect of age and breed size on Trichomonads infection, and impact of this parasite on feces quality.
262 dogs (188 puppies and 74 dams) from a large French breeding kennel were included in the study. Puppies were followed between 4 and 10 weeks of age. Their dams were followed during the same period of time. After a spontaneous defecation, feces quality was scored. Presence of Trichomonads was evaluated using a commercially available system "In PouchTM TF test" (BioMed Diagnostics, Oregon USA) as already described [1]. Positive culture systems were frozen and single-tube PCR assays were performed for 9 positive samples in order to sequence and identify the Trichomonads observed. According to the expected mean adult body weight, puppies were divided into two groups: small and large breed dogs (breed with a mean adult body weight < 25 kg or > 25 kg).
427 sampled were collected (288 in puppies and 139 in dams). Prevalence of Trichomonads was influenced by age and breed size. A significantly higher prevalence was observed in puppies compared to their dams (34% (73/288) vs. 11% (15/139); p<0.001). A higher prevalence of trichomonads was observed in large breed puppies compared to small breed puppies (48% (69/144) vs. 3% (4/144); p<0.001). Puppies with abnormal feces did not present a significantly higher prevalence of Trichomonads than dogs with normal feces (32% (23/73) vs. 27% (58/215); p=0.457). PCR assays performed on DNA extracted from 9 positive cultures identified PH.
These results show the poor specificity of the medium to distinguish TF from PH and the necessity to use PCR for precise identification of Trichomonads. All the dogs in this study lived in the same environment with contacts possible between them. So effects of age and breed size may be due to a sensibility of large breed dogs and young dogs to this parasite.

EFFECT OF ANTIPARASITIC TREATMENT ON THE IMMUNE RESPONSE OF BOVINE

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Helminthes penetrate and establish themselves in the host tissues, incorporate metabolites from the host, and modulate the host immune response. Proteins secreted by parasites and proteins expressed on their surfaces as membrane-bound proteins participate in a wide range of parasite functions (Rosenzvit et al., 2006).

While helminthic infestation is recognized as deleterious to the host, it is unclear whether anthelminthic treatment might be immunosuppressive. The effects of piperazine or trichlorphon administered to drinking water or fenbendazole administered in feed were insignificant in BALB/c mice. The induction of allospecific cytolytic T lymphocytes (CTLs) in vitro, influenza specific memory T cells in vivo, influenza specific antibody secretion *in vivo*, or influenza-specific helper T cells and CTLs in vitro were examined. Results of this study indicate that anthelminthic treatments did not interfere with immune responses (Reiss et al.1987).

These studies were accomplished on 15 calves of 4-6 months old of Holstein race, divided in 3 groups. I^{th} group with non-infected and anticolibacilary vaccinated animals. II^{nd} group - poliparasitated bovine (*Strongyloides papillosus, Neoascaris vitulorum, Eimeria spp*), treated with complex antiparasitary chemotherapy (Amprolium, Ivermectinum, Tylosinum 200) and vaccinated (anticolibacilary vaccine) after 15 days post-therapy. III^{rd} group - poliparasitated calves (*Strongyloides papillosus, Neoascaris vitulorum, Eimeria spp*), treated with complex antiparasitary chemotherapy (T-activinum, Amprolium, Ivermectinum, Tylosinum 200) and vaccinated (anticolibacilary vaccine) after 15 days post-therapy.

In result of calves vaccination of II^{nd} group, have found lower level of total lymphocytes decrease by 12.5% ($p<0.05$), B - 16.9% ($p<0.001$), T - 21.2% ($p<0.01$, Th - 38.2% ($p<0.01$), but increases level of null cells with 21.2% ($p<0.01$) and Ts - 20% ($p<0.01$). The phagocytosis activity decrease by 20.6% ($p<0.01$), and the phagocytosis index by 28.8% ($p>0.05$). The specific antibody level decrease at antigen K_{99} by 85% ($p<0.001$); F_{41} - 75.7% ($p<0.01$); K_{88ab} - 70% ($p<0.01$) and Att_{25} by 89.6% ($p<0.001$).

The total average level of specific antibody decreased by 82% ($p<0.01$) in comparartive with non-infected and anticolibacilary vaccinated animals (I^{th} group), it is may be a result of antiparasitary chemotherapy immunotoxic effects.

The parallel researches where proves, where in result of calves vaccination of III^{rd} group, have found lower level of total lymphocytes decrease by only 12.5% ($p<0.05$), T remains constant, but phagocytosis activity decrease by only 4.3% ($p>0.05$), and the phagocytosis index by 10.5% ($p>0.05$), but increase the B lymphocytes level by 9.5% ($p<0.01$), null cells - 13.2% ($p<0.05$) and Ts - 2.4% ($p>0.05$). These immune modifications, induce formation lower of antibody level at antigen K_{99} by 40.5% ($p>0.05$); F_{41} - 19.7% ($p>0.05$); K_{88ab} – 31% ($p>0.05$) and Att_{25} by 15% ($p>0.05$).

Therefore lower of antibody level by 16. 1% ($p>0.05$) in comparartive with control group, confirm the immunotoxic effect of Ivermectinum, but the fact that it is more increased in comparartive with infecteted, treated with complex antiparasitary chemotherapy (Amprolium, Ivermectinum, Tylosinum 200) and vaccinated animals, is due modulatory effect of T-activin.

Keywords: Polyparasitism, phagocytosis, immunotoxic, limphocytes.

STUDY OF THE FERTILITY OF *ECHINOCOCCUS GRANULOSUS* CYSTS IN RUMINANTS IN THE PROVINCE OF DJELFA (ALGERIA)

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The fertility of 1200 hydatid cysts (hepatic and pulmonary) collected after the inspection of 2367 carcasses (350 cattle, 1230 sheep and 787 goats) at slaughterhouses of Djelfa (Algeria) during the two years (2009 and 2010), been studied by microscopic examination. The number of cyst collected by species was 450, 630 and 120 cysts respectively for cattle, sheep and goats. The organ samples were preserved in formalin or alcohol 92° and hydatid fluid was aspirated by a sterile syringe and stored in boxes in a cool molded. We considered that all cysts containing protoscoleces (hydatid sand) was fertile and those without protoscoleces (acephalocystes) were sterile. Our results showed that the fertility rate was (44.44%, 50.80%, 22.50%) rates of sterility (27.10%, 38.1%, 77.50%) and rate of calcified cysts was (28.46%, 11.10%, 00%) respectively for bovine, sheep and goats species.
However, we have studied the morphological appearance and consistency of cysts found, it was noted that the form unilocular unicystiq was the most widespread. It was found that the form unilocular polycystic was observed in younger animals, while those with advanced age had calcified cysts or with the casein.
Keywords: hydatid cyst, Fertile; Sterile; protoscoleces; Morphology; Dimension.

MOLECULAR IDENTIFICATION OF *ECHINOCOCCUS MULTILOCULARIS* IN SMALL MAMMALS BASED ON MITOCHONDRIAL DNA IN RAZAVI KHORASAN PROVINCE, IRAN

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Alveolar echinococcosis is a zoonotic infection caused by the metacestodes of *Echinococcus multilocularis*. Many species of small rodents, including microtine and arvicolid rodents or *Ochotona* spp. are natural intermediate hosts of this cestode. The main object of the current study was to determine the presence of *E. multilocularis* infection in its natural intermediate hosts in Chenaran County, Razavi Khorasan Province, Iran. A trapping program was performed in five villages of Chenaran County that this tapeworm was recently reported in carnivores. Totally, the liver of 85 small mammals included 54 *Microtus transcaspicus*, 15 *Mus musculus*, 9 *Apodemus witherbyi*, 4 *Ochotona rufescens*, 2 *Crocidura gmelini* and one *Nesokia indica* were investigated for the presence of *E. multilocularis* infection using multiplex PCR of mitochondrial genes. *Echinococcus multilocularis* infection was identified in the liver of 30 small mammals (35.3%): 23 *M. transcaspicus*, 3 *O. rufescens*, 2 *M. musculus*, one *C. gmelini* and one *A. witherbyi*. Furthermore, the multiplex PCR detected *Taenia* spp. infections in the liver of 14 captured small mammals (16.5%); including 9 *M. transcaspicus*, 2 *M. musculus*, one *C. gmelini*, and one *A. witherbyi*. The only trapped *N. indica* species was infected with *Taenia* spp. Base on the present study, *M. transcaspicus*, *O. rufescens, M. musculus, C. gmelini* and *A. witherbyi* can role as natural intermediate hosts for the transmission of *E. multilocularis* in Chenaran County, northeastern Iran.

The high prevalence of *E. multilocularis* in small mammals indicated that the Chenaran County is an endemic area for *E. multilocularis* infection. Therefore, establishment of this life-threatening cestode in the studied area could be an important source for human alveolar echinococcosis in this region.

SY07/1

THE ROLE OF PROTEIN KINASE C AND MITOGEN-ACTIVATED PROTEIN KINASE SIGNALLING IN HOST DETECTION, INVASION AND DEVELOPMENT OF *SCHISTOSOMA MANSONI*

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Our work has focused on the role of protein kinase C (PKC), extracellular signal-regulated kinase (ERK) and p38 mitogen-activated protein kinase (p38 MAPK) in the human parasitic trematode *Schistosoma mansoni*. Here, we report on studies concerning free-living cercariae and developing schistosomula that aim to elucidate the underlying mechanisms of host detection, invasion, development and establishment in the host. Immunolocalization studies with cercariae revealed activated PKC, ERK and p38 MAPK predominantly associated with the acetabular glands, with p38 MAPK and ERK activity also found in the parasite surface and tail. The involvement of these kinases in mechanisms that underpin host detection and penetration were studied by means of a combined linoleic acid (LA) and CFDA-SE based assay designed to induce and monitor the release of cercarial gland components. LA is a skin lipid known to trigger release of cercarial secretions and CFDA-SE is a fluorescent amine-reactive dye known to react with acetabular gland components of cercariae. In the presence of LA, cercarial PKC activity was biphasic, with an initial increase in phosphorylation between 5 to 15 minutes returning to basal levels at 30 minutes, which were sustained until the end of the assay (120 minutes); in contrast, ERK phosphorylation was significantly reduced after 15 minutes. Pharmacological inhibition of cercarial PKC, ERK and p38 MAPK with 15 μM GF109203X, 1 μM U0126 or 1 μM SB203580, respectively, prior to LA challenge, partially blocked acetabular gland release measured by fluorescent microplate readings and confocal microscopy, while the PKC activator PMA accelerated release. The roles of these signalling enzymes in mechanically-transformed cercariae (schistosomula) were also investigated. Active PKC was predominantly localized in the tegument and gastrodermis of developing schistosomula, while p38 MAPK and ERK were found mainly associated with the parasite surface. During schistosomula development PKC phosphorylation significantly increased after 72 hours. Further characterization of ERK and p38 MAPK is currently underway. Preliminary growth factor transactivation studies suggest that host growth factors can modulate the activities of ERK and PKC in schistosomula. Future work will focus on suppression of these signalling enzymes to help understand their role in schistosome growth, development and homeostasis. Overall these data contribute significantly to our understanding of cell signalling in schistosomes and how this signalling regulates parasite invasion and development.

POSSIBLE ROLE OF EPIGENETIC MECHANISMS IN SEX CHROMOSOME EMERGENCE IN *SCHISTOSOMA MANSONI*, A HUMAN PARASITE

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To help elucidate the mechanisms that led to the emergence of sex chromosomes we compared the DNA sequence (genome) and the chromatin structure (epigenome) of male and female individuals of the leuphotrochozoan parasitic platyhelminth *Schistosoma mansoni*, a blood fluke. In

this phylogenetically basal species, male individuals are homogametic (ZZ) whereas females are heterogametic (ZW). Asexual reproduction takes place in the invertebrate host (larval stages), while sexual reproduction occurs in the vertebrate host (adult stages). The proportion of repeated DNA is around 47% with an unknown proportion of domesticated, immobile repeats. We used massively parallel DNA sequencing to (i) de-novo assemble the repetitive sequences, and (ii) to identify unambiguously Z-specific, W-specific and pseudoautosomal regions of the *S. mansoni* sex chromosomes. Here we show that about 90 % of *S. mansoni* W and Z are pseudoautosomal. The W-specific region is composed almost entirely of 36 different repeat families, 33 of which are novel satellite DNAs. Despite an exhaustive search, we did not identify any female-specific gene, strongly suggesting that the female-specific repetitive sequences play a role in sex determination. Transcription and chromatin status of female-specific repeats were correlated to life stage, e.g., if repeats were transcribed, transcription was restricted to the larval stages lacking sexual dimorphism. In contrast, in the sexually dimorphic adults no transcription from repeats was found. In addition, levels of histone modifications typically associated with transcriptionally active euchromatic decreased around the W-specific repeats, as assayed by ChIP and ChIP-Seq. Recombination was repressed in this region even though homologous sequences are present on both Z and W chromosomes. Our study provides evidence for the hypothesis that repeat-induced chromatin changes may have been an initial event in sex chromosome emergence, at least in organisms with ZW chromosomes.

A ROLE FOR EPIGENETICS IN THE ADAPTIVE EVOLUTION OF A HUMAN PARASITE, *SCHISTOSOMA MANSONI*, IN RESPONSE TO ENVIRONMENTAL STRESS

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All living organisms must be able to express different phenotypes to adapt or acclimatize to environmental variations that can sometimes represent a stress for the organism. The precise mechanisms of adaptive evolution remain still elusive because they result from the interplay between genetic and epigenetic variations under environmental pressure. That is the reason why these mechanisms must now be studied in an integrative manner and at the genome-wide scale. Recent technological developments such as Next Generation Sequencing (NGS) provide the tool for analyzing epigenetic, genetic and transcriptional changes on a whole-genome scale in populations undergoing experimental evolution protocols. The host/parasite interactions are particularly well-suited models because in these systems selective pressure is strong and evolution fast. The plathyhelminte *Schistosoma mansoni* is a parasite responsible for schistosomiasis (intestinal bilharziosis), an important parasitic human disease ranking second only to malaria in terms of parasite-induced human morbidity and mortality. *S. mansoni's* life cycle is characterized by passage through two obligatory hosts: the fresh-water snail *Biomphalaria glabrata* (and other *Biomphalaria* species, dependent on the geographical location) for the asexual larval stage; and humans or rodents as host for the sexual adult stage. We exposed clonal *S. mansoni* populations to an allopatric intermediate host, an environmental condition that represent a stress for the parasite, for three generations. We show here that this change in the environment lead to changes in life-history traits (such as prevalence and sex ratios), modifications in gene expression and alterations of the epigenome (changes in chromatin status). Our data indicate that epigenetics could be used by the parasite to produce phenotypic variants in response to environmental stress.

GENETIC BASES OF ADAPTIVE EVOLUTION OF *SCHISTOSOMA MANSONI* TO ITS INTERMEDIATE HOST: CLUES FROM NEXT GENERATION SEQUENCING DATA

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As all parasites, *Schistosoma mansoni* have to adapt to different components of its environment, among which the interaction with host partner is the most fascinating aspect. To complete its life cycle, *S. mansoni*, as a digenetic parasite, interact first with the intermediate mollusc host before infecting the definitive vertebrate one. In addition to a host range that is very strict concerning the intermediate host (i.e. only some *Biomphalaria* species can be infected), a compatibility polymorphism phenomenon is observed for this host parasite interaction: specific combination of host strains with parasite strains allows infection (they are compatible) and others do not. Comparative genetic and epigenetic studies have already highlighted significant differences between compatible versus incompatible strains, supposing differential selective pressure due to intermediate host genetic background. Recent advances in next generation sequencing (NGS) enable to rapidly and cost-efficient scan the whole genome of *S. mansoni* strains, enlarging the possibility to test new hypotheses. In this study, we compared compatible and incompatible strains to test for differential positive selection at the genome level. We resequenced the genome of both strains using Illumina technology and generated SNP data sets. We then identified synonymous and non-synonymous mutations within specific genes compared to reference genome and calculate the evolution rates on different genomic regions. Based on SNP density distribution, we searched for candidate genomic regions under positive selection. These SNP markers will also help to identify selective sweeps associated with intense selection on genome regions of *S. mansoni* and thus explain phenotypic specificities of both compatible and incompatible strains.

SY07/2

RESISTANCE TO RE-INFECTION WITH *SCHISTOSOMA MANSONI* IN MICE TREATED WITH MEFLOQUINE

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Schistosomiasis is the most wide spread water-borne disease of considerable health problem globally. Praziquantel the only available drug for schistosomiasis, effectively kills adult schistosomes but lack efficacy against schistosomulae (juvenile schistosomes) a fact that explain low observed cure rates and rapid re-infection rates of schistosomiasis. The antimalarial drug mefloquine proved to have interesting anti-schistosomal properties mainly on schistosomulae. In this study the effect of mefloquine to induce resistance to re-infection of *Schistosoma mansoni* infected mice after treatment was evaluated and compared with praziquantel. Mefloquine was given orally as single oral dose of 400 mg/kg body weight and praziquantel was given orally of 500 mg/kg daily for 2 days. The induced resistance was evaluated parasitologically; measuring worm burden, tissue egg load and oogram, status of egg maturation and viability pathologically by measuring granuloma size and numbers and using Electron Microscope. Batches of *Swiss albino* mice, weighing 20 + 2 gm were infected with Egyptian strain of *Schistosoma mansoni* cercariae (90 + 10 cercariae/ mouse) cutaneously. Mice were grouped into several parallel groups (non-infected and not-treated, non-infected treated, infected not treated, infected treated with mefloquine and infected treated with praziquantel). In conclusion: Resistance to schistosomiasis mansoni re-infection was observed in mefloquine treated mice as shown by reduction in the total numbers of mature worms, immature worms and eggs. This reduction was accompanied by anti-pathological effects compared to praziquantel. This model need to replicate in human individuals may be a promising treatment regimen prevents treatment failures and a strategy for *Schistosma mansoni* control programs.

IN VITRO STUDY: ANTI-*SCHISTOSOMA MANSONI* BIOLOGICAL ACTIVITY OF VOLATILE OIL OF *PERSEA AMERICANA* MILL. LEAVES (AVOCADO) ON DIFFERENT PARASITE STAGES

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Schistosomiasis still an important helminthic infection that seriously delay socio-economical promotion in developing countries, it is endemic in approximately 76 countries in tropical and subtropical areas especially in Africa, Asia and Latin America. Bearing in mind; arising of many reports showing development of Praziquantel resistance, which is believed to be the only available chemotherapeutic drug; this necessitates searching for a new efficient anti-*Schistosoma* agent. In our present study we tested in vitro anti-*Schistosoma* efficacy of latex of *Euphorbia pseudocactus* A. Berger, volatile oil of *Persea americana* L. (Avocado oil) & and leaves methanolic extracts of *Fatsia japonica* (Thunb.) Decne. & Planch on *Schistosoma mansoni* stages. The parasite stage viability and morphological alterations were evaluated after incubation with different dilutions of the plant oil/extract using inverted as well as scanning electron microscopy. Volatile oil of *Persea americana* showed the highest pan-stages anti-*Schistosoma* effects with calculated IC_{50} 9.73, 6.87 and 4.82 mg/100ml against miracidium, cercaria and schistosomula respectively, while *Fatsia japonica* extract showed comparable moderate inhibitory effect with IC_{50} 5.54 mg/100ml only against schistosomula stage. However, latex of *Euphorbia pseudocactus* showed the weakest lethal effect on all *Schistosoma mansoni* stages with IC_{50} 37.98, 31.38 and 24.80 mg/100ml against miracidium, cercaria and schistosomula respectively. The morphological alterations by scanning electron microscopy were in conformity with lethal potency of tested plants. This might be the first study to address anti-*Schistosoma* effect of volatile oil of *Persea americana* suggesting that it may contain a potent promising medicinal component against all stages of *Schistosoma mansoni*, which enforce further fractionation studies to evaluate different extracts and components of this plant.

ANTI-PARASITIC ACTIVITY OF BIOMPHALYSIN, THE FIRST BETA PORE FORMING TOXIN FROM *BIOMPHALARIA GLABRATA*

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Aerolysins are virulence factors belonging to the super-family of beta pore forming toxin (beta PFT) mainly described as secreted products of Gram positive and negative bacteria. They have cytolytic activity triggered by channel formation in target cell membranes. Indeed, these proteins bind with high affinity to the glycosyl-phosphatdyl-inositol anchored proteins located on the surface membrane of target eukaryotic cells. Their binding to receptor induces a proteolytic cleavage leading to an active from that oligomerizes, forming a channel that causes lysis of the target cell. To our knowledge beta PFT were mainly described in bacteria and only 2 beta PFT were identified and functionally characterized in eukaryotic organisms. An interactome approach was performed between *Schistosoma mansoni* and *Biomphalaria glabrata* extracts to identify immune complexes involved in parasite/host interaction. This approach revealed a putative cytolytic protein related to beta PFT family from *B. glabrata* named Biomphalysin. Our data show that Biomphalysin is a novel beta type pore forming toxin characterized by many beta-sheets and a transmembrane domain, signatures of aerolysin members. Interestingly, Biomphalysin protein is only expressed in haemocytes supporting its role in innate immune cellular defence. Biomphalysin is over expressed after bacterial challenges (Gram negative and Gram positive bacteria) but not after parasitic challenges (*Echinostoma caproni* and *S. mansoni*). Biomphalysin was shown to have haemolytic activity toward sheep red blood cells and to have cytotoxic activity toward *S. mansoni* sporocysts. Binding of Biomphalysin on sporocyst membrane was confirmed by immunocytology. These results

provide the first functional description of an effector protein involved in parasite killing in the Lophotrochozoan snail *Biomphalaria glabrata*.

SY07/3

PRELIMINARY INDICATION OF DIRECT PREDISPOSING RELATIONSHIP BETWEEN CHRONIC HUMAN *SCHISTOSOMA MANSONI* INFECTION AND HEPATOCELLULAR CARCINOMA

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Estimated 500,000 - 1 million cases of hepatocellular carcinoma (HCC) are reported to occur yearly worldwide, with a mean annual incidence of around 3 – 4% of global population. HCC is rapidly fatal in most patients; that make its incidence and mortality rates almost equal. In the last 5–10 years there were many alarming reports of sharply increased incidence of HCC. In Egypt, HCC reported to account for about 4.7% of chronic liver disease (CLD) patients, which has tremendous impact on socio-economic development in the country. Available data suggests indirect evidence of an association between *Schistosoma mansoni* and hepatocellular carcinoma, possibly through potentiation of hepatitis B and C infections. In present study we conducted cross-sectional comparative analysis on 60 patients diagnosed as HCC cases. Chronic schistosomiasis cases were confirmed by finding anti-*Schistosoma mansoni* antibodies IgG by ELISA. Hepatitis C viral infection was proved by detection of viral load by quantitative Real time PCR. Among the study group 56.6% (34/60) were dweller in rural and 43.4% (26/60) resident in urban areas in Fayoum governorate. Patients' age ranged 45- 70 years old. Within hepatocellular carcinoma cases 26.7% (16/60), and 33.3% (20/60) suffered pure chronic schistosomiasis and pure Hepatitis C (HCV) infections respectively, with no statistically significant differences ($p=0.37$), indicating comparable risk value of both infections in predisposing directly to HCC. Additionally; frequency of HCC patients with assumed potentiated HCV infection by chronic *Schistosoma mansoni* 6.7% (4/60) were statistically significant ($p<0.05$) less among total HCC patients included in this study, when compared to HCC patients proceeded by either pure chronic schistosomiasis 26.7% (16/60) or pure HCV infection 33.3% (20/60). Our present study is one of few, addressing the possibility of direct relation between *Schistosoma mansoni* & hepatic carcinoma, concluding an initial indication of equal risk value of both human chronic *Schistosoma mansoni* infection and hepatitis C viral infections in precipitating hepatocellular carcinoma among Egyptian patients. These data urge additional studies to explore further understanding of factors that could be beyond such relationship.

SY17/1

METAL BIOACCUMULATION AND PARTITIONING IN THE TISSUES OF THE ENDOPARASITE, *BOTHRIOCEPHALUS ACHEILOGNATHI*

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Recently, interest in the use of intestinal helminth parasites as bioindicators of heavy metal pollution in aquatic habitats has increased. Previous studies have indicated that acanthocephalans and cestodes in particular have a greater capacity for the accumulation of heavy metals than their host organisms and other known free living indicators. Further research into the ability of intestinal helminth parasites to accumulate heavy metals has revealed a partitioning in the metal concentrations between the anterior and posterior body segments in cestodes. The aim of this investigation was to firstly identify the metals present in the different body segments of the pseudophyllidean cestode *Bothriocephalus acheilognathi* (Yamaguti, 1934) in comparison to those

metals present in the tissues of the host fish *Labeobarbus kimberleyensis* (Gilchrist and Thompson, 1913). Secondly to determine whether a difference occurs in the concentrations of the metals present in the different body sections of the tapeworms. Largemouth Yellowfish (*L. kimberleyensis*) were sampled with the use of gill nets from the Vaal Dam, South Africa. The cestodes were obtained after dissection of the host fish and they were separated into three sections, namely the scolex, mature proglottids and gravid proglottids. Analysis of metal concentrations for the fish tissue was done with the use of ICP-MS and measurement of the metal concentrations in the cestode tissue was performed using Total Reflection X-ray Fluorescence Spectrometry. Selected cestode segments were stained with a fluorescent probe to visually demonstrate the heavy metals binding sites within the different body sections. The results for the metal concentration analyses shows that metals are sequestered differentially between the different body segments of the cestodes, also the cestodes accumulated more metals than the host fish and the environment. The use of the fluorescent probe showed that the metal ions were binding to the eggshells of the cestodes and to the embryo within the eggs. There was also some fluorescence of the organs (eg. uterus and testes) within the mature proglottids. We conclude from the study that 1) the tapeworms do indeed accumulate metals at greater concentrations than those present in the tissues of the host and the environment, 2) that there is partioning in the accumulation of metals in the different body segments, this was also visually demonstrated with the use of the Phen Green fluorochrome.

Funding for this project was provided by the NRF and the University of Johannesburg.

ACETYLCHOLINESTERASE SECRETION BY *ANISAKIS SIMPLEX* LARVAE (NEMATODA: ANISAKIDAE) - A BIOLOGICAL RESPONSE TO CHANGES OF ENVIRONMENTAL CONDITIONS

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Acetylcholinesterase (AChE) is one of the most important enzymes involved in nerve impulse transmission in both vertebrates and invertebrates. In addition to the presence of neuromuscular AChE, many parasitic nematodes synthesize acetylcholinesterase in secretory glands and release enzyme into their external environment. Secreted acetylcholinesterase plays an important role in host - parasite interactions. The objective of the study was to evaluate the activity of AChE secreted by *Anisakis simplex* larvae (L3) and to analyze host-parasite interactions in enzymatic activity. *A. simplex* larvae were obtained from herring *Clupea harengus*, sampled in several regions of the southern Baltic. The enzyme kinetic in E/S products and somatic extracts of larvae as well as herring muscle tissue was monitored using an Absorbance Microplate Reader. Generalized Linear Models (GLM) were applied to analyze the relationship between AChE activity and area of sampling as well as the biological parameters of host and parasites. Obtained results reveled that average AChE activity was approximately 4-fold higher in E/S products and 8-fold higher in somatic extracts of *A. simplex* larvae than in herring host muscle tissue. Acetylcholinesterase activity in nematodes was inversely related to the enzyme activity in their hosts: AChE inhibition, recorded in herring sampled in polluted waters was accompanied by the highest enzymatic activity of its parasites. Physiological function of AChE secreted by parasitic nematodes has been widely discussed in the literature and numerous roles for this form of enzyme have been suggested. The results of our investigation may indicate that AChE secretion by *A. simplex* larvae is a biological response to direct and/or indirect changes of environmental conditions.

LIFE CYCLE OF LAMPROGLENA CLARIAE UNDER LABORATORY CONDITIONS

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Lamproglena clariae (Fryer, 1956) is endemic to Africa and attaches to gill filaments of freshwater fishes of the Clariidae catfish family (Marx & Avenant-Oldewage, 1996). The adult stage of this parasite has been extensively studied and described (Fryer 1956, 1961, 1964; Marx & Avenant 1996). A brief description of the nauplius stages of this parasite was recorded by Fryer (1956). The only species in this genus whose life cycle has been described is *Lamproglena chinensis* Yü, 1937 by Kuang (1962) and Grabda (1963) provided detailed and comprehensive findings on the life cycle of *Lernaea cyprinacea*, a parasite belonging to a different genus of the same family of Lernaeidae. The current study aimed at determining the number of life stages of *L. clariae* and further studying and recording their morphology. Adult specimens of *Lamproglena clariae* were collected from the gills of fish. Egg sacs were collected and maintained in tap water to allow larvae to hatch. After the third naupliar stage, larvae were transferred to suitable hosts in aquaria in the laboratory. Fish were killed at different intervals, various larval stages of the parasite recovered and preserved in 70% ethanol. Preserved specimens were cleared in 90% lactic acid, mounted and drawn with the aid of a Zeiss light microscope equipped with digital imaging software. Three nauplii and four copepodite stages were observed. Nauplius stages are translucent, elliptical bodies filled with a mass of yolk, which is reduced as the nauplius develops. The first stage has three pairs of appendages; the antennules, antennae and mandibles. The second and third stages have maxillule, appearing as a papilla with a pair of setae. There is an increase in the number of setae on appendages as they molt from one stage to the next. Copepodite stage I develops 14-21 days after hatching, and in the absence of a host fish, the parasite larvae die before reaching stage II. Peristaltic movement of the gut commences with the emergence of Stage I. Significant morphological changes in copepodites with passage from one stage to the next are expressed mainly by increase in the number of free abdominal segments and appendages, increased segmentation of the appendages and in the general increase in body length. At each stage, comparisons were made with the corresponding stages of *L. cyprinacea* and *L. chinensis*.

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HUMAN INTESTINAL FLUKES *HAPLORCHIS TAICHUI* AND *HAPLORCHIS PUMILIO* IN THEIR INTERMEDIATE HOSTS, FRESHWATER SNAILS FAMILY THIARIDAE IN THAILAND

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The intestinal flukes *Haplorchis taichui* (Nishigori, 1924) and *Haplorchis pumilio* (Looss, 1899) (family Heterophyidae) are among the most important food-borne parasitic zoonoses found in Southeast Asia. Here, the infection rates of freshwater snails family Thiaridae in Thailand were reported. The snails were collected by handpicking and scooping every two months for one year at several locations between December 2004 and September 2009. The snail samples were examined for parasitic infections in the lab by shedding and crushing methods. Parasite infections were found in snails from 66 of 126 sampling sites. Six species of thiarid snails were collected in this study, viz. *Melanoides tuberculatus* (Müller, 1774) *Melanoides jugicostis* (Hanley & Theobald, 1876), *Thiara scabra* (Müller, 1774), *Sermyla riqueti* (Grateloup, 1840), *Neoradina prasongi* (Brandt, 1974) and *Tarebia granifera* (Lamarck, 1822). Five species (*M. tuberculatus*, *T. scraba*, *T. granifera* and *N. prasongi)* were found to be infected with *H. taichui*, while four species (*M. tuberculatus*, *M. jugicostis*, *T. scraba* and *S. riqueti*) were found to be infected with *H. pumilio*. The infection rates of *H. taichui* and *H. pumilio* were 0.22% (133/59,884) and 1.03% (619/59,884), respectively.

Keyword: Intestinal Flukes, *Haplorchis* sp., Freshwater Snails, Thiarid snail.

This work was supported by the research and development institue Silpakporn University and Parasitology and Medical Malacology Research Unit, Silpakorn University (PaMaSU), Thailand.

EVALUATING THE STATUS AND IDENTITY OF SNAILS AS INTERMEDIATE HOSTS OF TREMATODES OF GENUS *MELANOIDES* OLIVIER, 1804 (GASTROPODA, THIARIDAE, *MELANOIDES*) IN THAILAND

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Genus *Melanoides,* which are widely distributed with many congeneric species and constituent populations in Thailand and the Southeast Asia. Two species, *M. tuberculata* and *M. jugicostis* were recorded in Thailand (Brandt, 1974). They were reported serve as the first intermediate host of parasitic trematodes. Given the importance in the infectious diseases and public health, it is essential to understand the taxonomy and distributions of the genus *Melanoides.* The aims of this study were investigation of systematics, evolution diversity and genetic variation of snails genus *Melanoides* in Thailand and comparison with the other regions (Lake Malawi, Madagascar Island, Lao, Vietnam and India). For Thai samples, the snails were collected from 141 localities various water source; such as waterfall, streams, ponds, rivers and brooks; between 2006 and 2010. Identify techniques based on the shell morphology (adults and juvenile shells), radulae, biogeography, geometric morphometrics, and phylogenetics. Analyses of shell parameters (height of shell, width of snail, length of aperture, width of aperture, height of last body whorl, and number of whorl), morphological, anatomical and genetic data (16S and COI) were studied. All samples of *M. jugicostis* had very similar soft-part anatomy with *M. tuberculata*, they were no specific differences in qualitative traits for anatomical characters. Radular study, the thiarid radula is Taenioglossan pattern, *M. tuberculata* had formula form 3 or 4 lateral cusps on the both sides and 1 central teeth (3-4/1/3-4) but *M. jugicostis* had the central teeth distinct 2 or 3 triangular cusps on both sides (2-3/1/2-3). In addition, the embryos and juveniles shells of *M. jugicostis* were very similar *Plotia scabra* (out group samples). On the fourth whorl of *M. jugicostis* was distinguished more sculpture and form knobs where they were crossed by spiral ridges. Whereas, the juveniles of *M. tuberculata* did not have knop appearance on the shell. Hitherto, *M. tuberculata* and *M. jugicostis* are important host parasites of human and some domestic animals in Thailand, therefor; the research of snail species is one of the most important knowledge for parasitic control.
Keywords: Freshwater snail, Thiaridae, Intermediate host, Trematode, *Melanoides* Olivier, 1804.

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SY17/2

EARLY SPRING: GOOD FOR PARASITES AND BAD FOR HOSTS OR BAD FOR BOTH?

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Theory predicts increasing frequency of extreme weather events associated with the anthropogenic climate changes. These were shown to result in extreme demographic fluctuations

threatening population survival in free-living organisms. Here we provide field evidence for the effects of the unusually warm early spring of 2011 on the populations of larval trematode parasites and their snail hosts in a natural reserve in Central Europe. As part of a longer time-series we have collected 20 samples of *Lymnaea stagnalis* and 17 samples of *Planorbarius corneus* at same dates in June, August and September of 2010 and 2011 at three sites within the Bohdanecsky Pond Reserve (Czech Republic). Totals of 17 and 10 trematode species were identified in component communities in *L. stagnalis* and *P. corneus*, respectively; of these 8 and 7 species, respectively, were common. The data on trematode richness and prevalence were examined in a 'before-after' design with respect to the warm spring of 2011. Contrasts between years for each sampling date revealed significant differences in component community richness and overall prevalence for *L. stagnalis*. Trematode communities sampled just after the warm spring in 2011 were characterised by much higher richness and overall prevalence than those sampled after the previous spring. Thereafter, the total number of species decreased but the prevalence was distinctly higher than in the samples of summer and autumn 2010. Although trematode communities in *P. corneus* exhibited no marked differences in overall prevalence between years for all three sampling dates, species richness was much lower in those sampled during summer and autumn 2011. The changes in prevalence and richness translated into substantial differentiation of trematode community composition and structure. Snail density exhibited a similar pattern for both host species, demonstrating a substantial decrease after the warm spring of 2011. Our findings illustrate that a substantial boost of trematode transmission by extreme heat in early springs may lead to local extirpation of the intermediate snail hosts. We suggest that extreme weather events can result in significant demographic fluctuations with possible effects on population persistence of trematodes and hosts that might lead to cascading effects on the local food webs.

LIVING IN A LAGOON: EFFECT OF ENVIRONMENTAL FACTORS ON LARVAL EMERGENCE RATES OF TWO OPECOELIDS IN *GIBBULA ADANSONII*

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Lagoons are important habitats for the transmission of digeneans and are characterised by variable abiotic conditions. However, experimental studies on the effect of environmental factors on transmission of lagoonal species are lacking. In a study of digeneans parasitizing molluscs in Els Alfacs lagoon (Ebro Delta, Spain, Western Mediterranean) we found heavy infections with sporocysts emitting two types of cotylocercous cercariae in the snail *Gibbula adansonii* (Gastropoda: Trochidae). We identified the cercariae molecularly using ITS ribosomal DNA sequences as the larval stages of *Cainocreadium labracis* and *Macvicaria obovata* (Digenea: Opecoelidae) and elucidated aspects of their life-cycles. Here we report the results from experiments designed to assess the effects of temperature, salinity, water level and photoperiod on the rates of cercarial emergence of the two species. Emergence rates were quantified and analyzed using general linear model repeated measures ANOVAs. Temperature had a significant effect on cercarial emission in the two temperature gradients tested (increasing and decreasing), with mean rates being typically higher at higher temperatures. However, the patterns of variation in cercarial emergence of the two species in response to the temperature gradients differed. Emergence rates of both *C. labracis* and *M. obovata* decreased with decreasing temperatures but only those of *M. obovata* increased when temperature was increased again. Both species had similar emergence rates at elevated salinity levels (45 psu) but showed a differential response to the salinity gradient (45-25 psu). There was a rapid decrease of cercarial emergence for *C. labracis* at decreased salinity levels whereas no significant effect was observed for *M. obovata*. Rates of cercarial emergence of the two species differed with regard to water level (high/low) with no significant changes for *C. labracis* and significantly higher rates at low water levels for *M. obovata*. There was a significant effect of both photoperiod (light:dark 12:12 hr and 15:9 hr) and

light-dark conditions on cercarial emergence of *C. labracis*, with rates being higher during the 12:12 hr cycle and during light periods. On the other hand, cercarial emergence rates of *M. obovata* did not differ between the two photoperiods but were distinctly higher during the dark periods. These differential responses in emergence rates of the two species to the tested environmental factors indicate an adaptation to specific transmission strategies for infecting the next hosts, with *C. labracis* infecting small benthic fishes with diurnal activity and *M. obovata* using as second intermediate hosts snails with presumably nocturnal activity.

DENSITY- AND TIME-DEPENDENT PROCESSES IN THE POPULATION GROWTH OF *GYRODACTYLUS SALARIS* ON ATLANTIC SALMON STOCKS

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Due to direct transmission and a short life cycle with in situ reproduction, gyrodactylid monogeneans are excellent models in which to study the evolution of host specificity and pathogenicity. Over the past 25 years, NHM Oslo has systematically examined the response of Norwegian salmon stocks to the highly pathogenic *Gyrodactylus salaris* using a standard methodology, in which individual fish are isolated and maintained individually within a common environment with no opportunity for parasites to move between them, although they share the same water supply. The growth of the parasite population is then followed through time until the fish has either recovered or died. This has generated a large number of datasets with many strains of salmon, which are available for reanalysis to test for density-dependence, time-dependence or immunological phenomena using improved modern statistical methodologies. Reanalysis of 17 of these datasets using Generalised Linear Modelling (GLM) demonstrates unequivocally that parasite population growth rate declines throughout the experiment in more than 60% of datasets, and that exponential population growth does not occur. It is not clear whether the decline in population growth rate is due to density-dependent or time-dependent processes, and we use an agent-based model of gyrodactylid population growth to distinguish between these alternatives. There is considerable difference between replicate experiments using the same stock of salmon; this may indicate substantial environmental effects influencing the outcome of infections, or differences in the genetic composition of salmon stocks between experiments. However it is clear that, even in the most susceptible stocks of Norwegian salmon, there are limits on potential parasite population growth rate.

SITE SELECTION IN *DOLOPS RANARUM,* A BRANCHIURAN FISH PARASITE

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Previous research by our group confirmed that helminths and Crustacea is site specific. We showed for instance that *Bothriocephalus ageilognathi* prefers the first 20% of the intestine*; Cichlidogyrus philander* attached to gill arch 2 and 3 and occurred dorsally on the distal ends. In the copepods *Lamproglena clariae* parasites occurred predominantly on the fourth gill arch and specifically the median part of the gill arch for attachment; the size of the parasite was directly correlated to the size of the host. However, in *Lamproglena hoi* the parasites attach close to the gill arch. The branchiuran *Dolops ranarum* occurs inside the buccal cavity and gill chamber of *Oreochromis mossambicus* (tilapia), however, it occurs mostly on the skin of *Clarias gariepinus* (African catfish). It was also observed that the parasites are able to move around freely on the host, lay their eggs of the host and is able to relocate a host after egg depositing. A catfish was placed in a 120mm diameter glass tube at an angle to allow an air space for air breathing. A constant water flow of oxygenated borehole water was pumped in at 300ml/min to force the fish to swim slowly creating water current around the host. Ten *D. ranarum* specimens were inserted into

the tube and their movement was monitored for one week at intervals, initially every 30 minutes. It was noted that the parasites initially attach at random but then move around the host to attach selectively and stay in the same position for most of the experimental period. It is deducted that attachment is not a random process but presumably driven by availability of food and shelter in *Branchiura* but that optimal distribution of eggs influence attachment in copepods additionally.

INVOLVEMENT OF SUBTILISIN-LIKE SERINE PROTEASES SUB3 IN THE ADHERENCE OF *MICROSPORUM CANIS* TO HUMAN AND DIFFERENT ANIMAL SPECIES EPIDERMIS

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Microsporum canis is a filamentous fungus responsible for the most cases of dermatophytosis in domestic carnivores, especially in cats, its natural host. This zoophilic dermatophyte can also infect other animal species and is responsible for a dermatozoonosis whose incidence is increasing in several countries. Since dermatophytes are generally confined to keratinized structures of the epidermis and its appendages, keratinolytic proteases from *M. canis* are considered as potential factors of pathogenicity. Recently, a keratinolytic subtilisin-like serine protease (Sub), Sub3, has been identified as essential for the adherence of the fungus to feline corneocytes.
The aim of this study is to assess whether Sub3 is also involved in the adherence of *M. canis* to human and other animal species epidermis, in addition to cat.
The adherence to epidermis of an *M. canis SUB3* RNA-silenced strain (IHEM 22957) was compared to that of the wild-type control strain (IHEM 22958), using an *ex vivo* adherence model and skin explants from humans, dogs, horses, rabbits, mice, guinea pigs and cats. The adherence assays were adapted from the one developed in the laboratory on feline epidermis (Baldo et al., 2010). Briefly, skin explants were conditioned and then inoculated with 5×10^4 arthroconidia of each strain for 4 hours at 37 °C under 5% CO_2 and humidified atmosphere. After washing with PBS-Tween 0.1% for 10 minutes, adherent arthroconidia were counted by culture. For each tested animal species, three independent experiments were performed using skins explants from three different animals in quadriplicates.
The results indicated that the adherence *M. canis SUB3* silenced strain is reduced in proportion of 84 to 92% when compared to the control strain set up as 100%, according to the type of epidermis used and whatever the animal species tested.
In conclusion, Sub3 is a keratinolytic protease that seems essential for the adherence of *M. canis* to human and various receptive animal species epidermis.

SY19/1

THE CURRENT SITUATION OF RARE HELMINTHIASES IN THE RUSSIAN FEDERATION

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At the present time is difficult to determine the number of nosological forms of helminthiasis, registered in the country. In Russian Federation only 11 nosological forms of helminthiasis is included in the official statistics. The rest of the helminthiasis, the incidence of which not more than 1.0 per 100 thousand population, is regarded rare in Russia and their record in a group of "other" helminthiasis. In this regard, it is difficult to identify the list of identified helminthiasis in the country. In the mid-2000s, we have found that in Russia reported 17 nosological forms of rare helminthiasis. The group of rare helminthiasis varies in the biological and epidemiological aspects and consists of

17 nosological forms. In the structure of rare helminthiasis largest share clonorchiasis - 63, 0±1, 9%. The area of clonorchiasis is located in the Far East, which revealed 99, 0±0,5% of autochthonous cases, so it cannot be considered a rare disorder in this region of the country. The share of dirofilariasis have to 15, 6±1, 4%, strongyloidiasis - 9, 9±1,2%, fascioliasis - 2, 5±0,6%, trichostrongylosis - 2, 0±0, 6%, hookworm diseases - 1, 7±0,5%. Sporadic cases of metagonimosis, nanophyetosis, anisacidosis, dipylidiosis, hymenolepidosis, sparganosis, cysticercosis, urinary schistosomiasis, paragonimoses, dioctophymosis, dicrocoeliosis are registered. Rare helminthiasis in Russia were found in 49 out of 83 subjects. Diagnosis of rare helminthiasis greatly depends on the level of knowledge of doctors of these diseases. The availability of international travel has led to an increase in visits of Russians in different countries. This travel increases the risk of imported parasitic diseases that do not know the doctors and they cannot make the correct diagnosis for a long time. In Russia, reported 9 imported nosological forms from 28 countries in 2006-2008 years. Our study showed the distribution of rare helminthiasis in Russia.

THE USE OF THE DIPLOZOON AS A SENTINEL ORGANISM FOR METAL POLLUTION

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Many studies have already been conducted on the use of endoparasites (Acanthocephalans & Cestodes) as sentinels for heavy metals. These studies revealed that endoparasites accumulate metals in concentration far above that of their respective host. There has been no conclusive result regarding the use of ectoparasites as such sentinels. When using an endoparasite as a sentinel the respective host has to be sacrificed, this is not the case with ectoparasites which can be removed with little damage to the host. Thus the use of a Diplozoon, which is a monogenean found on the gills of *Labeo umbratus* (Smith, 1841), as a sentinel species for metal pollution was evaluated. *Labeo umbratus* where collected during January and March 2011 from the Vaal Dam (South Africa). *Labeo umbratus* was chosen as a test fish because they have a high prevalence of diplozoons and they have been shown to accumulate metals. This parasite-host combination was deemed appropriate for the biomonitoring of the Vaal Dam due to its wide distribution throughout this system. The Vaal Dam was chosen as the study site due to its vast economic importance to three of South Africa's major industrial provinces. This dam also provides drinking water to more than 10 million people within the greater Pretoria-Witwatersrand-Vereeniging area. Five different tissue types (muscle, gills, liver, kidney and spinal cord) as well as the parasites where removed from the fish for metal analysis. Tissue samples were analysed using an inductively coupled plasma-mass spectrometry (ICP-MS) and the parasites where analysed using Total reflection X-Ray fluorescence (TXRF). This study has revealed that the Diplozoon species does in fact accumulate certain metals such as Fe, Cu, Ni & Zn in concentration above those found in the fish host and ambient environment. These results illustrate the potential use of such a parasite for the biomonitoring of metal contamination within the aquatic environment.

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CAPILLARIA PHILIPPINENSIS A NEWLY EMERGING PARASITIC CAUSE OF PROTEIN-LOSING ENTEROPATHY IN EGYPT

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Capillaria philippinensis, a tiny nematode parasite of fish eating birds; was identified as a cause of human disease for the first time in the Philippines in the year 1964. The disease was also reported in Thailand and the nearby countries in the Far East. Few sporadic cases have been reported in

other countries in Asia, Africa, Europe and South America. In Egypt, the first case was diagnosed in 1989, followed by many cases, until we were able to report 127 cases with12 deaths. Lack of knowledge of the disease and the parasite may cause a lot of undiagnosed missed cases. Egypt is considered the country with the highest number of cases outside the endemic area. The mode of infection is the ingestion of infective larvae in small non-eviscerated fish, eaten raw or undercooked. Cases presented with chronic diarrhea. The duration of diarrhea ranged from one month to two years. It is usually accompanied with vomiting and mal absorption manifested by electrolyte imbalance, hypoproteinemia, dehydration, vitamin deficiency and weight loss. Most of the patients showed lower limb edema, sometimes with ascites and/or pleural effusion. Death occurred as a result of irreversible hypokalemia that caused heart arrest or as a result of superimposed bacterial infections. Diagnosis was done by examination of stool specimens, sometimes more than once. A provocative test have been developed and used in our laboratory successfully. Cases have to be differentiated from a wide group of parasitic, bacterial, viral, immunological diseases as well as intestinal malignancy. This presentation will stress on the main points of the disease epidemiology after more than 20 years in Egypt and will show different stages of the parasite isolated from our cases.

SYNANTHROPIC DOGS AND CATS AS PARASITIC POLLUTION SOURCE OF URBAN ECOSYSTEMS IN CHIŞINĂU

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Preventing the spread of zoonotic invasions in Chişinău urban ecosystems requires a broader knowledge of the amount of pollution sources. Parasitological studies included pathologic material samples obtained from 955 synanthropic canine and 279 feline. The studies accomplished during 2006-2011 aimed the estimation of parasitism structure by location on / in the host, emphasizing the parasites with zoonotic circuit, determining major zoonoses and their evaluation in seasonal dynamics and by age groups in infected animals.
The obtained results confirm:
In **synanthropic canine** from urban ecosystems of Chişinău 14 species of zooparasites were registered: *Isospora canis*, *Sarcocystis spp*, *Dipyllidium caninum*, *Diphyllobothrium latum*, *Toxocara canis*, *Toxascaris leonina*, *Ancylostoma caninum*, *Trichuris vulpis*, *Rhipicephalus sanguineus*, *Sarcoptes canis*, *Demodex canis*, *Otodectes cynotis*, *Ctenocephalides canis*, *Trichodectes canis*. 10 (71, 4%) species from the mentioned above can determine infestations in humans; 6 (44, 4%) species are ectoparasites; 8 (55, 6%) species determine permanent endoparasitism with cavitary habitat (62, 5%), cavitary/tissular (12, 5%), tissular (12, 5%) and intracellular (12, 5%). In parasitism structure the species *Toxocara canis* (parasite with zoonotic circuit) has a dominant position (35, 7±5, 62%) with increased incidence in winter (38, 5±10, 41%). The increased invasion of the puppies (53, 9±4, 38%) is higher due to the 3 infestation ways (intrauterine, galactogenic, digestive).
In **synanthropic feline** from urban ecosystems of Chişinău 12 species of zooparasites were registered: *Isospora felis*, *Toxoplasma gondii*, *Dipyllidium caninum*, *Diphyllobothrium latum*, *Toxocara cati*, *Toxascaris leonina*, *Ancylostoma caninum*, *Demodex cati*, *Notoedres cati*, *Otodectes cynotis*, *Felicola subrostrata*, *Ctenocephalides felis*; 9 (75, 0%) species from the mentioned above can determine infestations in humans; 5 (42, 0%) species are ectoparasites; 7(58, 0%) species are endoparasites with cavitary (71, 3%) and intracellular (28, 7%) location. The major parasitozoonoses (toxocarosis/dipilidiosis) shows a higher incidence in summer (41, 2±4, 58%) and in autumn (41, 3±7,2 5%). The distribution of toxocarosis/dipilidiosis cases in subadult feline (0-12 months old) constitute 37, 6±6, 53%. Cats are susceptible to infection since postnatal period (digestive, galactogenic ways).
Thus, synanthropic canine and feline in urban ecosystems of Chişinău, which constitute an important source of parasitic environmental pollution, represent a real danger for infestation in humans.

SY19/2

EIMERIA OOCYSTS IN SOIL AND FAECES ON NATURALLY INFECTED PASTURES AND THE ROLE OF SEASONAL EFFECT

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Eimeriosis is the disease caused by pathogenic species of the intestinal parasite *Eimeria* that play an important role in cattle health, mainly in young stock. Little is known about the ecology of this parasite. This study examined the seasonal presence of *Eimeria* species after time from early summer and fall to the following spring on southern Estonian pastures. Three pastures were selected and visited 3 times: June 2010 (T1), October 2010 (T2), and April 2011 (T3). During the first two visits, fresh faecal samples were collected and the sampling locations were recorded using a global positioning system (GPS). On the third visit, sites sampled previously were relocated by the GPS coordinates and soil samples were taken. Quantitative concentration and flotation technique followed by microscopic investigation was used to identify *Eimeria* species in faecal samples. An in-house flotation method was used for recovering oocysts from soil. Oocyst counts per gram soil (T3) were 0.2% and 7.1% of the oocysts per gram faeces (OPG) from the same site sampled the previous season in T1 and T2, respectively. Fewer samples [OR: 0.27] at sampling time T2 were found positive for *Eimeria* compared with T1, and the average OPG of T2 amounted to only 1/3 of what was observed in T1 (p<0.001). Similarly, a third of soils (T3) were positive for *Eimeria* oocysts when re-sampled from T1 in contrast to half of the soils from T2. Pathogenic species (*E. alabamensis*, *E. bovis*, and *E.* zuernii) were found in 40% of the faecal samples in combination with OPG>500, which may indicate clinical coccidiosis. Either *E. bovis* or *E. zuernii* were found in 21% of the samples with OPGs above 500. Significantly higher (p<0.001) *E. alabamensis* and *E. zuernii* OPG scores were found in faeces at T1 than later in the year, atT2. On the other hand, *E. alabamensis* and *E. bovis* were recovered from the soil in higher (p<0.01) numbers on re-sampling after time from T2 than from T1. Under natural conditions, bovine *Eimeria* oocysts seem to be recoverable from the same locations in higher numbers and more frequently if they are shed late rather than early during the grazing season. Different species may not survive equally well. Estonia seems to have quite high numbers of cattle infected on pastures at levels that could indicate clinical coccidiosis, possibly due to the farmers not rotating cattle on pastures.

IMPORTANCE OF GENTLE HANDLING OF *EIMERIA BOVIS* OOCYSTS RECOVERED FROM SOIL SAMPLES AND TIMING OF OOCYSTS ENTERING THE SOIL ON THE PRESENCE IN THE FOLLOWING GRAZING SEASON

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Eimeria bovis is an intestinal parasite that can cause diarrhoea in calves. It has a direct life cycle including an exogenous sporulation phase in the environment which renders the oocysts infective. *Eimeria* oocysts can persist in the environment under various kinds of natural stress, but very little is known about their survival on and within the soil. Therefore we studied the effect of natural environmental conditions on the presence of *Eimeria bovis* oocysts in the soil. Furthermore, the importance of mechanical handling during diagnosis was evaluated in regard of its influence on the recovery rate of oocysts from soil. Soil samples were spiked with 100,000 *E. bovis* oocysts in July 2010 (T10) or with 50,000 oocysts in October 2010 (T7), respectively. The samples were stratified into soil samples that were added the oocyst solution plus 1 ml of uninfected cattle faeces material (T10: N=60; T7: N=29) or only the oocyst solution (T10: N=52; T7: N=14). The soil samples were left at ground level to be exposed to natural conditions until their analysis in April 2011. A subset of the samples was analysed immediately after spiking (T0). An additional experiment was conducted to evaluate the handling of samples by shaking the samples during analysis 0 (N=10), 1 (N=10), 5

(N=10), or 10 (N=10) times. The oocysts were recovered using a gentle in house flotation method and counted in a reading chamber. Calculations of oocysts per gram soil were adjusted to the dry matter content. Oocyst counts dropped to 0.30% for T10 compared to the initially spiked dose whereas the oocyst count was higher for T7 (< 3%). The presence of faeces did not affect the oocysts counts in any of the samples. The number of soil samples with detectable oocysts was significantly higher in T7 compared to T10 (p<0.05). Gentle sample handling (0 shakes) resulted in significantly higher recovery rate of oocysts compared to analyses with 1 or more shakes (p<0.001). Under experimental Estonian conditions, more soil samples contain detectable *E. bovis* oocysts in spring if the soil has been infected in autumn rather than in summer and at higher levels. This indicates sporulated oocysts on pasture late in the grazing season may be the more important contributor to next seasons pasture infections. Methods for oocysts recovery with non-gentle mechanical steps may result in significant losses entailing significantly lower detection sensitivity.

SY13/1

INDUCTION OF PROTECTIVE IMMUNITY IN MICE TO *TRICHINELLA SPIRALIS* USING A 30 MER PEPTIDE OF THE 43 KDA *T. SPIRALIS* ANTIGEN OR TSL-1 ANTIGENS WITH DEFINED ADJUVANTS

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Trichinellosis is a public health hazard and an economic problem in porcine animal production and food safety for humans. Therefore the development of methods for its prevention and control are most relevant. Protection against *T. spiralis* in experimental models has been achieved using a variety of muscle larvae (ML) antigens such as TSL-1 antigens (TSL-1 Ag), recombinant proteins or peptides expressed in live vectors using different adjuvants and DNA immunization. Our group has designed different immunization protocols which include the use of: a 30-mer peptide from *T. spiralis* 43 kDa antigen expressed in a live vector (*Salmonella enterica* serovar Typhimurium) together with the *T. spiralis* recombinant protein, the protein Lumazine synthase (LS) of *Brucella* sp. and the Transfer Factor (TF) obtained from crocodile together with TLS-1 antigens to immunize BALB/c mice previous to the challenge infection with *T. spiralis* ML. In general the results showed an approximately 60% reduction in ML burdens in the immunized animal with some variations according to the protocols used. Animals immunized with the peptide expressed in the live vector and boosted with the *T. spiralis* recombinant protein or with TSL-1 antigens and Lumazine synthase a 45-70 % reduction in adult worms was observed. Cytokines profiles determined in the intestinal fluids of immunized mice with TLS-1 antigens and LS or TF showed an early induction of Th1 type cytokines followed by a transitory increase of Th2 type cytokines. In animals immunized with TLS-1 antigens and LS an early increased expression of activated macrophages markers (Fizz 1 and Arg1) and a decreased of Tregs cells in the mesenteric lymph nodes was observed. All together these results suggest that the use of the 30-mer peptide expressed in *S. enterica* ser. Typhimurium together with a boost with the *T. spiralis* recombinant protein or TSL-1 Ags with different immunization protocols induced a significant protection against *T. spiralis*. Therefore these protocols may be used with other *T. spiralis* antigens to potentiate their capacity to induce protective responses in the host against this parasite.

TRICHINELLOSIS IN CROATIA

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No region has experienced a more marked threat of trichinellosis than that of Southeastern and Eastern Europe, especially Croatia. The following prevalence rates from Croatia clearly demonstrate the high risk for humans with traditional food habits of eating cured pork products. The disastrous spread of trichinellosis happpened in late 1990s. Before that time the incidence of T. spiralis in grazing pigs was very high, 21.76% (170 examined) but most of positive animals were lightly infected (0.016-0.02 l/g). While out of 2394 pigs raised in small private farms, 1.67% were positive with high infection rates, none of the pigs raised on a modern breeding and fattening farm were seropositive. Due to war and post war conditions in Croatia the trichinellosis spread ou tremendously. In the period between 1997 and 1999, 600 240 slaughtered pigs were tested for Trichinella and 0.16% were found to be positive. A decade later (2009) the prevalence showed a significant decrease whereby only 0.01% of 950 000 pigs tested positive. It has to be stressed that in 2011 only 25 pigs were found infected with Trichinella spiralis. This decrease was a result of 10 years of government funded intensive monitoring and control activities. The greatest success within the eradication program was achieved through continuous rodent control at all sites where infected pigs were detected, prompt disposal of infected swine carcasses and compensation to the owners for condemned pigs.

INCIDENCE OF *TRICHINELLA* INFECTION IN PORK AND WILD BOAR SAMPLES IN TRANSYLVANIA – FEATURES ON THE INVESTIGATION METHODS

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Many aspects of the biology and epidemiology of *Trichinella* infection in pork and wild boar are described in a number of research articles, but the fool impact and prevalence of this parasite in Transylvania area are still not known. The purpose of this research was to assessed the incidence of *Trichinella* in pork and wild boar meat samples using three major techniques of detection (trichineloscopy, digestion method and molecular methods). For this purpose, the positive samples for *Trichinella* infestation in pork and wild boar samples received in DSVSA Cluj and DSVSA Mureş were reexamined by these three methods. With trichineloscopy, reliable results were obtained but still the most effective method was the artificial digestion. In year 2011, from the total of 57 samples analyzed by direct trichineloscopy and artificial digestion, a single case was detected with the calcified form of *Trichinella* spp. .Although the classical compression method is not currently used in UE, it has the advantage of identifying other parasitic forms with zoonotic character. The identification of *Trichinella spp*. by molecular methods has revealed two species of *Trichinella* with the highest prevalence in the area studied: *T. spiralis* and *T. britovi.* The prevalence of this infestation with *Trichinella* in pigs between the years 2007 – 2011 was of 0.12% in Transylvania area, being lower than the one found in wild boar meat samples (3.36%). In year 2011, from the total of 338 pork meat samples examined in Cluj county, 15 were positive and in wild boar samples from a number of 308 samples, 3 were positive. Romania, and especially Transylvania area, remains the country with the highest prevalence of *Trichinella*. The traceability from a geographic point of view is a must which can ease the work of veterinarians in case of *Trichinella* epidemics. As a routine diagnostic procedure, the trichineloscopy and digestion methods were found to be possible reliable methods for detection, but the most sensitive one remains the molecular identification.

THE ANTI-TUMOR POTENTIAL OF *TRICHINELLA* EXPERIMENTAL INFECTION

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Helminths have been experimentally associated with protection against a number of autoimmune and allergic disorders. *Trichinella spiralis* has a powerful cytotoxic effect against some tumoral cells, the infection activating immunological competent cells to produce cytokines that can inhibit tumor growth. The hypothesis tested in the present study is the ability of *Trichinella* infection to develop in host body a certain protection against tumor invasion. The supposition was tested in a rat model, using two species of parasite (*Trichinella spiralis* and *Trichinella britovi*) and Walker 256 carcinosarcoma as an easy reproducible tumor. The rats were initially infected with different doses of *T. spiralis* and *T. britovi*. 3 weeks p.i. the rats were subcutaneous grafted with Walker 256 solid tumor. Three month later, after clinical examination, the rats were sacrificed; the tumors were collected and weighted. Microscopical sections were performed and these were stained with Haematoxilin-eosin and Tricrom-Masson method. No significant statistical differences were observed in total body weight between the groups. The microscopic aspects of the tumors collected from controls revealed: tumor enclosed by a thin layer of conjunctive tissue, epithelial tumor cells without clear intercellular limits, nuclear and cellular pleomorphism, vascular invasion, basophilic cytoplasm, cells organized in nests separated by a thin layer of conjunctive tissue, pleomorphic cells, hyperchromatic with vesicular nucleus, large nuclei, sometimes more than one, infiltration of inflammatory cells, specially eosinophils and lymphocytes. The microscopic aspects of the tumors collected from *Trichinella* infected groups revealed: thick conjunctive capsule and dystrophic calcification of the tumor tissue. Both *T. spiralis* and *T. britovi* infection protected the hosts against tumor invasion, but *T. spiralis* developed a more powerful effect.

SY05/1

SEROPREVALENCE OF *TOXOPLASMA GONDII* AND *NEOSPORA CANINUM* IN DOGS FROM THE MEDITERRANEAN ISLAND OF CORSICA

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Toxoplasma gondii is an important zoonotic intracellular protozoan parasite, which can affect all warm-blooded mammals and birds throughout the world, including humans. *Neospora caninum* is a protozoan parasite of animals, which represents a major cause of abortion in cattle. Dog is the definitive host for this parasite, shedding oocysts in its feces. In recent years, surveys of

Toxoplasma gondii and *Neospora caninum* infection in dogs have been reported worldwide, including France. However, little is known about the prevalence of *T. gondii* and *N.caninum* in dogs in Corsica Island. Two hundred eighty one serums samples have been collected in the south department of Corsica (Corse du Sud, 2A), mainly from adult and hunting dogs (respectively 86 and 87%). Modified agglutination test (MAT) has been performed for detecting antibodies against *T.gondii* and *N.caninum*. Among the 281 sera tested, 65.12% were positive for *Toxoplasma gondii* with a range dilution from 1/6 to 1/768, while 10.32% were positive for *Neospora caninum* at a 1/6 dilution. The seroprevalence of *T.gondii* was variable from one village to another going from 43% up to 92%. 150 serum samples were positive for *T.gondii* and negative for *N.caninum*, while only 23 samples presented a mixed infection underlying the discriminatory power of the MAT test in the diagnosis of this type of infections, in spite of sharing common antigens.
The present results on *T. gondii* seroprevalence in dogs confirm that the zoonotic parasite is widely present in Corsica, as it has been previously shown in studies on wild boar (Richomme et al., 2010). To our knowledge, the serological results on *Neospora* are the first one attesting of the risk of bovine neosporosis on the Mediterranean Island. Extensive field investigations in the dog population of the whole island are requested in order to confirm the present results, while more dilutions are necessary for the detection of *N.caninum* infection in order to avoid false-negative results.

PREVALENCE OF *TOXOPLASMA GONDII* AND *ENCEPHALITOZOON CUNICULI* ANTIBODIES IN DOMESTIC RABBITS IN THE CZECH REPUBLIC AND SLOVAK REPUBLIC

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Rabbit meat is commonly used for human consumption. The aim of this study was to evaluate prevalence of *T*. *gondii* and *E*. *cuniculi* antibodies in domestic rabbits kept in different housing conditions as important information for veterinary practice and human health. Blood samples from commercially breed rabbits were collected in a slaughterhouse; other samples were obtained individually on small family farms from marginal ear vein of animals or after slaughtering. Totally was collected 1883 samples of rabbit sera, 902 samples from 6 commercial rabbit farms and 981 sera from 29 small family farms to determine presence of IgG and IgM antibodies of *T*. *gondii* and *E*. *cuniculi* with using of in-house ELISA tests. *T*. *gondii* antibodies were detected in 5, 5 % of rabbits on 2 commercial farms and on 24 family farms. There was a significant difference between prevalence of *T*. *gondii* on commercial farms (0, 4 %) and on small family farms (10, 1 %). All tested farms were *E*. *cuniculi* positive. *E. cuniculi* antibodies were presented in 36, 2 % of all animals. 20, 51 % animals on commercial farms and 50, 66 % animals on small family farms were infected with *E*. *cuniculi*. We are reporting a significant decrease of *T*. *gondii* prevalence in rabbits from family farms in comparison to previously reported results. This reveals a big decrease of toxoplasmosis in rabbits apparently caused by a change in breeding of animals, using anticcocidials and complete feed mixtures similarly to intensive production systems. *E*. *cuniculi* prevalence 20, 51 % in rabbits from commercial farms and 50, 66 % in rabbits from family farms and is comparable to other studies.

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ENDEMIC *TOXOPLASMA GONDII* GENOTYPE CAUSES FATAL INFECTIONS IN ANIMAL HOSTS IN EUROPE

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Toxoplasma gondii is a successful parasite of domestic animals, wildlife, and humans. The effect of *T. gondii* genotype on the outcome of infection has recently gained substantial interest in human toxoplasmosis research. As a part of my PhD project, our group has been characterizing the *T. gondii* strains that had proved fatal to different animal hosts following naturally acquired infections. The genotyping method adapted to our laboratory is based on length polymorphism of seven microsatellite markers, and we perform the genotyping of the parasites directly from the tissues rich in parasites, without a bioassay step that could have a selective effect. In retrospective studies by our group, naturally acquired toxoplasmosis has been confirmed as the cause of death of 14 (8.1%) of 173 European brown hares, 4 (2.7%) of 148 mountain hares, 3 (15.8%) of 19 Eurasian red squirrels, and 6 (3.1%) of 193 cats, but none of 167 Eurasian lynx in Finland. All the cases were caused by *T. gondii* parasites belonging to the genotype II, which is typically nonvirulent in mice, and considered endemic in Europe. Interestingly, very similar results have been reported by other European researchers as well. Taken the recent studies together with ours, *T. gondii* parasites belonging to the endemic genotype II has caused altogether 32 fatal infections in altogether five different animal host species. This further affirms that no especially virulent *T. gondii* strain is required to kill a host. Moreover, all of these were naturally acquired infections, implying the infection doses have been reasonable and the infection routes probably the ones these hosts should be most adapted to. All these infections were naturally acquired, which confirms the endemic status of *T. gondii* genotype II in Europe. In particular, not only animals, but undoubtedly also humans can encounter *T. gondii* even in the northernmost parts of Europe.

USING MAGNETIC CAPTURE AND REAL-TIME PCR FOR DETECTION OF *TOXOPLASMA GONDII* IN TISSUE SAMPLES OF EXPERIMENTALLY INFECTED GOATS AND PIGS

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Toxoplasma gondii infections are widely distributed in humans and in many warm-blooded species. One of the most common sources of *T. gondii* infection in humans is ingestion of undercooked meat containing tissue cysts. The standards for detecting *T. gondii* in meat samples are bioassays, but they are not applicable for screening large numbers of samples. Other preventive tests for detection of the contamination level of different types of meat are still missing due to lack of appropriate methods for detection of *T. gondii* in tissue samples. Magnetic capture (MC) is a new molecular method enabling detection of *T. gondii* in a large tissue sample and, in combination with real-time PCR for the 529 bp repeat element, allows quantification of *T. gondii* DNA concentration. In comparison with conventional methods of DNA isolation utilizing maximally 50 mg tissue samples, MC handles up to 100 g of the tissue. The aim of this study was to determine *T. gondii* distribution and predilection sites in food animals (goats and pigs) after experimental infection using MC qPCR technique. Goats were administered with 20000 oocysts p.o., pigs were administered with 5000 oocyst p.o. using the tiger isolate, genotype II. Goats euthanized at day 30 and day 90 after infection, and pigs euthanized at day 76 after infection were used in this study. Twenty to hundred grams of brain, lung, heart liver, spleen, kidney, both fore limbs, both hind limbs and dorsal muscles were tested using MC and qPCR. The difference of contamination level in

tissues and the variance between two groups of goats was compared using a Man-Whitney test. Lungs and brain were identified as the *T. gondii* predilection sites with highest *T. gondii* concentrations in goats and in pigs the brain. A significant increase of *T. gondii* bradyzoites in goats 30 days post infection compared to 90 days post infection was revealed only in liver and dorsal muscle tissue. Our results confirm the suitability of MC qPCR method for the detection of *T. gondii* in tissue samples. Furthermore, we conclude that MC qPCR can also be used for the assessment of the distribution of the tissue cysts of *T. gondii* and quantitative determination of *T. gondii* predilection sites in food animals.

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SY05/2

EVIDENCE AND PARTIAL CHARACTERIZATION OF A METALLOPROTEASE FROM *TOXOPLASMA GONDII*

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Toxoplasma gondii is an obligate intracellular protozoan parasite belonging to the Apicomplexa family affecting all warm-blooded organisms. It is now well recognized that metalloproteinases play a major role in the invasion and the biology of parasites. Importantly, the penetration of parasites within host cells is essential for their survival. In the Apicomplexa family, parasites such as *Plasmodium falciparum* and *Cryptosporidium parvum*, metalloproteases have already been described. To date, only five toxoplasmic metalloproteases have been described in *T. gondii*: an aminopeptidase N (M1 peptidase family, aminopeptidase N (*Homo sapiens*)), two toxolysin (M16 peptidase family, pitrilysin (*Homo sapiens*)), a leucine aminopeptidase (M17 peptidase family, leucyl aminopeptidase (*Bos taurus*)) and FtsH1 peptidase (M41 peptidase family, FtsH peptidase (*Escherichia coli*)). In a previous investigation, we identified a parasitic protease in *T. gondii*-infected monocytic cells exhibiting both gelatinolytic and elastinolytic activities.
The objective of this study was to purify and characterize this *T. gondii* metalloprotease. We demonstrated, by gelatin zymography, that it displays an optimal pH of activity between 7.5 and 9 and is maximally active at physiological temperature. To assign its classification, different inhibitors were used: PMSF (serine protease), NEM and iodoacetamide (cysteine protease), EDTA, 1-10 phenanthroline (divalent ion chelator) and, phosphoramidon (general Metalloproteinase inhibitors), BB 94 and ilomastat (more specific inhibitors of Matrix MetalloProteinases, i.e., MMP). The gelatinolytic activity is decreased on average by 80% using either BB94, EDTA, 1-10 phenanthroline or ilomastat, suggesting that this enzyme belongs to the metallo-endopeptidase family and presents some analogy with the MMP clan. To confirm these results, DQ-gelatin, a natural substrate, is used by spectrofluorimetry. This allows us to know precisely the coefficient of inhibition for each inhibitor metalloprotease. The protease was purified by three consecutive steps of chromatography: gel filtration (1), gelatin-agarose affinity chromatography (2) and zinc-chelating chromatography (3) and its identification within the parasitic whole protease families is in progress.

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IDENTIFICATION OF PARASITE PROTEINS INTERACTING WITH THE TRANSCRIPTION FACTOR UHRF1 IN *TOXOPLASMA GONDII* INFECTED CELLS

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Toxoplasmosis, a disease caused by the parasite *Toxoplasma gondii*, is one of the most common infections in France: about 50% of the adult population is infected and an estimated 200 000 to 300 000 new infections occur each year. There is no effective treatment available for the intracellular forms of the parasite. It is therefore necessary to find new ways to prevent parasite proliferation and its intracellular persistence. The parasite persists in the cells by interfering with many host cell signalling pathways (apoptosis, cell cycle regulation, inflammatory cytokines) to ensure its survival. These interactions remain largely unknown. We have identified a novel transcription factor, UHRF1 (Ubiquitin-like PHD and RING finger Containing domains, 1), whose expression is modulated during infection and who is essential for parasite proliferation. Activation of uhrf1 induces cell cycle dysregulation of the infected cells and modulation of the host cell genome (Brunet et al., 2008). However, the parasitic factors responsible for uhrf1 activation remain unknown. The objectives of this work are to determine the parasitic factors that regulate UHRF1 in cells infected with a type I strain and to compare UHRF1 expression and regulation in cells infected with type I, type II and atypical strains. *Toxoplasma* belongs to the phylum of the Apicomplexa, defined by the presence of an apical complex consisting of secretory organelles among which the rhoptries (ROP) and the dense granules (GRA). During parasite invasion, rhoptries discharge their contents into the cytoplasm of the host cell. Among the rhoptry proteins, ROP16 is interesting because: i) it is a serine-threonine kinase that can activate and induce the phosphorylation of the transcription factor STAT3, this resulting in a decreased production of proinflammatory cytokines induced by the macrophages, ii) it also has a nuclear localization, iii) it is polymorphic and associated with parasite virulence. We will analyse the role of ROP16 in the early activation of uhrf1. For this we will use: i) a parasite strain knock-out for ROP16, ii) plasmids of ROP16 and various mutants. Interaction between UHRF1 and its partners (activity, phosphorylation) will be reviewed by a luciferase reporter gene, Western blot, immunoprecipitation, and two-hybrid assays. ROP16 being highly polymorphic, the experiences will be repeated using strains of variable virulence. These studies will allow us to better understand the molecular mechanisms that lead to *T. gondii* persistence in the host cell and draw new therapeutic approaches.

SPATIAL GENETIC STRUCTURE OF TYPE II *TOXOPLASMA GONDII* STRAINS INVOLVED IN HUMAN CONGENITAL TOXOPLASMOSIS IN FRANCE

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Congenital toxoplasmosis involves type II strains in 95% of cases in France. Thanks to their high discriminatory power, microsatellite sequences are the markers of choice for studying the population structure of genetically closely related strains, such as type II strains. We used spatial principal component analysis (sPCA) to investigate the spatial genetic structure of *T. gondii* type II strains involved in 240 cases of congenital toxoplasmosis in France over the 2002-2009 period. All strains were genotyped with 15 microsatellite markers. Mailing addresses of patients were geo-referenced a posteriori in decimal degrees and categorized into urban or rural areas of residence. No spatial genetic structure was found by the sPCA for type II strains involved in mothers living in urban areas, but a global spatial genetic structure was found for those collected in mothers living in a rural environment. This global structure clearly separated the strains associated to rural environments in the northwestern part of France (Bretagne, Pays de la Loire, and Normandie) from

those associated to rural areas in the rest of the country. Our results suggest that sources of infection with *Toxoplasma gondii* in France may be different according to the type of habitat. In rural areas, the major way of infection is likely to involve strains that are circulating locally in the region where people live. This suggests that oocyst-based sources of infection with contaminated vegetables, fruits or water are of major importance in rural areas. On the other hand, the absence of spatial genetic structure of strains involved in the contamination of women living in urban areas suggests that these strains are originating from different geographical regions. These data support cyst-based sources of infection with meat products purchased in supermarkets as the predominant way of infection with *Toxoplasma gondii* in urban areas. Overall, these results may advocate for targeted messages in the prevention of toxoplasmosis according to the type of habitat (rural versus urban) of susceptible people.

SY13/2

THE ROLE OF BIRDS OF PREY IN THE TRANSMISSION OF *TRICHINELLA PSEUDOSPIRALIS*

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Recently, *T. pseudospiralis*, the only *Trichinella* species infecting birds, has been detected increasingly reported in Europe. The presence of *T. pseudospiralis* in the Central Europe was for the first time recorded in 2003 in Eastern Slovakia and in following years repeatedly diagnosed in sylvatic animals from this area. Moreover, molecular analyses revealed distinctive genetic relationship of Slovak isolate with those from Finland and Sweden, suggesting the potential role of migratory birds in spreading infection from endemic foci in Scandinavia. The aim of our study was to investigate raptors, owls and corvids for the presence of *T. pseudospiralis* to illuminate the potential role of birds of prey in transmission of the parasite. During 2006-2012, using artificial digestion of pectoral muscle samples, we examined 103 individuals from Slovakia and Sweden, belonging to 14 species of Falconiformes, 2 species of Strigiformes, and 4 corvid species. In two *Strix aluco* from Sweden we detected muscle larvae, most likely of *T. pseudospiralis* species. Molecular evidence will be preformed and possible role of birds of prey in transmission of *Trichinella pseudospiralis* in nature will be discussed.

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CYSTS CALCIFICATION OF *TRICHINELLA SPIRALIS* IN FARM SWINE *(SUS SCROFA DOMESTICA)*

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The research basis has been made up from meat samples, coming from swine slaughtered in slaughter houses and in individual farms, of various races and ages, grown in farmhouse or intensive systems, as well as meat samples from experimentally infested pigs. In our experiences and observations, modifications noticed in cysts and larvae of *Trichinella* sp. have been taken into consideration. In the examination of infested meat slices, the classical procedures of clarification have been used, with hydrochloric acid 4%, ordinary vinegar, sodium hydroxide 3% or phenolated glycerine 5%, according to case.

The research revealed a large variety of *T. spiralis* cysts, morphopathological aspects, relating to their essential components (larva and capsule), as well as of the parasited muscle tissue. The calcification process in the case of the farm pigs, regularly begins after six months after the animal's infestation. The calcification is preceded or not by the apparition of a reaction cellular infiltrate, made up by polymorphonuclears, initially localized in the polar zone of the cyst, with an extension around or inside it. The calcification occurs in the cyst central zone, covering the devitalized larva, in various proportions. Sometimes, the calcium salts deposits appear like a black spot, which may include partially or totally the larva and the inner space of the cyst, resulting in a large diversity of morphopathological aspects. Some authors consider certain phases of this calcification as "atypical" or "pathological". Yet, actually, this is a normal calcification process, characteristic to farm pigs. In certain cases, the larva calcification occurs even before the capsule formation.
The *Trichinella* massively calcified cysts appear very much alike the *Sarcocystis* spp. calcified cysts. In order to set the differential diagnosis, the aforementioned classical clarification methods can be applied or, alternatively, the artificial slow digestion method can be used.
Keywords: *Trichinella*, calcification.

OCCURRENCE OF *TRICHINELLA* SPP. IN THE SYLVATIC CYCLE IN GERMANY

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Trichinellosis is a food-borne zoonotic disease caused by nematodes belonging to the genus *Trichinella* (T.). In Europe, *T. spiralis*, *T. britovi*, *T. pseudospiralis* and *T. nativa* may occur in domestic and/or wild animals depending on the geographic region. This study aimed to evaluate German data of *Trichinella* meat inspections in domestic and wild animals obtained over the last decade. Furthermore, trichinellosis cases in humans reported in Germany were assessed. From 2001 to 2010 approximately 464 Mio domestic pigs were subjected to meat inspection with only five *T. spiralis* positive findings (prevalence 1x10-8). Out of about 3.44 Mio wild boars tested from 2001-2010, 89 animals were *Trichinella* positive with an average prevalence of 0.003%. Larval burdens for *T. spiralis*, *T. pseudospiralis* or *T. britovi* ranged from a few to more than several hundreds of larvae per gram of muscle (median 17.6). Results from sporadic monitoring in different areas of Germany show that *Trichinella* prevalence in foxes (*T. spiralis*, *T. britovi*, *T. pseudospiralis* and surprisingly *T. nativa*) and raccoon dogs (*T. spiralis* and *T. pseudospiralis*) varied from low (<0.1%) up to 1% to 5%, respectively. Findings for the larval burden ranged from a few up to more than one hundred larvae per g (median 9.7). During the past years, the raccoon dog population has grown dramatically in Germany, especially in the north eastern Federal States Mecklenburg-Western Pomerania (MWP) and in Brandenburg (BB). Notified human cases in Germany are rare (on average 6 cases/year) and mostly related to "imported cases" from countries where trichinellosis is still a problem especially in domestic pigs. Infrequently, autochthonous outbreaks occur, e.g., in 2006 where 16 people from the district Uecker-Randow (MWP) were reported with trichinellosis. Pork was identified as the source of infection but the origin of the meat was not reliably identified. The data shows that the risk of *Trichinella* infection in pigs is negligible in Germany. This confirms the possibility of a risk based meat inspection for fattening pigs kept under intensive farming conditions. Conversely, the sylvatic *Trichinella* cycle is prevalent in wild boars and in wild carnivores (raccoon dogs and foxes) which play an important role as natural reservoir. From the perspective of consumer protection, wild boars and domestic pigs kept outdoors are at a higher risk for infection and must be examined for *Trichinella* without exception.

THE ROLE OF DOGS IN THE CIRCULATION OF *TRICHINELLA* IN THE FRENCH MEDITERRANEAN ISLAND OF CORSICA

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In 2004 *Trichinella britovi* was detected for the first time in a remote valley in the centre of the French Mediterranean Island of Corsica considered until then as *Trichinella* free. First investigations in the surrounding wildlife revealed one infected fox. An epidemiological survey in wildlife was thus conducted in the entire island from 2006 to 2008. On the 1881 wild boars and 74 foxes sampled, no *Trichinella* larvae were recovered by the digestion method. However, the serological tests implemented in wild boars samples revealed a seroprevalence of 2% in wild boars, suggesting that Corsican wildlife had been exposed to *Trichinella*.
In February 2010, the parasite was found again in outdoor pigs, living at less than 10 km from the positive herd of 2004. Further to local investigations revealing that a roaming dog put down had probably been eaten by the infected pigs, a serological survey was conducted on farm and hunting dogs living in the same remote valley (Haut Taravu).
An in-house E/S ELISA was set up using 444 negative serum samples from urban dogs collected in the National Veterinary School of Alfort. Then, 297 sera collected from dogs living in the Haut Taravu valley and 68 sera from dogs living in the main village of the neighboring valley (Bastelica) were tested. A Western blot was also carried out to confirm serum samples doubtful or positive by ELISA. Out of 365 dogs collected in both valleys, 13 dogs were seropositive by ELISA of which 8 were confirmed positive by Western blot. The 8 dogs doubtful by ELISA were not positive by Western blot. These results reveal a serological prevalence of *Trichinella* infection in dogs of 2% (95% CI: 0.7%-3.6%). Among the seropositive dogs, one came from Bastelica and had a high serological response. All seropositive dogs were hunting dogs and older than 1 year.
Additionally, nine other pig carcasses have been seized for trichinellosis since the end of 2011. Those pigs came from the Gravona valley, a neighboring valley of Bastelica. Local investigation indicated that stray dogs and foxes had been shot and left on the premises and probably consumed by pigs.
These findings suggest that dogs play a major role in the circulation of *Trichinella* in the studied area and in the contamination of pigs in Corsica.

SY13/3

IMPACT-UPDATE OF HUMAN TRICHINELLOSIS -A RETROSPECTIVE EPIDEMIOLOGICAL STUDY IN BRASOV COUNTY-ROMANIA DURING 1998-2012, FOR RISK MANAGEMENT IN FOOD SAFETY AND ECOSANOGENESIS

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The importance of establishing the diagnosis of trichinellosis, early treatment and the supports of the disease prevention derive from the extended medical,social and economic implications, of this parasitic ailments. Clinical and epidemiological investigations you sign up on line diagnostic orientation, the scientific substantiation through diagnosis set, to clarify the sources of human infestation, please include the type and level of in the environment and in the body of the host

pollution, natural environmental conditions and measures that influence the evolution of invasions; possibilities of dynamic infestation; for seasonal dynamics and age; dynamics of clinical manifestations or complications installed. Were carried out studies and researches on the epidemiological process in trichinellosis, its share in maintaining the endemic character of this disease, compared with the annual outbreaks from different incidents, in relation to the presented data at the national and international level for a period of 15 years. These objectives shall be characterized by interdisciplinarity, by pluridisciplinarity and transdisciplinarity, by including the same target group several categories of specialists: biologists, physicians, veterinarians, chemists, economists, ecologists, chemists, which participate in the 21st century to ensure the food resources of mankind while respecting the principles, while respecting the principles of bio-economy and eco-economy necessary to ecosanogenezis . It is very important knowledge of risk management safe food in the event of massive infestation and the danger that the population may be exposed.

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PRODUCTION AND CHARACTERIZATION OF MONOCLONAL ANTIBODIES AGAINST A SERINE PROTEASE FROM NEWBORN LARVAE STAGE OF *TRICHINELLA SPIRALIS*

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Trichinella spiralis is an intracellular parasitic nematode of mammalian skeletal muscle, causing a serious zoonotic disease. Detecting the *T. spiralis* infection sensitively and specifically in pig is essential for preventing transmission of the disease. In a previous work, an immunodominant *T. spiralis* serine protease, named NBL1, was obtained by suppression subtractive hybridization cDNA library of *T. spiralis* Newborn Larvae stage. Attempts have made to utilize the immunodominant region of NBL1 (NBL1-C, the C terminal part of the protein) for immunodetection of pig trichinellosis and vaccine development. The aim of this study was to produce and characterize monoclonal antibodies (mAbs) against the recombinant NBL1-C protein (rNBL1-C), in order to develop a sensitive and specific immunodetection tool for an early detection of pig trichinellosis. Five mAbs directed against rNBL1-C were produced on mice. All mAbs were found to be of IgG1 isotype and shown by western-blot to specifically recognize the rNBL1-C with a unique band at the expected molecular weight of 15 kDa. Epitope mapping with 15 overlapping peptides containing whole length of NBL1-C indicated that all mAbs recognized three overlapping peptides containing a common motif of 10 amino acids (PSSGSRPTYP). Two mAbs named 6C3 and 5D4 were further characterized using antigens from various developmental stages of *T.spiralis.* Western blot revealed that a band of 50 kDa was observed with Adult and Newborn larvae mixed antigens as well as with Newborn larvae stage alone. On the other hand, both mAbs failed to recognize muscle larvae somatic antigens. Thus, the 50 kDa protein identified represents the native NBL1. Indirect immunofluorescence analysis using cryosections of different *T. spiralis* stages revealed that both mAbs intensely stained only the embryos within the gravid females and the cuticle of newborn larvae. The produced mAbs could be useful candidates for the development of a competitive ELISA for early detection of *T. spiralis* infection in pigs. Moreover, a perspective is now open for the characterization of a major antigen of *Trichinella* involved in the invasion of the host.

SEROEPIDEMIOLOGICAL INVESTIGATIONS ON *TRICHINELLA* SPP. ANTIBODIES IN CATS FROM ROMANIA

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A number of 246 serum samples from cats, belonging to different rural or urban areas from 8 different counties from Romania were analyzed in this study. The cats ageing between one month and 17 years had a healthy clinical status. The serum samples were examined by ELISA (*Enzyme Linked Immuno Sorbent Assay*) in order to identify the IgG anti-*Trichinella* antibodies. A *Trichinella spiralis* Antibody Test Kit Microwell ELISA (Bio-Rad) was used. The ELISA kit was intended for in vitro test in swine. The kit conjugate was replaced with a peroxidase-conjugated anti-Cat monoclonal antibody. For the controls two kittens, 2 months old, were used. The positive control was obtained by experimental infection of one cat with 2000 *T. spiralis* muscular larvae. The second kitten was kept as negative control. The samples were organized on different categories including age (kittens, young, adults, old), gender, breed, habitat (indoor/outdoor), location type (urban/rural).
From the total of 246 analyzed samples, 19 (7.70%) were positive. The highest seroprevalence was registered in old cats (13.3%), and in cats from urban areas (8.2%). Neither of the studied variables (age, gender, breed, habitat (indoor/outdoor), location type (urban/rural)) was statistical relevant.
This is the first seroepidemiological study regarding *Trichinella* infection in cats, from Romania, and one of a few worldwide.

SY16/1

SAMPLING STRATEGIES FOR VETERINARY PARASITOLOGICAL SURVEILLANCE

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The aim of the work is to review different informative sampling strategies for parasitological surveillance.
We take advantage of two existing surveys conducted on the Province of Benevento (2.071,2 Km^2 1354 farms; Campania Region, Southern Italy). A GIS of the region was developed using Arc-GIS 9.x GIS software. All the sheep farms in the Province were georeferenced.
Spatial sampling in Parasitological Surveillance is usually applied following a systematic grid sampling. In our example a systematic sampling strategy over-imposed a 10 km x 10 km grid on the entire Province (29 quadrants) to uniformly sample the farms. We use data from this first survey and a GIS to estimate parasite infection probability surface and a prediction standard error surface using Bayesian Geostatistics. We concentrate on *Fasciola hepatica* and *Dicrocoelium dendriticum*.
We consider this information to design a second survey. To illustrate the benefit of using information from previous surveys we show the results of four strategies listed as decreasing cost: a systematic sampling strategy with 5 km x 5 km grid (112 quadrants); a spatially balanced with unequal inclusion probabilities proportional to predicted infection probabilities; a spatially balanced with unequal inclusion probabilities proportional to prediction uncertainty; a systematic sampling strategy with 10 km x 10 km grid.

We analyze the results of each strategy in terms of number of positives/negatives, costs and prediction accuracy with regard to the most demanding design.
In all surveys the centroid of each quadrant was identified. Among all the geo-referenced farms the one closest to the centroid of each quadrant and with more than 50 animals was selected. Rectal fecal samples were collected. On each ovine farm, the animals were divided into two age groups: lambs (4–18 months) and adult sheep (>18 months). Five individual fecal samples were collected from lambs, and 15 from adults. At laboratory, fecal samples were pooled into 4 groups of composite samples for each farm (3 composites from adults and 1 composite from lambs). Each composite sample was formed from 5 equal parts by weight of individual fecal samples. Copromicroscopic examinations were performed using the FLOTAC technique.
The originality of the present work stands on the integration of Bayesian Geostatistics (Posterior Prediction Probabilities and Prediction Uncertainty) with Spatial Sampling Design. The relative advantage of the different designs depends on the cost of false positive/negative which is subject specific, and on the underlying spatial pattern which affect estimate precisions.

The research leading to these results has received funding from the European Union Seventh Framework Programme FP7-KBBE-2011-5 under grant agreement n° 288975.

A NEW BAYESIAN KRIGING MODEL TO ESTIMATE THE PROBABILITY OF PARASITIC INFECTIONS IN THE CAMPANIA REGION (ITALY)

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The advantage of mapping the location of the farms and of studying the spatial distribution of parasitic infections is clear since it enables focused intervention strategy and could address important scientific clues. Model-based geostatistics and Bayesian approaches are appropriate in the context of Veterinary Epidemiology when point data have been collected by valid study designs. The aim is to predict a continuous infection risk surface. Little work has been done on predicting infection probabilities at unexplored locations. In this work we developed a Bayesain Kriging model to obtain an estimate of infection probability for each one of the 8794 geo-referenced sheep farms of the Campania region (Southern Italy). Data were derived from a cross-sectional study carried out to study the spatial distribution of selected helminths. A grid sampling was performed to select the farms for coprological examinations. Faecal samples were collected for 121 farms and the presence of 23 different helminths was investigated using the FLOTAC dual technique. Each helminth infection was modelled separately. The spatial distributions of each helminth infection were very different and the distribution of the posterior predicted probabilities very heterogeneous. We proposed a probit Bayesian kriging model to obtain the map of posterior probability of infections for each one of the georeferenced farms of the region. In particular, we report the geographical distribution and the posterior predicted probability obtained only for two selected parasites which are very different in terms of prevalence: *Fasciola hepatica* (12.4%) and *Dicrocoelium dendriticum* (66.9%). The predicted posterior probabilities for *F. hepatica* ranged between 1.6% and 89.5%. The median was 7.47% and the 75% of the predicted data had a probability of infection below 19.3%. The range of estimated probabilities for *D. dendriticum* ranged between 2.7% and 99.8% and only 25% of the predicted probabilities had a value below 46.8%.
This approach represents a useful tool to communicate with field researchers and to address targeted infection control treatments in the region.

The research leading to these results has received funding from the European Union Seventh Framework Programme FP7-KBBE-2011-5 under grant agreement n° 288975.

SPATIAL ANALYSIS OF *TOXOPLASMA GONDII* INFECTION IN GOATS IN SERBIA

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A result of the current global expansion of the goat industry, instigated by an increase in the market share of goat cheese and other milk products, is the heightened interest in goat health. A potentially important goat infection is toxoplasmosis, both as a cause of animal pathology and, through products including meat and milk, as a threat to human health. We undertook a seroepidemiological study including spatial analysis of *Toxoplasma* infection in Serbia.
A cross-sectional survey of goats involved a total of 400 serum samples obtained between January 2010 and September 2011 from 110 goat farms from throughout Serbia. The farms were selected to reflect the goat population in the country; only two were large farms with an intensive raising system, from which 30 and 40 animals were sampled, respectively, while the vast majority (n=90) involved traditionally raised household herds including one to five animals, from which an animal or two were examined. *Toxoplasma*-specific antibodies were detected by the modified agglutination test. The cut-off titre was set at 1:25. The overall seroprevalence was 70%, ranging from 43.8% in the Belgrade area to 95.8% in Western Serbia. At the farm level, at least one animal tested positive in 83% of the farms; of these, one large farm tested 100% (40/40) positive. Conversely, no animals (0/30) were infected in the other large farm, which had only recently imported the animals (from France).
Age, gender, herd size, farm type, husbandry, feeding and watering practices, presence of other animals and cats on farms, kidding, health status, origin and geographical region, were all analyzed as risk factors for infection. Logistic regression analysis revealed farm location in Western Serbia (OR=14, 95% CI=2.9-67.4, p=0.001), and external acquisition of animals vs. raising on farm (OR=2.5, 95% CI=1.5-4.4, p=0.001) as risk factors, while flock size from 10 to 30 animals (OR=0.406, 95% CI=0.214-0.771, p=0.006) was protective. Western Serbia, an area with more rainfall and humidity and higher elevation than the rest of the country, also had the highest prevalence of *Toxoplasma* infection in sheep and cattle. The results were mapped using geographical information systems (GIS) and spatial analysis performed according to environmental factors (elevation, temperature, rainfall, humidity). The spline modeling method showed an increase in the risk of infection with elevation.
The presented data show an overall high level of *Toxoplasma* infection in goats in Serbia, and specifically point to areas in which preventive measures in goat husbandry should be urgently applied.

SPATIAL DISTRIBUTION OF SOIL AND ANIMAL CONTAMINATION BY *TOXOPLASMA GONDII* IN A RURAL AREA

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Toxoplasma gondii is a protozoan parasite that infects humans and all warm-blooded animals. Its life-cycle includes felids (notably domestic cats), the only definitive hosts excreting oocysts in the environment, and numerous intermediate hosts (notably rodent species). Human infection can occur through ingestion of either meat containing cysts or soil contaminated with oocysts. Identifying factors responsible for intermediate hosts and soil contamination by *T. gondii* is essential to estimate human infection risk. Our aim was to investigate the spatial distribution of *T.*

gondii in rodents and soil. We especially focused on the influence of the presence of domestic cats, distance to the village centre and soil type. Positives were expected to be found close to the village or in any other areas highly used by cats. The study area (1.4 km2) was located in the Ardennes (North-Eastern France) and focused on a small village and its surroundings. The local population of domestic cats has been monitored since 2008, with trapping sessions occurring twice a year. In September 2011, eight species of rodents were trapped and 243 samples of soil were collected. A total of 153 cats and 194 rodents were tested for the presence of *T. gondii* using a modified agglutination test for antibodies. Soil contamination by *T. gondii* oocysts was assessed using a method based on concentration and quantitative PCR. The overall seroprevalence was 56.4 % in cats and 8 % in rodents, while 30 % of soil samples were positive. Preliminary analyses suggested that the proportion of positive soil samples varied with the distance to the village and cats presence. By contrast, we did not detect any effect of soil type, even when considering potential interaction with the distance to the village. Prevalence in rodents varied between 1.5% and 28.4% according to the species, with a spatial distribution of positive animals not restricted to the village. By including animal infection and soil contamination data, this study showed that environmental contamination by *T. gondii* in a rural area is more largely distributed than previously believed. In addition, all types of soil are concerned. The risk of toxoplasmosis for humans caused by oocysts infection should then be reassessed.

SY16/2

INCREASED VISUAL APPROACHES IN SCIENCE COMMUNICATION

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The development of near real-time surveillance systems, based on geographic information systems (GIS), global positioning systems (GPS) and remote sensing, facilitates the establishment of accurate, up-to-date early-warning systems (EWS). Reliance on cartographic representations of the burden of diseases are increasingly needed in a world where infectious diseases can cross continents in as little time as it takes a passenger aeroplane. Access to interactive, computer-generated map applications represent a straightforward way of visualizing large numbers of epidemiological datasets in a geographical context. For example, frameworks bundling spatial data from virtual globes together with GIS packages, can assemble separate, state-of-the-art modules like 'Lego bricks' resulting in user-friendly platforms for evaluation, prediction and risk profiling. In this way, epidemiological information can be shared in real time, not only with decision-makers in the Ministries of Health, but also with independent scientists as well as the individual researchers who produce the evidence base. This progress is encouraging and will no doubt contribute to new approaches for establishing and communicating datasets. New publication models, relying on hybrid methodologies no longer limited to printed literature but cutting across different media, are leading to improved health management information systems, in particular with respect to surveillance and response. Derived from the idea that visualization can transfer information in a way that written text alone is incapable of, printed narratives mixed with interactive graphs and audio-visual representation have started to appear. This approach empowers a broader range of people than scientists to both understand and mentally process voluminous amounts of material in a relatively short time. By strengthening visualization and making it an integral part of the scientific communication, relationships, objects, phenomena and even complex ideas can be communicated in a more easily understood form. Video clips, presenting concepts, methodologies and results can be both entertaining, coherent and transfer important information. Thus, entering the new millennium, the old adage that a picture is worth more than a thousand words confirms that publication in this field should indeed have a strong graphic/visual bias.

SY16/3

SHORTCOMINGS IN OUR WORK TO DESCRIBE ASPECTS OF GEOSPATIAL HEALTH ISSUES

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The Global Network for Geospatial Health, GnosisGis, has now existed in 11years and six volumes of its the well recognised international Journal of Geospatial health has been published successfully. A high number of different aspects of geospatial health have been published specifically within infectious diseases. But it has also been obvious that very few papers has been submitted and published within the vector distribution of the diseases though it is clear that in order to understand distribution now and in the future knowledge of vectors is an important prerequisite to understand the epidemiology and distribution of infectious diseases.
The future WHO strategy aims for elimination of schistosomiasis, which implies a complete interruption of transmission of the infection; in humans as well as in the environment. Investigating transmission by examining schistosomes in humans becomes difficult and less effective as infection rates among people decrease. Therefore for a disease like schistosomiasis and an emerging disease like fascioliasis which is becoming more and more a problem for humans it is regrettable that so little has been done within mapping and modelling the vector snails which are crucial for the transmission of these diseases. The same can be said for mosquito borne diseases. On this background it is prudent to pursue the missing knowledge for these diseases.
In the present paper the studies on this subject are being reviewed and shortcomings pointed out.

MODELLING QUESTING *IXODES RICINUS*: SPATIAL DISTRIBUTION IN ROMANIA

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Understanding the ecology and distribution of ticks is a key step in epidemiological studies of tick-borne zoonoses. Among European ticks, *Ixodes ricinus* is considered to be one of the most important vectors of human and zoonotic pathogens.
Modelling geographical distribution is one of the main steps in understanding both species range and abundance and also the most cost-effective approach.
Statistical and spatial models are based on two years survey at national scale, data being collected in randomly chosen forest habitats for an overall uniform geographic distribution. *Ixodes ricinus* was present in 97.7 % (n = 180) of locations of the dragging sites, occurring exclusively in 41.7 % of the locations, whereas it was the dominant species in 38.8 % of the other locations, accounting for over 70 % of the total tick community.
The distribution and abundance of *Ixodes ricinus* suggested by our models, together with its wide host specificity, high frequency in humans and significant vectorial capacity, suggest its paramount importance as a zoonoses vector in Romania.

SY20/1

COMPARATIVE STUDY OF TRICHINELLA SPP INFESTATION IN WILD AND DOMESTIC FAUNA IN THE HUNEDOARA COUNTY

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Trichinellosis was and still is a important zoonosis in Hunedoara county because it signals further cases of illness in humans. Research objective was the correlation of the appearance and maintenance of outbreaks of *Trichinella* in domestic swine with *Trichinella* outbreaks in wild animals under the influence of geographical area and the human factor (like hunter and creating habitats that favor the existence and reproduction of rats - a species potentially invasive).
During 01.01.2008 - 30.06.2012 I analyzed by trichinoscopic exam and artificial digestion 201 muscle samples (pillar, tongue, masseter) from 201 corpses of foxes from 36 hunting funds and found 34 positive samples from foxes of 21 hunting funds; 7 muscle samples (pillar) from 7 bears of 6 hunting funds and found two positive samples from the same hunting fund. However were examined 617 samples muscle tissue (diaphragm pillars) from the wild boars, of which 3 were positive.
Even if in this period in slaughtered pigs have not been reported positive cases at pigs slaughtered in the traditional system in households still found infestations with *Trichinella* spp in localities in approach of the hunting funds with *Trichinella* outbreaks in wild animals.
Dynamics outbreak of *Trichinella* in domestic swine within Hunedoara county shows that there may be a correlation between the synanthropic cycle and silvatic cycle (especially in populations of wild boars and foxes). An increasing number of positive cases in foxes hunted hunting funds on most of the area of Hunedoara county, from silvatic fauna the fox especially by means of the rats may be the source of infection for domestic pigs.

TRICHINELLA (NEMATODA, TRICHINELLIDAE) OF WILD ANIMALS IN UKRAINE

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Trichinellosis is one of the most dangerous helminthic diseases common to humans and animals. More than 1,500 cases of human trichinellosis were reported in 1971-2000 (Ukrainian Sanitary-Epidemiological Service Reports). Infected pork used to be the main source of human *Trichinella* infection in past, but the consumption of infected game caused most recent human trichinellosis in Ukraine. The aim of this work was to study the *Trichinella* prevalence and species composition in wild animals. Materials were collected during the hunting seasons in 2002–2010 in 14 regions of Ukraine. The muscle tissue samples from 200 wild boars, 650 carnivores (wolf, fox, marten and badger) were studied. *Trichinella* has been found in all studied regions. Wolves and foxes were found to be the main reservoir of *Trichinella*. Our studies demonstrate the presence of tree species of *Trichinella* in Ukraine. *T. britovi* is found in wild boars, wolves, foxes and martens from the Carpathians (the Transcarpathian region), Polesie and Steppe. At the same time, *T. nativa* is found only in the foxes from the Chernihiv Polesie. *T. spiralis* is found in the domestic pigs from the Kyiv Polesie. No cases of mixed infection were recorded. Our data suggest that the domestic and sylvatic cycles of trichinellosis are relatively isolated from each other in the studied areas. The *Trichinella* prevalence was 3.2% for ungulates and 15% for carnivores. The intensity of infection was the highest in the animals from the Carpathians (the Transcarpathian region) and the lowest in the animals from the Chernihiv Polesie. This suggests that the mountain regions are more suitable for the maintenance of *Trichinella* foci in Ukraine. The *Trichinella* prevalence of wild carnivores is on its raise over the past 30 years in Ukraine: from 3.5% in 1976, to 8.8% in 1989 and 15% in our study. Also, the Ukrainian Carpathians and Polesie may be declared an endemic region for the *Trichinella* infection.

NUTRIA AND MUSKRAT AS INTERMEDIATE HOSTS OF *ECHINOCOCCUS MULTILOCULARIS* IN A NEW ENDEMIC AREA, OF THE WEST PART OF FRANCE

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Nutria (*Myocastor coypus*) and muskrat (*Ondatra zibethicus*) are large semi-aquatic invasive rodents, naturalized throughout European countries. They are regarded as pests, and they can be infected with several pathogens and parasites transmissible to livestock, pets, and humans.
In Europe the life cycle of *Echinococcus multilocularis* involves red foxes (*Vulpes vulpes*) as definitive hosts and common voles as intermediate hosts. The possible role of Nutria and muskrat in this cycle is not clear, even if data demonstrate some larval infection in liver.
We have taken opportunity of a large study on sanitary status of nutria and muskrat in 12 departments of 3 westerner regions of France to investigate the larval liver infection on captured animals.
Five water sites (river or pond) by department were selected and a total of 914 animals were catched. The sample design was built to calculate nutria and muskrat densities on each site. After autopsy, we have sampled liver cyst, in 43 nutrias and 108 muskrats, which have been analysed by PCR on the mitochondrial cox1 gene for identification of cystic hepatic parasite infection especially alveolar echinococcosis. Several parasitic were identified: *T. taeniaformis* (63.6%), *Taenia mustelae* (7.9%), *Taenia polyacantha* (7.9%), *Taenia martis* (0.7%) and *E. multilocularis* (2.6%). Only 4 cysts from 2 nutrias and 2 muskrats were positive for *E. multilocularis* infection. One of these cysts in muskrats contained protoscolex that confirmed the fertility and the possible active role in *E. multilocularis* life cycle. These 4 animals were originating from 3 departments (Calvados, Manche, Orne) where foxes have been found recently positive for *E. multilocularis*.
The first results of the study assess that aquatic rodents are infected by *E. multilocularis* in west part of France. The possible contribution of nutria and muskrat in the distribution and dispersal of this parasite is still controversial. Our results confirm that in a new endemic area, the aquatic rodents are rapidly contaminated and this is the proof of a contaminated environment, which indirectly revealed a human risk.

TOXOPLASMA GONDII PREVALENCE IN ISRAELI CROWS AND GRIFFON VULTURES

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A cross-sectional *Toxoplasma gondii* seroprevalence study was performed on Israeli free range-crows (*Corvus cornis*, *C. monedula*, *C. splendens*) and Griffon vultures (*Gyps fulvus*) in order to assess exposure to this infection in scavenger birds that feed on animal carcasses and their possible role in the epidemiology of toxoplasmosis. Using the Modified Agglutination Test (MAT) with a minimum cut-off titre of 1:25, 52 of 122 crows (42.6%) and 40 of 101 Griffon vultures (39.6%) were found to be *T. gondii* seropositive. Crow *T. gondii* seroprevalence was significantly higher in northern areas of Israel (p = 0.007) where annual precipitation is higher and annual summer maximum temperatures are lower than in the drier and warmer south. Seroprevalence in crows is positively associated with higher human population densities possibly related to the increased cat population in these areas. PCR analysis of brain extracts from crows resulted in the detection of *T. gondii* DNA in 1 positive crow from northern Israel. Genetic analysis of DNA from

the positive crow brain confirmed infection with *T. gondii* type 2 using a multiplex multilocus nested PCR-RFLP (Mn-PCR-RFLP) of the SAG1, 5-3' SAG2, alt.SAG2, SAG3, BTUB, GRA6, C22-8, c29-2, L358, PK1 and Apico loci. The high *T. gondii* seroprevalence in these bird species suggests that infected carrion may be responsible for widespread infection of carcass scavenger birds which may further transmit infection to other carnivorous intermediate hosts or feline definitive hosts when consumed post-mortally.

SY20/2

TRYPANOSOME POLYPARASITISM AND THE DECLINE OF THE CRITICALLY ENDANGERED AUSTRALIAN POTOROID, THE BRUSH-TAILED BETTONG, *BETTONGIA PENICILLATA* (GRAY, 1837)

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For the last 7 years the Brush-tailed Bettong (*Bettongia penicillata*) (*alias*: Woylie) in Western Australia has been on the verge of extinction. An extensive conservation effort involving government agencies, zoos and universities has had minimal influence, and the remaining woylies have recently experienced an alarming 93-95% reduction in numbers. The reasons for the population decline are still unknown. As part of an investigation to understand this dramatic decline, it was discovered that the trypanosomes in the blood of the woylie can be grouped into three morphologically distinct trypomastigote forms, encompassing two different species. The larger of the two species, *T. copemani* exhibits pleiomorphic trypomastigote forms, with the two different phenotypes being distinguished primarily by the significant distance between the kinetoplast and nucleus. The morphology of the smaller species, being reported for the first time here, is about 5% the size of *T. copemani*. It has a singular trypomastigote form in the blood during the subacute phase of infection. Here we describe the morphology of this new species and the supporting molecular characterisation. The results of a longitudinal study show that during the subacute phase of a mixed infection, the blood trypomastigote forms dominate. The parasites then appear to migrate from the blood and enter the chronic phase, where they infect the tissues of the host. Each phenotype exhibits a predilection for certain tissues, where they can display *T.cruzi*-like pathology at the time of autopsy. Results indicate that the trypanosomes of woylies may have contributed to their decline and highlights the need for extensive health checks at the time of translocation of endangered animals.

CO-INFECTION AND GENETIC DIVERSITY OF TICK-BORNE PATHOGENS IN ROE DEER IN POLAND

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The presence of wild ungulate species, such as roe deer, has been shown to be essential for maintaining and amplifying of tick populations and, consequently, the tick-borne diseases. The aim of our study was to estimate the prevalence and rate of co-infection of *Babesia, Bartonella* and *Anaplasma phagocytohilum* in roe deer and to evaluate the molecular diversity of tick-borne pathogens in game reservoir. DNAs of all investigated parasites were detected; almost half of tested samples (35/71) were infected with at least one species. *A. phagocytophilum* (35%) was the most common and *Bartonella* spp. (12%) the rarest. A total of 18.3% (13/71) of all positive samples of roe deer were infected with at least two pathogens, and one-third of those (4/13) were coinfected with *A. phagocytophilum*, *Bartonella* and *Babesia* species. On the basis of multilocus molecular studies we conclude that: (i) two different genetic variants of *A. phagocytophilum*, zoonotic and non-zoonotic, are widely distributed in roe deer population; (ii) roe deer is the host for zoonotic *Babesia* isolates (*B. venatorum*, *B. divergens*), closely related to strain/species found in human; (iii) our *B. capreoli* and *B. divergens* isolates differed by the conserved two bases difference at positions 631 and 663; (iv) this is the first description of *Bartonella schoenbuchensis* infections in roe deer in Poland. We present one of the first complex epidemiological studies on *Babesia, Bartonella* and *A. phagocytophilum* prevalence in naturally infected populations of roe deer. The role of these game animals as reservoir host of the tick-borne pathogens seems to be important but the pathogenicity and zoonotic potential of parasite isolates require further investigation.

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ANISAKIDAE INFECTION IN FISH OF THE AEGEAN SEA

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The fish-borne parasitic zoonoses have been limited for the most part to populations living in low-middle income countries, but the geographical limits are expanding because of growing international markets and the increasing popularity of undercooked seafood, the improved transportation systems, and the demographic changes such as population movements. Nematode worms of the family Anisakidae are the causative agents of infections in humans when fish is consumed raw and of serious allergies up to the death, when fish is consumed raw or cooked by previously sensitized people. This is why, anisakiasis is considered to be a serious public health risk in many countries. Although there are various reports on the circulation of these zoonotic parasites in the Mediterranean Sea, there are no reports on human anisakiasis in Greece and on the prevalence of Anisakidae worms in commercial fish species from the Aegean Sea. The prevalence was investigated in 462 fish belonging to 26 species, fished in three areas of the Aegean Sea. Anisakidae larvae were detected in 87 (18.83%) fish of 13 species. These larvae were identified by morphology as the third-stage larvae of the genera *Hysterothylacium* sp. or *Anisakis*. Larvae of the genus *Anisakis* were identified by PCR-RFLP as belonging to *A. simplex* s.str., *A. pegreffii*, or as hybrids between *A. simplex* s.str and *A. pegreffii*. Our results are in agreement to previously published works carried out in the Mediterranean Sea, confirming the presence of the parasite and identifying *A. pegreffi* as the most prevalent species. However, to our knowledge, no human cases have been reported in Greece, possibly due to diagnose failure, asymptomatic infections, and/or spontaneous healing. Changes of feeding habits as consequence of the globalization combined with the high recorded prevalence in the Aegean Sea could represent a risk to acquire food-borne parasitic infection/allergies in the near future.

SY20/3

CRYPTOSPORIDIOSIS IN OVERWINTERING EUROPEAN HEDGEHOGS (*ERINACEUS EUROPAEUS*)

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Apicomplexan protists of the genus *Cryptosporidium* are important pathogens infecting broad range of vertebrates including humans. To date, only few records of *Cryptosporidium* infection in insectivores (1x bat, 3x shrews and 6x hedgehogs) are available. All these findings described only of *C. parvum* or *C. parvum*-closely related genotypes. In our report, we described cryptosporidiosis in semi-tamed overwintering European hedgehogs (*Erinaceus europaeus*) of different age and origin. Four young hedgehogs were hospitalised for anorexia and malodorous diarrhoea in January 2012. Immediate parasitological examination revealed presence of Cryptosporidium sp. and *Isospora rastegaievae* oocysts, and *Capillaria* spp. eggs in faeces in one of the hospitalised hedgehog only. Another 3 hospitalised hedgehogs were negative in coproscopy, but started to shed *Cryptosporidium* oocysts two days later. Whole group of 15 hedgehogs have been coproscopically monitored for 4 month till their releasing back to wild. Except *Cryptosporidium*, other parasites such *Isospora*, *Capillaria* and *Crenosoma* were also detected in faeces of some individuals. Eleven hedgehogs shed *Cryptosporidium* oocysts (4.74 ± 0.46 ×3.88 ± 0.46) with patent period 2 – 23 days. Repeated shedding of *Cryptosporidium* oocysts occurred in some animals in very low level after 2-3 months since the first outbreak. Clinical signs were not observed in the second period of shedding. Four hedgehogs were coproscopically negative for *Cryptosporidium* oocysts in whole monitored period. Comparison between microscopic and molecular detection of *Cryptosporidium* oocysts is given. PCR and sequencing of a part of the *Cryptosporidium* oocyst wall protein (COWP) gene and the 18S rRNA gene suggested the presence of *C. parvum*. Surprisingly, subadult SCID mice inoculated with 1 × 103 fresh oocysts originated from positive hedgehogs did not produce detectable infection. Zoonotic potential of *Cryptosporidium* hedgehog's isolates is so far unknown and will be discussed.

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PREVALENCE OF *NEOSPORA CANINUM* AND *TOXOPLASMA GONDII* INFECTION BY PCR IN RED FOXES (*VULPES VULPES*) FROM ROMANIA

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Neospora caninum and *Toxoplasma gondii* are two protozoan parasites that can affect many species of wild and domestic animals worldwide. No study regarding prevalence of *N. caninum* and *T. gondii* in red foxes was done our country. The purpose of this study was to determine the prevalence of *N. caninum* and *T. gondii* in brain tissues from red foxes. 182 red foxes (*Vulpes vulpes*) were collected from 6 counties from Centre and North-West of Romania. Genomic DNA extraction was performed on all brain samples. DNA was extracted from 40 mg tissue using a commercial kit (Bioline, UK), according to the manufacture's protocol. PCR protocol was conducted using primers from the Nc-5 region of the genomic DNA for detecting *N. caninum*. Also, 18s gene of *T. gondii* was amplified. The prevalence of *N. caninum* DNA was confirmed in 0.5% (1/182) red foxes. The positive sample for *N. caninum* was collected from Satu-Mare county. *T. gondii* DNA was confirmed in 6.6% (12/182) red foxes. The positive samples came from all 6 counties but the

highest prevalence of *T. gondii* DNA was obtained in Alba county 25% (2/8) followed by that obtained in Harghita county 14.3% (1/7). The result obtained in the present study confirmed that *N. caninum* and *T. gondii* are prevalent in Romanian wildlife carnivores, showing that red foxes are involved in *N. caninum* and *T. gondii* life cycles.

MICROHABITAT SELECTION AND HOST SPECIFICITY OF THE DIGENEAN *PHOLETER GASTROPHILUS* (HETEROPHYIDAE) IN TWO CETACEAN SPECIES

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Pholeter gastrophilus is a digenean that has been reported in at least 17 cetacean species worldwide, occurring mainly in 3 stomach chambers, namely the fundic stomach (FS), the connecting channel (CC) and the pyloric stomach (PS) of its hosts. The distribution of *P. gastrophilus* differs significantly among stomach chambers depending of host species. To account for these differences, two hypotheses have been put forward: (1) *P. gastrophilus* is a complex of cryptic species, each one adapted to a specific host species; (2) *P. gastrophilus* is a single species and its chamber distribution is passively driven by the duration of the digestion process, which differs among host species according to the average energy content of their prey. A corollary of the latter hypothesis is that selection of stomach chamber entails no fitness consequences. We investigated these issues in Mediterranean bottlenose dolphins, *Tursiops truncatus* (in which *P. gastrophilus* is known to favor the FS) and striped dolphins, *Stenella coeruleoalba* (in which *P. gastrophilus* has not apparent selection for any chamber). We confirmed that specimens of *P. gastrophilus* infecting both dolphin species were conspecific using 28SrDNA, ITS2 and CO1 as molecular markers. We measured body area, the uterus area filled with eggs, and egg area of 184 individuals of *P. gastrophilus* occurring in the FS and the PS from 7 striped and 2 bottlenose dolphins. Only the uterus area filled with eggs was significantly larger (1) in individuals of *P. gastrophilus* from the FS vs. PS, regardless of host species, and (2) in striped dolphins vs. bottlenose dolphins regardless of chamber. These results represent *prima facie* evidence that all stomach chambers would not be equally suitable microhabitats for *P. gastrophilus* and, therefore, they raise the question over whether the distribution of the parasite among chambers is just a side-effect of host's digestive physiology. Also, *P. gastrophilus* appears to exhibit significant differences regarding host specificity. This is an interesting example of an apparently generalist parasite that would exhibit subtle differences of compatibility among hosts even though they belong to a similar trophic guild and are both regularly infected with *P. gastrophilus*.

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MOLECULAR PREVALENCE AND GENETIC DIVERSITY OF *BORRELIA BURGDORFERI* SENSU LATO IN WILD CANIDS AND FELIDS FROM ROMANIA

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Lyme disease, caused by the spirochete *Borrelia burgdorferi*, is one of the most important tick-borne diseases. In Europe, Lyme borreliosis endemic areas are maintained trough complex interactions among different tick species, a variety of *Borrelia* strains belonging to the complex *B.burgdorferi* sensu lato and a large number of vertebrate hosts upon which ticks feed. Wildlife

could play a role in the emergence of tickborne diseases and also in the maintenance of tick burdens. With this view and considering the fact that no previous reports of the seroprevalence of antibodies to *B.burgdorferi* in Romanian wild canids and felids are available, this study aims to bring epidemiological data necessary to elucidate the role of this medium-sized wild mammals as reservoirs or dispersal agents.
Between August 2010 and December 2011, 242 tissue samples (heart and liver) have been collected from 121 dead animals, legally hunted or road killed, from various localities in Romania. The animals belonged to 5 species as follows: *Canis aureus* (5), *Canis lupus* (12), *Felis silvestris* (6), *Lynx lynx* (4), V*ulpes vulpes* (93). From each tissue sample, for PCR analysis the DNA was extracted by DNeasy® Blood & Tissue Kit (Qiagen) according to the manufacturer's instruction. Molecular detection of *Borrelia burgdorferi* s.l. was achieved by nested PCR using OspA primers. Genospecies identification was done by RFLP. The prevalence for *Borrelia burgdorferi* s.l. was 2.5% (3). All the positive cases belonged to *Vulpes vulpes* species. For all the three positive animals, heart samples were positive, while the liver samples were negative in two cases. The genospecies were identified as *Borrelia afzelii* (2) and *Borrelia burgdorferi* s.s. (1).
This is the first study that evaluates the prevalence and genetic diversity of *B. burgdorgeri* s.l. in various wild vertebrates from Romania.

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ROLE OF WILD BIRDS AS HOST OF HARD-TICKS IN ROMANIA

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Wild birds are hosts for several species of tick, contributing to the maintenance of their population in a certain area. Diversity and abundance of ticks parasitic on birds was assessed for a month in April, in two different locations in Romania. The first location located in the Danube Delta Biosphere Reserve, Grindul Wolves is an important refuge for birds nesting and feeding and the second location located in NW Romania. The aim of this study was to assess the distribution of tick species in different climatic zones in the same season and to understand the role of bird species are host for ticks. Tick species parasitizing birds included *Ixodes ricinus, I. arboricola and I. redicorzevi.* Tick prevalence and intensity of infestation differed between study areas and was higher in birds from the first location where a large population of wild animal may support tick population. Ixodes ricinus was the most abundant tick collected in the first location. The most parasitized bird species were similar between the two areas, and were those that forage mostly on the ground and/or low shrub vegetation, such as Sturnus vulgaris and Turdus merula.

THE PARASITES OF EUROPEAN BISON, *BISON BONASUS* (LINNAEUS, 1758) – THE REVIEW

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The first descriptions of European bison *Bison bonasus* parasitofauna origin from the beginning of XXth century. The regular monitoring of infectious and parasitic diseases is conducted from the late 50ties, from the leaving of first bisons to open area, by some scientific institutions. The study concerns free-living animals, as well as closed in reserve. Among protozoan and metazoan parasites, there were detected: 20 species of protozoa: *Trypanosoma wrublewskii, Giardia* spp*., Sarcocystis cruzi, S. hirsuta, S. hominis, Neospora caninum, Toxoplasma gondii, Cryptosporidium*

spp., *Eimeria cylindrica, E. subspherica, E. bovis, E. zuernii, E. canadensis, E. ellipsoidalis, E. alabamensis, E. bukidnonensis, E. auburnensis, E. pellita, E. brasiliensis, Babesia divergens*; 4 species of trematodes: *Dicrocoelium dendriticum, Fasciola hepatica, Parafasciolopsis fasciolaemorpha, Paramphistomum cervi*; 3 species of cestodes: *Taenia hydatigena (larvae), Moniezia benedeni; Moniezia* sp., 36 species of nematodes: *Ashworthius sidemi, Cooperia oncophora, C. pectinata, C. surnabada, C. punctata, Haemonchus contortus, Mazamastrongylus dagestanicus, Nematodirella alcidis, Nematodirus europaeus, N. helvetianus, N. roscidus, Ostertagia antipini, O. kolchida, O. leptospicularis, O. lyrata, O. ostertagi, Spiculopteragia asymmetrica, S. boehmi, S. mathevossjani, Trichostrongylus axei, T. capricola, T. vitrinus, Dictyocaulus filaria, D. viviparus, Setaria labiatopapillosa, Onchocerca lienalis, O. gutturosa, Aonchotheca bilobata, Bunostomum trigonocephalum, Gongylonema pulchrum, Chabertia ovina, Oesophagostomum radiatum, O. venulosum; Thelazia gulosa, T. skrjabini, Trichuris ovis*; 5 species of mites: *Demodex bisonianus, D. bovis, Chorioptes bovis, Psoroptes ovis, Sarcoptes scabiei*; 3 species of *Ixodidae ticks: Ixodes ricinus, I. persulcatus, Dermacentor reticulatus; 1 Mallophaga* species - *Bisonicola sedecimdecembrii*; two species of *Hippoboscidae* flies: *Lipoptena cervi*, *Melophagus ovinus*. It is evident, that there are few monoxenous parasites specific for European bison. These are *Trypanosoma wrublewskii*, *Bisonicola sedecimdecembrii* and *Demodex bisonianus*. There are many parasites typical for cattle, and many new parasite species acquired from Cervidae. This fact can be connected with dramatically history of *B. bonasus* species, restituted from some specimens breed in close farms. Other characteristic fact is the increase trend in biodiversity of parasites, as well as in prevalence and intensity of infections.

SY03/1

FIRST DETECTION OF *LEISHMANIA* MAJOR-LIKE IN NATURALLY INFECTED *SERGENTOMYIA MINUTA* IN PORTUGAL

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Zoonotic leishmaniasis caused by *Leishmania infantum* is endemic in Portugal being *Phlebotomus perniciosus* and *P. ariasi* the proven vectors. In addition, *L. major* / *L. infantum* hybrids have already been identified in four Portuguese autochthonous human leishmaniasis cases. The existence of these hybrids allowed hypothesizing the existence of sand fly species responsible for their circulation under natural conditions. Phlebotomine sand flies from the genus Sergentomyia are widely distributed throughout the Old World and are considered vectors of *Sauroleishmania* sp. We report a survey carried out to update the distribution, abundance and vectorial roles of phlebotomine sand flies in Algarve Region (AR). From March to November 2007, sand flies were captured by CDC miniature light traps. Kinetoplastid DNA-PCR and the internal transcribed spacer regions of *Leishmania* ribosomal DNA -PCR were used to screen female sand flies for *Leishmania* infections. A total of 1663 sand flies from *P. perniciosus*, *P. ariasi*, *P. sergenti* and *Sergentomyia minuta* were collected. The highest phlebotomine density was observed in July. The predominant species was *P. perniciosus* and it was found infected with *L. infantum*. In addition, in one *S. minuta* specimen L. major-like DNA was detected. These results reinforce the previous statement that *P. perniciosus* is the main vector of leishmaniasis in AR. Although up to now no autochthonous cutaneous leishmaniasis due to *L. major* has been detected in Portugal, they probably exist since cutaneous leishmaniasis cases are often not diagnosed and very rarely the parasite was identified from skin lesions. As far as we are aware this is the first time that a sand fly from *Sergentomyia* genus was found infected in Europe with parasites belonging to *Leishmania* sp. This finding challenges the dogma that Leishmania genus is exclusively transmitted by species of Phlebotomus genus in the Old World. On-going surveillance with systematic epidemiologic surveys on *Leishmania* reservoir hosts and vectors is crucial since the increased migration and travelling flow

elevate the risk of introduction and spread of infections by *Leishmania* species which are only sporadically endemic or non-endemic and their possible transmission by sand flies species that normally are considered as non-permissive.

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PROTEOMIC APPROACH FOR COMPARISON OF *IN VITRO* CULTURED TRYPANOSOMATIDS: *CRITHIDIA LUCILIAE* AND *LEISHMANIA INFANTUM*

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Family Trypanosomatidae consists from several heteroxenous and monoxenous genera, where heteroxenous genera like *Trypanosoma, Leishmania* and *Phytomonas* cause serious human, animal and plant diseases, unlike monoxenous genera like *Leptomonas, Crithidia, Blastocrithidia and Herpetomonas* which inhabit the insects. Members of the genus *Leishmania* are heteroxenous trypanosomatids, causative agents of cutaneous, mucocutaneous and visceral leishmaniasis in whole world. *Leishmania infantum* is a causative agent of leishmaniasis in humans and dogs in Mediterranean region and locally in southern parts of Croatia. Source of antigen for serological testing are *in vitro* grown promastigotes, which are known to be infective for humans during the manipulation and are nutrient demanding.
Monoxenous trypanosomatids are in general apathogenic for humans and animals, although they can be pathogenic in immunocompromised human patients. *Crithidia* genus involves monoxenous parasites which inhabit the digestive tract of insects. *Crithidia luciliae* is a flagellate in the digestive tract of insects such as common green bottle fly (*Lucilia serricata*), and house fly (*Musca domestica*). In general, monoxenous trypanosomatids are less nutrient demanding for in *in vitro* growth than the pathogenic heteroxenous trypanosomatid species.
The cross-reactivity in family Trypanosomatidae is well known phenomenon. A heteroxenous plant trypanosomatid *Phytomonas serpens*, apathogenic for mammals, share the same antigenic epitopes which are recognized by chagasic sera and are partly protective in *Trypanosoma cruzi* experimental infection. On the other hand, sera from chagasic patients react with *C. luciliae* antigen tested with immunofluorescence antibody test and western blotting. Also, sera from *Leishmania*-infected animals and humans reacted with *C. luciliae* and *L. infantum* antigen at the same level when comparatively tested with immunofluorescence antibody test and dot-enzyme linked immunosorbent assay.
Promastigotes of all *Leishmania* species contain on their membrane a glycoprotein known as leishmanolysin or promastigote surface peptidase or major surface peptidase. Homologues of membrane peptidases were described either on the cell surface or being released by apathogenic trypanosomatids belonging to the genera *Crithidia, Herpetomonas, Blastocrithidia* and *Phytomonas.*
Due to the high level of cross-reactivity and non-specificity in serological tests we compared the proteome profiles of two different species *C. luciliae* and *L. infantum* by means of 2-DE coupled with mass spectrometry.

MOLECULAR TITRATION OF *LEISHMANIA* PARASITES BY CLONED KINETOPLAST SEQUENCE IN REAL TIME PCR

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Leishmaniasis is a worldwide infectious diseases affecting people, domestic and wild animals in temperate, subtropical and tropical regions caused by diphasic protozoans of the genus *Leishmania*.
In the Mediterranean basin, dogs are the main reservoirs for human visceral leishmaniosis due to *L. infantum* and a great proportion of infected animals do not develop a clinical disease. Canine leishmaniosis can produce a wide spectrum of lesions and clinical signs, but some of them are considered highly suggestive of the disease in endemic areas. Diagnosis can be conduct by different tool application (microscopy, culture isolation, serology, PCR) and the choice of one or more of them depends on the goal of testing: epidemiology or management of clinical cases. The *Leishmania* genome has a variable number copy of repetitive extra chromosomal elements defined kinetplast. Due to the minicircle fraction of them represent a good target for sensitivity amplification test. We developed a rapid and accurate method for titration of the parasites by quantitative PCR. The objective of this study was to detect the exact content of the minicircle kDNA element in the parasite. Using the appropriate software, primers and probe were designed to a discrete region of the kinetoplast DNA. The kinetoplast fragment was cloned in a pGEM plasmid and transferred in *E. coli* competent cells; the recombinant plasmids were recovered by adapt exctraction system and quantified as copy number. Serial dilutions of this plasmid were created to employ in the real time test. In an introductory experiment we compared the quantitative real-time PCR test targeted both to DNA directly extracted from cultivated parasite and to cloned 116-bp kinetoplast DNA fragment. Two different kind of dilution curves were created and analysed in our experimentation. We found the Ct value at the equivalent point between the parasite and plasmid copy number serial dilutions. A 10-fold dilution series of 6 standard DNA obtained from *L. infantum* zymodeme MON-1 promastigotes (log phase concentration, 1×10^6 parasites/ml) was used as calibrators, allowing the plotting of a standard curve. Each dilution was tested in triplicate and the mean value was represented in the curve. The limit of detection of the real time PCR was assessed using a serial dilution from 1×10^{-3} to 1.7×10^{-4} parasites per reaction. The inter-assay reproducibility was estimated by testing 10-fold serial dilutions, being the experiment repeated 10 times. In this kind of test the results were expressed as the number of parasites per ml and kDNA copy number.
The obtained data were used to validate the quantitative detection method and to standardize the diagnostic tool.

PHLEBOTOMINE SANDFLIES IN THE MALAGASY SUBREGION (MADAGASCAR, ARCHIPELAGOS OF COMOROS AND SEYCHELLES): SETTLEMENT AND ENDEMISM

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Phlebotomine sandflies from the Malagasy subregion have been poorly studied in the past, according to the lack of autochthonous transmission of leishmaniasis in this area. Ten years ago, the first *Phlebotomus* was reported from Madagascar. To date, fourteen species and subspecies of phlebotomine sandflies are recorded in Madagascar, in the Union of Comoros and in the Seychelles. A lot of phlebotomine sandflies have been caught during field trips carried out in this area during the last decade. The samples have been processed in order to be analysed using both morphology and molecular biology (sequencing of the second Internal Transcribed Spacer of rDNA and/or the cytochrome b and cytochrome oxidase I of the mtDNA). Moreover, the interpretations at the origins and especially of the affinities of this fauna group have never been studied by lack of

precise knowledge of the origins of different lineages of these sandflies, its regional differentiation and its spatial distribution in the Malagasy subregion. The authors have analysed the results based on the morphological and molecular data: morphological traits and molecular phylogenies. At the light of the results obtained including molecular characterization and phylogenies, the authors discuss the endemism concerning Phlebotomine sandflies in the studied islands, the relationships of Malagasy *Phlebotomus* with species from Africa and Asia, and propose an hypothesis explaining the settlement of these islands by Phlebotomine sandflies, including an old one related to the isolation of Madagascar from the Gondwana following a generalised track, and more recent ones explaining the settlement of the Comoros and the Seychelles.

DETERMINATION OF SPECIES AND INTRA SPECIES GENETIC VARIATIONS OF *LEISHMANIA* PARASITE BY ITS1 REAL TIME PCR ASSAY

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Leishmaniasis caused by *Leishmania* parasites are seen in two clinical forms as cutaneous (CL) and visceral (VL) in Turkey. *Leishmania* (L.) *tropica* and *L. infantum* were determined as CL agents, while *L. infantum* was incriminated as VL agent in the country. Canine leishmaniasis (CanL) is widely common throughout the country and *L. infantum* is the responsible agent of the disease. In the present study, internal transcribed spacer (ITS1) region of nuclear DNA was chosen as the target of a real time PCR assay with the aim of developing a fast and standardized molecular diagnostic tool together with species identification and determination of intra species genetic variations. Clinical samples obtained from CL (n=289), VL (n=47) and CanL (n=66) cases and culture stocks of *Leishmania* isolates (n=87) were included to the present study. DNA was isolated from the clinical samples (blood in EDTA, bone marrow smear, lesion aspiration smear, lymph node aspiration smear) and culture stocks. All DNA samples were examined by the ITS1 real time PCR assay using newly designed primers and probes differentiating *L. donovani* complex, *L. tropica* and *L. major*. Genotyping of the samples was performed according to the melting temperature peak analysis Species identification was succeeded in 341 (84.8%) out of the 402 clinical samples by ITS1 real time PCR assay, while two simultaneous peaks were detected in 61 (15.2%) samples. Species identification of 84 isolates was resulted as 68 *L. tropica* and 16 *L. donovani* complex, while analysis of 3 isolates were resulted with 2 peaks. Real time PCR products of 134 samples were sequenced as the confirmational analyses. As a result, a standard and fast diagnostic method was developed and species identification and genotypic variations of *Leishmania* parasites in Turkey within species and intraspecies were determined in the present study.

This study was supported by a project (n=107S154).

IN VITRO EFFECT OF LISOQUINOLINE ALKOLOIDS OF *BERBERIS* HERB ON PROMASTIGOTES OF *LEISHMANIA MAJOR* AND TACHYZOITES OF *TOXOPLASMA GONDII*

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Toxoplasmosis and leishmaniasis are one of the more common parasitic zoonoses worldwide protozoans, are organisms that the numbers of them infected tissue and Cause disease.and important infectious complication which of the requires prolonged treatment. The purpose of this research was determine effect alkaloids of Berberis herb on promastigote *Leishmania major* and tachyzoite *Toxoplasma gondii*. With attention to chemical drugs harms in treatment above

diseases. Nowadays much attention has attracted to the use of herbal medicines. Material and methods: In this study used the promastigotes of Leishmania major isolated from RPMI1640 culture. Then were added to sample by different concentrations of aqueous and alcoholic extracts Berberis, with density 10ml, 20ml, 50ml, 100ml, 200ml, during 24 hours, 48 hours, 72hours. Also by this review were used tachyzoite of *Toxoplasma gondii* isolates from Peritoneal of mice, Then were added to sample different concentrations of extracts Berberis during 24 hours, 48 hours, 72 hours. The results were studied by using of neobar slides and light microscope. Result: Findings from this study showed alcoholic extract of barberry with 100 µg/ µl dilution, during 72 hours the reduction of *Leishmania major*, also extract was greatest effect on the reduction of parasite *Toxoplasma gondii* with 50 µg/ µl diluted in 72 hours.
Alcoholic extract of barberry contain many types of chemicals substance called "alkaloid isokinolin" and can toxic effect on the protozoans. By attention to increasing resistance of the various antibiotics and chemical drugs. Is essential for plant material be used in order treatment infectious diseases by more research and Knowledge.
Keyword: Alkaloids, Berberis, *Leishmania*, *Toxoplasma*.

SY03/2

VISCERAL LEISHMANISIS IN BOYER AHMAD DISTRICT, SOUTH OF IRAN

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This study aimed to determine the last condition of visceral leishmaniasis in Boyer Ahmad district in south of Iran. Visceral leishmaniasis is an endemic zoonotic disease in some parts of Iran caused by *Leishmania infantum*.
The information of new cases of visceral leishmaniasis collected from health centers. Serum samples were taken from 1628 children up to 10 years old and from 170 ownership dogs from different areas in the district by multi-stage cluster sampling. All samples were tested by Direct Agglutination Test (DAT). Fifteen infected dogs necropsied and parasitology study conducted by use of impression smear of liver and spleen. Nested Polymerase Chain Reaction (PCR) examination conducted on smears and tissues of liver and spleen to determine the molecular characterization.
The number of new visceral leishmaniasis cases during last 6 years (2007-2012) was 17 cases (11 male and 6 female). Seroprevalence rate among the children was 3.1% with antibody titre ≥1:3200. There was no significant difference in seropositivity between the sexes (2.8% males and 3.3% females). The seroprevalence in dogs was 10% (95%C.I, 5.4%-14.6%) with antibody titre 1≥320. No statistical significant difference was found between male (10.7%) and female (8.3%) seroprevalence (*p*=0.781). *Leishmania* amastigote was seen in 13 smears of liver and spleen (13 cases). The agent of disease in 14 dogs determined as *Leishmania infantum* by nested PCR method.
It seems that Boyer Ahmad district is an important area for visceral leishmaniasis in Iran which the disease is going to distribute. The majority of seropositive dogs (58.8%) that were lived in this district were asymptomatic. It seems that all symptomatic and asymptomatic infected dogs are the most important risk factors for human infection in VL endemic areas.
Keywords: *Leishmania infantum,* Visceral Leishmaniasis, Serologic test, Owner ship dogs.

DO WE REALLY KNOW *PHLEBOTOMUS* (*LARROUSSIUS*) *PERFILIEWI*?

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Phlebotomus perfiliewi Parrot is a proven vector of *Leishmania infantum* in some endemic foci of visceral leishmaniasis in Asia, Europe and North Africa. It belongs to the subgenus *Larroussius Nitzulescu*. Its first description made by Parrot was based on two males caught in Crimea. With the passing of the years, three sub-species have been recognized: i) *P.perfiliewi perfiliewi* Parrot 1930, ii) *P.perfiliewi transcaucasicus* Perfiliev 1937 and iii) *P.perfiliewi galilaeus* Theodor 1958. According to the literature, the taxonomic status of these taxa has never been discussed; despite there are some conflicts and doubts in their description and their identification mainly based on their ascoid formulas and the morphology of their aedeagus. A collection of *P. perfiliewi* s. l. has been processed for morphological and/or molecular studies. The sampling includes type-specimens and also populations from Iran, Turkey, Greece (continent and Crete), Cyprus, Israel, Italy (continent and Sardinia) and Algeria. The morphometrical measurements were coupled with the sequencing of the mitochondrial Cytochrome b and ribosomal Internal Transcribed spacer 2 fragments. The sequences show different lineages more or less in agreement with the previous taxa and geographical distribution. The authors suggests a taxonomic revision of this species which needs to be previously discussed with specialists, taking into account that the type specimens are different from the *P. perfiliewi* recorded in the western Mediterranean basin and identified by Parrot himself.

Keywords: *Phlebotomus perfiliewi*, visceral leishmaniasis, morphological and molecular approaches.

NATURALLY ACQUIRED AND LABORATORY INDUCED RESISTANCE TO ALLOPURINOL IN CANINE LEISHMANIA INFANTUM ISOLATES FROM ISRAEL

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There are 23 different *Leishmania* species, most of them zoonotic with animals serving as reservoirs for infection. Dogs and wild canines are considered the main reservoirs for human visceral *L. infantum* infection and they also suffer from a severe and fatal disease caused by this pathogen. A growing concern over the last decade has been the large number of drug resistant *Leishmania* isolates infecting humans, and an effort is being made to uncover the mechanisms of resistance. Very little information has been available on resistance to anti-leishmanial drugs in dogs. Allopurinol**,** alone or in combination with meglumine antimoniate or miltefosine, is the main drug used for treatment of canine leishmaniasis. Although it is helpful in improving the clinical manifestations of infection in treated animals, relapse of disease despite treatment is possible. The objective of the study presented here was to test whether reduced susceptibility to allopurinol exists, and whether it may play a role in disease relapse. *L. infantum* parasites were isolated and cultured from 3 groups of infected dogs: 10 newly diagnosed untreated dogs; 5 infected clinically healthy allopurinol treated dogs; and 4 dogs that showed clinical relapse during treatment. Susceptibility to allopurinol was evaluated by a viability assay using alamarBlue® (AbD Serotec, Oxford, UK), adapted to measuring promastigote proliferation at different drug concentrations. IC_{50} values for the treated relapsed group were 3-fold higher than comparable values from the non-treated or clinically healthy groups (p=0.03). In an *in vitro* model for induction of resistance under increasing drug pressure, a 2-10 fold increase in susceptibility

was recorded over 3 months. This is the first laboratory-based report of possible resistance to allopurinol in isolates from dogs. These results substantiate the suspicion that allopurinol resistant parasites can develop in treated dogs, likely due to natural selection of resistant parasites in response to treatment by itself or to a combination of host and parasite factors. Furthermore, these results suggest that the reduced susceptibility to allopurinol may be linked to a clinical relapse. Further analysis of the mechanisms of drug resistance is important in the development of effective treatments for this major zoonotic infection.

This study was supported by the U.S. Middle East Regional Cooperation (MERC) Project NIH-NIAID project TA-MOU-08-M27-072 and grant from the Agricultural Faculty Centre for Animal Husbandry and Health, The Robert H. Smith Faculty of Agriculture, Food and Environment, The Hebrew University.

SY09

MASSIVE LARVAL GROWTH ALLOWS SOME CESTODES TO SKIP GROWTH IN THE DEFINITIVE HOST

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Complex life cycles occur in various taxa and pose evolutionary questions, like how life history strategies are optimized when occupying separate niches during ontogeny. Many parasitic worms (helminths) are transmitted tropically between hosts before reproducing in large, high-trophic-level hosts. Parasites are likely to grow faster and safer to a larger reproductive size in bigger hosts, which explains why the majority undergo considerable growth in their definitive host before reproduction. One well-studied group of tapeworms (*Schistocephalus*, *Ligula*, and *Digramma* spp.) is a notable exception; they reproduce semelparously without any growth in their avian final hosts. Arguably, the gastrointestinal tract of birds is an environment conducive to parasite growth, so why don't these worms grow in the final host? We explored theoretically the conditions leading to this counterintuitive no-growth strategy and established that it is favored when the optimal larval size is greater than or equal to the optimal size for reproduction. We tested and found support for this expectation with cross-species comparative analyses. The sizes attained by no-growth tapeworm species in their last intermediate host (fishes) are comparable to or larger than the adult sizes reached by species that grow in similar definitive hosts (piscivorous birds). Moreover, no-growth species are already as larvae relatively large for the mass of their definitive hosts. We speculate that massive larval growth in these species may be facilitated by transmission between large-bodied hosts, occupation of the intermediate host body cavity, and infecting definitive hosts that swallow food whole.

OPTIMALITY, MICROEVOLUTIONARY, AND MACROEVOLUTIONARY PERSPECTIVES ON THE LIFE CYCLE OF *SCHISTOCEPHALUS SOLIDUS*

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Transmission from one host to the next is a critical life history transition in complex life cycle parasites. The optimal size and age at transmission is thought to be shaped by the growth and mortality rates experienced by parasites in successive hosts; the timing of transmission should maximize lifetime growth while simultaneously minimizing mortality. I provide growth and mortality rate estimates for the tropically-transmitted tapeworm *Schistocephalus solidus*, and I explore their

expected consequences at both micro- and macroevolutionary levels. I focused on the transmission of the worm from its copepod first host to its stickleback second host. The parasite experiences far higher growth rates and far lower mortality rates in fish, suggesting any growth in the first host is, from a life history perspective, wasted time. Nonetheless, the worm spends about two weeks growing and developing in copepods before becoming infective to fish. Using an experimental evolution approach, I was able to select worms for faster development in copepods, indicating that there is not an absolute genetic constraint on the amount of time worms need to spend in copepods. In a cross-species comparative analysis, I found that other helminth species that have evolved transmission from copepods to fish exhibit very similar growth and developmental rates, suggesting that there may be a universal, optimal life history strategy for a copepod-fish life cycle. The incoherence between predictions from optimality models, quantitative genetics, and interspecific comparisons suggests that there may be important, overlooked constraints in the evolution of life history strategies in complex life cycle parasites.

SY07/4

HUMAN FASCIOLIASIS IN RUSSIA (2006-2011)

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In the Russian Federation, fascioliasis is regarded a rare helminthiasis. We analyzed data on notified cases in 2006-2011 years. During this time there were reported 28 cases of fascioliasis caused by Fasciola hepatica. Of these, 20 cases were autochthonous. 8 imported cases, mostly reported in people who have come to Russia from Tajikistan, Uzbekistan, Korea, Georgia. According to the Federal Agency for Tourism in the mid-2000s, in Russia increased the number of visitors from Uzbekistan and Kyrgyzstan to 1,7 times, from Tajikistan to 1,6 times. An increase in travel increases the risk of imported parasitic diseases in the country. Two Russian citizens were infected in Ukraine and Belarus. Local Russian cases were found in 20 patients. Cases were reported from 19 administrative territories of 11 subjects of Russian Federation: Irkutsk, Murmansk, Kirov, Penza, Kaliningrad, Tyumen, Saratov, Orenburg Regions, Republic of Sakha, Perm, Moscow. The risk of infection increases in rural areas where people have livestock and use farm animal dung for vegetable production. In Russia 14 cases of infection occurred in rural areas: in the Tyumen (7 cases), Orenburg (2 cases), Penza, Kirov, Arkhangelsk Regions, Republic of Bashkortostan, Stavropol (1 case in every subject) and 6 cases in urban areas: in the Tyumen (2 cases), Kaluga, Kaliningrad, Perm and Krasnodar. The patient age varied from 2 to 74 years. Adults were more affected (23 patients). Most affected age was 20-39 years (13 persons). No sex prevalence was noted (male – 15, female – 13). High proportion of patients was unemployed (13). According to our data, 6 patients with clinical symptoms did not refer to a doctor for more than a month. It took from 1 day to 4 months to make the correct diagnosis. Late diagnosis is probably explained with the lack of knowledge of physicians about fascioliasis. High prevalence of animal fascioliasis was reported from some territories of Russia (Chechen Republic, Republic of North Ossetia-Alania, Altai and others). It is contrasted with only sporadic of human fascioliasis reported cases. Thus, the apparent connection between human and animal fascioliasis is not reported yet. It is of great importance to establish a real situation of fascioliasis in Russia.

HUMAN FASCIOLIASIS IN ARGENTINA: A MULTIDISCIPLINARY ANALYSIS

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Human fascioliasis is currently emerging worlwide and endemic regions are recognized in Latin America, Europe, Africa and Asia. The highest known prevalence and intensities of human fascioliasis occur in Latin America in Andean regions of Bolivia and Peru. In Argentina, fascioliasis has always been considered an important disease affecting animals and national slaughterhouse reports are available, yet human fascioliasis is not of obligatory declaration and has never been adequately addressed despite the proximity and the geographical and climatic similarities with the endemic areas. The only information of human cases is that available in published and unpublished written reports; a previous review accounted for 85 human cases. The purpose of this research is to provide an in-depth analysis of the results obtained in a thorough bibliographical search of human fascioliasis in Argentina. In total, 58 reports were identified which described 619 cases. The majority (97.7%) from mountainous regions concentrated in central and western Argentina with very few cases in the rest of the country, not matching the distribution of animal fascioliasis. The age of the patients ranged from 3 to 95 years (mean 37.1) and 55% of the patients were female. Wild watercress ingestion was the main risk factor, described in 214 patients, during recreational, weekend or holiday activities. Eleven family outbreaks involving 63 persons are described. Diagnosis mainly relied on egg finding (288), followed by serology (82), intradermal reaction (63), surgery (45), and erratic fluke observation (6). A delay in diagnosis (average 3.5 years) and high lithiasis proportion and surgery reports suggest that many patients are frequently overlooked. In cases when the month of the appearance of the first symptoms was noted ,most of the cases occur during summer and early fall correlating significantly with monthly precipitation, monthly maximum temperature and monthly minimum temperature a with a time lag of 3 months which fits with the logical delay between infection moment and symptom appearance and diagnosis. This also coincides with field activities during summer holidays and would explain the second peak observed in June related to the Easter Holidays. Emetine appears as the drug most used (186), replaced by triclabendazole in recent years (21). Surgery reports are numerous (27.0%). High seroprevalences found in recent random surveys suggest human endemic situations. This analysis highlights that human fascioliasis may have been overlooked in the past and may be currently underestimated.

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SY07/5

DETERMINATION OF SNAILS WITHIN THE GENUS RADIX AND THEIR ROLE IN *FASCIOLOIDES MAGNA* LIFE CYCLE

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Snails of the genus *Radix* are well known among parasitologists due to their ability to transmit trematodes. Although many representatives of the genus *Radix* participate in the life cycles of veterinary and medically important pathogens, their taxonomy is still unclear and confusing. There are two major approaches in species delineation: traditional methods taking into account shell morphology and anatomy of reproductive organs, and modern ones based on molecular (DNA) analyses. In our study we applied both approaches to snail populations collected mainly in the

Czech Republic. For phylogenetic analyses two genes were characterized - mitochondrial 16S rDNA and nuclear ITS2 rDNA. In the samples 5 species of the genus *Radix* (*R. auricularia*, *R. labiata*, *R. lagotis, R. peregra* and *R. ampla*) were confirmed, nevertheless, some disagreement between the trees constructed for both genes was observed. For morphological determination the method based on ratios of 11 conchological characters was designed. When applied to unknown samples, delineation of *R. labiata* and *R. lagotis* appeared to be a problem. *Radix auricularia* was well distinguished from *R. labiata* and *R. lagotis* (with one exception). *Radix ampla* was well separated from all remaining species. *Radix peregra* was not evaluated in this experiment. For determination by means of reproductive organ morphology, the shape and position of bursa copulatrix, and its duct were used. According to these criteria, *R. auricularia* was distinguishable from *R. labiata*, *R. lagotis*, *R. peregra* and *R. ampla*. Similar morphological characters were observed in the pairs of *R. labiata* - *R. ampla* and *R. lagotis* - *R. peregra*. In addition, susceptibility of Radix species to the infections by *Fascioloides magna* and *Trichobilharzia regenti* was also determined. Experimental infections and observations in the field show that *R. lagotis* and *R. labiata* may represent susceptible "vectors" of *F. magna*. *Radix peregra* was proved as the intermediate host of *T. regenti*.

INTERACTIONS BETWEEN *ALARIA ALATA* MESOCERCARIAE AND THE PARATENIC HOSTS

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Among the latest emerging trematodes in Europe, one of the most recent is *Alaria alata*. This parasite is the only known trematode for which the mesocercarial stage (also called DMS) can infect man. In France, the compulsory search of *Trichinella* sp. in wild boar carcasses has led to DMS finding and discarding for human consumption more than a hundred of positive carcasses (Portier et al., 2011). The main risk of human contamination resides in missed diagnosis or inadequate inactivation of the parasite in meat due to the lack of knowledge on this parasite.
Using the *Alaria* migration technique described by Riehn *et al.* (2010) as a new more effective method for the search of DMS in boars' meat, we identify elective sites in wild boars. A total of 15 different tissues were sampled on 6 carcasses. Investigations give parasite burdens of over 500DMS/100gr in throats, tongues and pillars of the diaphragm, showing that samples used for *Trichinella* inspection (tongue and pillars of the diaphragm) are perfectly suitable for the search of DMS.
Tests were carried out to assess the resistance of DMS to freezing and cold temperatures. Results show that DMS are eradicated from pillars of the diaphragm after 4 days freezing at -16°C at heart confirming preliminary published results. DMS persisted well at 4°C, burdens being maintained after two weeks at these temperatures in decaying tissues of different types.
In order to evaluate the viability of DMS from wild boars and their transmission to another host, a laboratory model was elaborated by feeding mice with 30 DMS from boars. These infestations were successful with up to 27 DMS retrieved after one month. Distribution in mice was close to that observed in boars. A mouse to mouse infection was also performed and revealed that DMS were able to infect several paratenic hosts successively.
During mice dissection, DMS were observed encapsulated and free. We succeeded in sustaining live DMS in different media for over 60 days and observed the production of the said capsules hence showing that this encapsulation is not a production of the host.
These experiments have led to a more precise assessment of the risk for humans and to changes in the French legislation regarding boar carcasses bearing DMS.

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SY07/6

FASCIOLA HEPATICA TRANSMITTED BY *LYMNAEA NEOTROPICA* IN ARGENTINA

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Fascioliasis is widespread in livestock in Argentina with important endemic areas. It not only affects cattle, but also sheep and goats, particularly in the arid, marginal regions where these animals can be the only means of subsistence. There are reports of human cases and the proximity to endemic regions in Chile and Bolivia is cause for concern. Mendoza, an Andean province in Midwestern Argentina is currently making efforts for its control due to the very high prevalence reported in animals. Traditionally *Lymnaea viator* was considered the only vector in the region, but its identification was based solely on morphological criteria. Lymnaeid snails have great intraspecific variability regarding shell morphology and uniformity of their anatomy, thus, these characteristics do not suffice for correct species identification. Sequences of molecular markers amongst the nuclear ribosomal DNA and the mitochondrial DNA appear to be the most useful tools for specimen classification purposes. In a long term initiative to evaluate which are the fascioliasis areas of most concern, studies were performed in a recreational farm in Mendoza located at the foot of the Andes which included: a) coprological studies of cattle, goats, horses, donkeys and a llama to detect *Fasciola hepatica* eggs by the rapid sedimentation technique b) classification of the lymnaeid vector present and c) verification of natural transmission of fascioliasis by identification of the intramolluscan trematode larval stages. Lymnaeid and trematode classification was verified by means of nuclear ribosomal DNA and mitochondrial DNA marker sequencing. Coprological studies showed that all mammal species of the farm were affected by *F. hepatica* infection: 3 infected of 4 cattle analyzed, 3 of 4 goats, 3 of 7 horses, 4 of 4 donkeys, and 1out of 1 llama. Complete sequences of 18S rRNA gene and rDNA ITS-2 and ITS-1, and a fragment of the mtDNA cox1 gene demonstrate that the lymnaeid belongs to the species *Lymnaea neotropica*. Redial larval stages found in a *L. neotropica* specimen were ascribed to *F. hepatica* after analysis of the complete ITS-1 sequence. The finding of naturally infected L. neotropica is the first of this lymnaeid species not only in Argentina but also in Southern Cone countries. Its presence in relation to so many species of animals with *F. hepatica* together with the recent report of *Galba truncatula* in the same province, adds to the complexity of the epidemiology of fascioliasis transmission in the region.

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A NEW BASELINE FOR HUMAN AND ANIMAL FASCIOLIASIS TRANSMISSION IN CHILE

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Human fascioliasis is a parasitic disease that is emerging in many areas of the world and presents different patterns in its epidemiology and transmission. In Chile, the medical impact appears yearly stable and mainly concentrated in the central regions of the country, where the veterinary problem is highlighted by higher animal prevalences. Studies were undertaken by means of the sequencing of the rDNA ITS-2 and ITS-1 and mtDNA *cox*1 markers to clarify the specific status of the freshwater lymnaeid snail species, their geographical distribution, and fascioliasis transmission capacity in Chile, by comparison with other American countries and continents. The lymnaeid fauna of mainland Chile shows to be poor, including only two authochthonous species, *Lymnaea*

viator and *Pectinidens diaphana*, and a third introduced species of Palaearctic origin, *Galba truncatula*. Both *Lymnaea lebruni* and *Lymnaea patagonica* proved to be synonyms of *P. diaphana*. *Galba truncatula* appears to have always been confused with *L. viator* and seems distributed from Región VI to Región IX, overlapping with human endemic areas. DNA sequencing results suggest that the absence of correlation between remote sensing data and disease prevalences could be due to transmission capacity differences between *L. viator* and *G. truncatula*. Results obtained furnish a new baseline on which to undertake future appropriate studies on transmission, epidemiology and control of human and animal fascioliasis in Chile.

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SY12/1

DIROFILARIOSIS IN SLOVAKIA – RESULTS OF THE FIRST FULL-AREA MONITORING

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Canine dirofilariosis was in Slovakia for the first time diagnosed in 2005. In 2007, the initial epidemiological survey was performed which results revealed nearly 35% of dogs originating from climatically warm regions of southern parts of Slovakia being infected with *Dirofilaria repens*.
Since 2007, more than 2700 dogs from all regions of Slovakia have been examined for *Dirofilaria* infection using modified Knott test and PCR method.
Microfilariae were detected in more than 300 dog blood samples that represent an overall prevalence over 12%. In all infected dogs *D. repens* was identified as etiological agent of infections. In 8 dogs mixed infection with *D. immitis* was detected.
Monitoring results show significant regional differences in *Dirofilaria* distribution. In southern regions of Slovakia the mean prevalence reaches more than 20%, whereas in northern part only 2–4% of dogs are infected.
Transmission of *D. repens* to people was also confirmed in Slovakia. Since the first finding of the parasite in dogs 4 human cases of subcutanenous dirofilariosis have been recorded; in 3 of them the autochthonous origin of infection was unambiguously documented.

The work was realized within frame of the project “Protecting of the environment from parasitozoonoses under the impact of global climate and social change“(code ITMS: 26220220116), supported by the Research & Development Operational Programme funded by the ERDF (0.5) and by the Science Grant Agency VEGA 2/0011/12 (0.5).

PREVALENCE OF *DIROFILARIA IMMITIS* AND *DIROFILARIA REPENS* IN 310 STRAY DOGS IN BULGARIA

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Routine testing of dogs for *Dirofilaria immitis* in Bulgaria is becoming more popular among veterinarians and dog owners. This is still not the case with testing for *Dirofilaria repens*, a pathogen with zoonotic potential, still under looked and with unknown prevalence. The aim of this study was to confirm the presence of *Dirofilaria immitis* and *Dirofilaria repens* and to estimate their prevalence. Subject of the study were stray dogs from two big cities in Bulgaria. Stray dogs are maximally exposed to the vectors by living outdoors and are rarely or never treated with heartworm

preventives, which made them suitable to prove prevalence of dirofilarial infection in the chosen areas.
A total number of 310 dogs were tested. 280 dogs were tested by immunochromatographic antigen test (Anigen Rapid Canine Heartworm Ag Test Kit 2.0 *) and by modified Knott test. Another 30 dogs were tested only by modified Knott test. 240 of the whole blood samples were collected in April 2011 from dogs in Sofia, the source of the dogs was a county dog shelter operated by a nonprofit organization called Animal Rescue Sofia. Another 70 dogs from the town of Ruse were tested in September 2011, all of them confined to the local county dog shelter.
The dogs from Sofia, the capital of Bulgaria, had prevalence of 8.75% for *Dirofilaria immitis* and 7.5% for *Dirofilaria repens*. All dogs were tested by the Canine Heartworm Ag Test and by modified Knott test. A large number of the dogs had known history of living in a certain neighborhood of the city, which made it possible to draw a map of distribution of infection. Fewer cases were seen in the south, south-west part of the city, close to mountain Vitosha.
The dogs from Ruse, a town in north-east Bulgaria, on the river Danube, showed prevalence of 15.7% for *Dirofilaria immitis* and 8.6% for *Dirofilaria repens*. 40 dogs were tested by the Canine Heartworm Ag Test and by modified Knott test. 30 dogs were tested by modified Knott test only. The positive dogs with known origin were evenly distributed looking at the map.
The numbers confirm the presence of *Dirofilaria immitis* and *Dirofilaria repens* in these two areas which are different in climatic conditions and vectors. The percentages of *Dirofilaria repens* positive dogs are high enough to alert about the potential threat to human health.

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HUMAN DIROFILARIASIS: EMERGENT DISEASES IN ROMANIA?

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Introduction: Human dirofilariasis, produced by the tissue location of the nematode worm *Dirofilaria* spp., is spread in tropical, subtropical and recently in temperate regions, including Unated States, Europe, Japan. Based on the presence or absence of external longitudinal cuticular ridges, there are two subgenera currently described: *Dirofilaria dirofilaria and Dirofilaria nochtiella*. *Dirofilaria* species infecting humans are: *D. immitis, Dtenius, D. repens, D ursi, D. subdermata, D. striata.* The most common locations of immature filarial worms are the connective tissue, lungs, eye.
During the last few years, the parasite seems to become more common in Romania.
Objective: to evaluate the recent cases of human dirofilariasis, confirmed in Romania, during the last decade.
Methods: A retrospective analyze of the 22 human cases discovered and confirmed in Romania is done during the last 15 years (January 1997-April 2012). The diagnosis was done in University of Medicine and Pharmacy Carol Davila and in Eco-Para-Diagnostic clinic, Parasitology lab and, recently, it was confirmed by Institute of Parasitology, Switzerland. Clinical cases discovered in different clinics, were referred for monitoring to Eco-Para-Diagnostic Medical Center and Colentina Hospital. *D. repens* and *D. immitis* are responsible for human disease in our cases. Most of the patients (12) came from rural areas and recognized the contact and life in the vicinity of dogs, as

well as the presence of mosquitoes in their vicinity. None of the patients traveled or lived outside the country. The number of the cases increased every year. The patients were aged between 6 and 70 years. The residence of the cases was in Bucharest - 8, Ilfov - 4, Teleorman - 3, Cluj-Napoca - 1, Tulcea - 1, Valcea - 1, Galati -1, Brasov -1, Ialomita - 1, Giurgiu - 1case. Ocular - 12 (palpebral, subconjunctival, orbit), lungs - 3, soft tissue 6 and nostrils – 1 case, are the most common locations.
Conclussion: Human dirofilariasis became more common in Romania. The relationship with dogs and mosquitoes vectors, in both rural and urban areas, represent the main risk factors for human transmission on one side and, probably associated with the climate changes and the lack of spreading of insecticides, on the other side, are major concerns for the human transmission. We estimate a higher number of cases, many of them remaining unrecognized. In the future, medical attention should be focused on these parasites and their possibility of human transmission.

DIROFILARIASIS – CASE FILE

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Dirofilariasis is a zoonotic infestation with nematodes of the *Dirofilaria* spp. The usual hosts of the parasites are domestic and wild carnivores. Transmission occurs through a vector, as *Culex*, *Aedes* or *Anopheles* mosquitoes. The two species, *Dirofilaria immitis* and *Dirofilaria (Nochtiella) repens,* are increasingly recognized as human pathogens. Humans are accidental hosts and represent a dead end for the lifecycle of the worm. Human dirofilariasis typically manifests as either subcutaneous nodules or lung parenchymal disease, in many cases asymptomatically. Apart from pulmonary and cardiovascular manifestations; periocular, intraocular as well as orbital involvement have been described. In the last years, sporadic cases of human dirofilariasis have appeared in northern Italy, Hungary and Armenia, human disease being more frequent in areas with a warmer climate. At temperatures below 14°C, *Dirofilaria* stops developing, explaining the predominant occurrence of dirofilarial infections in warmer climatic zones.
We recently have identified a new case of *D. repens* infection in a young patient, female, 20 years old, living in Tamasda, Bihor county. The disease started by the appearance of a pruriginous erythema on the left forearm, initially neglected. After 2 months, a tumor appeared in that particular area, growing progressively (5cm in diameter), slightly painful, initially interpreted as a lipoma. The patient was operated and subcutaneously was discovered a cyst with rigid walls which contained a worm, 14cm long and 0.5mm thick, yellowish-white, with active movements. Biopsy and histological sections of the parasite were performed and the laboratory samples were examinated. The parasite was histologically identified as a *Dirofilaria repens* larva, in an advanced maturation state. No more larvae were found in other organs; the patient was functionally and imagistically examined.
The human dirofilarial infection occurred in a rural area because there are vagrant dogs, a possible source of infection with animal filaria in humans. Although the climate in Bihor county is inopportune for this parasitosis, there also exists the danger of human infestation in other vicinal areas, because of the precarious epidemiologic conditions. The epidemiology of human dirofilariasis is related to the prevalence of canine dirofilariasis, the presence of suitable mosquito vectors, and human activities that lead to exposure.

SS01/1

CYSTIC ECHINOCOCCOSIS INFECTION DYNAMICS IN LIVESTOCK IN GREECE

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Cystic echinococcosis caused by *Echinococcus granulosus* is one of the oldest recognised zoonoses, often threatening seriously food safety which was why it has long been the target of intensive, eradication programs and mandatory meat inspections. Throughout the 20th century, these efforts were successful in many countries, especially those in Europe. Recent reports however, indicate that cystic echinococcosis is re-emerging due to changes in human activities, the parasites' biology or both. The aim of our study was to describe the current epidemiological status of cystic echinococcosis in Greece by defining a) its incidence in as many animal species as possible in a strategically selected area; b) the intensity of the infection and the cyst fertility and c) the genotypes present. The study was carried out in Northern and Central Greece in a total of 9 abattoirs scattered in 4 different regions (Thrace, Thessaly, Central and Western Macedonia). Those regions were selected because of a) their significant livestock population and b) neighbouring with other Balkan countries. Moreover the specific abattoirs were selected on the basis of the number and the variety of animals slaughtered per month. During the inspection, all infected organs were recorded, removed and transported to the laboratory, in each organ the number and the location of the cysts was recorded and the hydatid cysts were ranked as fertile, sterile or calcified/caseous cysts . In total, 1707 animals (sheep, goats, buffaloes, wild boars and deer) has been checked during slaughter and 329 of them were found infected by *E. granulosus* (19.3%), while the infection rates per animal species were 30.2% (272/898), 7.86% (38/483), 42% (16/38), 1.1% (3/273) and 0%, respectively. The results obtained from the molecular analyses showed that all samples analysed provided - for the 2 genes cox1 and nad1 – similar high sequence identity levels with the G1 (/G2/G3) genotype complex, thus demonstrating that the conventional species *Echinococcus granulosus* sensu stricto appears to be dominant in Greece. As already reported in other parts of Europe the G1/G2/G3 genotype complex has always been considered as the major source of human contamination.

HIGH EXCRETION OF *CRYPTOSPORIDIUM UBIQUITUM* IN ADULT GOATS AROUND PARTURITION

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Cryptosporidium is an important agent of neonatal diarrhea in goat kids. Little is known about its molecular epidemiology in adult goats. This longitudinal study was set up to identify the species excreted by adult goats around parturition.
Individual faecal samples were collected from 20 adult pregnant goats between one and four years of age in one flock. Samplings began 1 month before the estimated kidding date and were done weekly until kidding and weekly for 2 weeks after kidding. Fecal samples were taken directly from rectum with sterile plastic gloves, transported to the laboratory in a cool box and stored at 4°C before being analyzed. *Cryptosporidium* oocysts were concentrated from 15 g of feces using a cesium chloride method. Oocyst output was determined using a direct immunofluorescent antibody test (IFAT) (MeriFluor® *Cryptosporidium*/*Giardia*, Meridian Bioscience Europe). Total DNA was extracted from each CsCl-cleaned fecal sample positive by IF (Maxwell® 16 Tissue DNA

Purification Kit, Promega). The samples were submitted to a nested PCR-RFLP on the SSU rDNA in order to identify the isolates to the species level.
According to their kidding date, goats were sampled between 4 and 8 times. 2 goats didn't kid and were excluded from the analysis of oocyst excretion. 16 goats out of 18 were found positive at least at one sampling date. Prevalence of excretion was maximal 14 days before kidding with half of the goats excreting oocysts at this date. Excretion was higher before kidding than after kidding. Individual oocyst excretion ranged from 6 to 247000 OPG. All isolates were identified as *C. ubiquitum* by PCR-RFLP.
This trial confirms the previous results showing an increase of excretion of oocysts of *Cryptosporidium* before kidding and confirms that adult goats excrete *Cryptosporidium ubiquitum*.

A. Rieux is grateful recipient of a grant from Anses/Region Poitou-Charentes.

SSO1/2

EPIDEMIOLOGICAL STUDY IN *TOXOPLASMA GONDII* AND *NEOSPORA CANINUM* INFECTIONS IN SMALL RUMINANTS FROM ROMANIA

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T. gondii and *N. caninum* are two protozoan parasites with worldwide distribution which can produce abortion and reproductive problems in small ruminants. The aim of this study was to determine the seroprevalence of *T. gondii* and *N. caninum* infection in goats and sheep from centre and north-west of Romania. 753 serum samples from goats and 2650 serum samples from sheep were tested for anti - *T. gondii* antibodies and 512 serum samples were tested for anti - *N. caninum* antibodies. All serum samples were tested by ELISA technique. For *T. gondii* was used Chekit Toxotest, IDEXX Laboratories, Switzerland, and for *N. caninum* was used Chekit *Neospora caninum* Antibody Test Kit, IDEXX Laboratories, Switzerland. The seroprevalence of anti - *T. gondii* antibodies, IgG type, in goats was 50.7% (382/753). The seroprevalence obtained in adult goats (55.9%; 380/680) was significantly higher than in kids (2.7%; 2/73) ($p<0.001$). The seroprevalence was significantly higher in household goats (74.5%) than those from herds (47.2%). In sheep, the seroprevalence of *T. gondii* infection was 53.5% (1417/2650). The seroprevalence of *T. gondii* infection obtained in adult sheep (61.1%; 1263/2067) was significantly higher than in lambs (26.4%; 154/583) ($p<0.001$). The seroprevalence of anti - *N. caninum* antibodies, IgG type, in goats was 2.3% (5/512). All positive serum samples were collected from goats reared in herds. This study showed that *T. gondii* and *N. caninum* are prevalent in small ruminants from Romania.

SY23/2

CRYPTOSPORIDIOSIS: AN IRISH PERSPECTIVE

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Cryptosporidium is a ubiquitous parasite affecting a wide range of animals including humans. Cryptosporidiosis poses a significant threat to public health in Ireland as several recent waterborne outbreaks have shown. The hydrogeological situation in Ireland, combined with high stocking rates of livestock and the absence of filtration from regular water treatment, render it particularly

vulnerable to large-scale outbreaks. Cryptosporidiosis is a notifiable disease in humans and Ireland and has one of the highest reported incidence rates in Europe, with between 8.7 and 14.4 cases/ 100,000 of population a year since 2004.
Over the last 8 years our team has used molecular tools to investigate the epidemiology of *Cryptosporidium* in both humans and farm animals in Ireland. Faecal samples were collected from 5 piggeries (n=342); 6 cattle (n=467) 7 sheep (n=651) and 3 horse (n=181) farms and examined by Auramine-O or IFAT staining. A subset of positive samples were further analysed by nested PCR of the 18s rRNA region followed by sequencing. *Cryptosporidium parvum* was identified in 2 pig, 13 cattle, 16 sheep and 6 horse samples. In addition *C. suis* (pigs, n=14), *C.* pig genotype II (pig, n=11), *C. muris* (pig, n=1), *C. xiaoi* (cattle, n=12; sheep, n=7), *C. ryanae* (cattle, n=30; horse; n=12; sheep, n=7), *C. bovis* (cattle, n=23; horse, n=6; sheep, n=17); *C. ubiquitum* (sheep, n=2), *C. andersoni* (cattle, n=3; sheep, n=2) and *C. hominis* (sheep, n=1) were also detected.
Cryptosporidium-positive human stool samples (n= 278), collected between 2000 and 2008, were typed to species level. In addition, gp60 subtypes were identified. *C. parvum* accounted for over 80% of all cases. All but 2 of the remaining samples were *C. hominis*. One case each of *C. meleagridis* and the *C. ryanae* and one mixed *C. parvum* and *C. hominis* infection were also identified. Almost all *C. parvum* isolates belonged to gp60 allele family IIa with a strong predominance of allele IIaA18G3R1. Most *C. hominis* isolates belonged to the geographically widely distributed allele IbA10G2.
Our study indicates that by far the majority of *Cryptosporidium* spp. found in animals are not considered major zoonotic agents. *C. parvum* was only found in 17% of all the animal samples tested. However, with 99% all subtyped human *C. parvum* isolates belonging to the zoonotic allele family IIa would indicate that more complex transmission patterns are at play in Ireland.

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ZOONOTIC TRANSMISSION OF *CRYPTOSPORIDIUM MELEAGRIDIS* ON A SWEDISH FARM

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Cryptosporidium meleagridis infects birds as well as mammals including humans. We report the first confirmed native case of human *C. meleagridis* infection and the source tracking in farm animals.
Within one week, three of the personnel on a 4H farm became ill with diarrhoea. One sought medical help due to prolonged diarrhoea and was diagnosed with *Cryptosporidium* and *Campylobacter* infection. The farm was run as an organic farm, with cattle, sheep, goats, pigs, horses, hens and broiler chickens. There was a café and a farm shop. The patient reported that eggs were washed in the kitchen sink. No hygiene barriers between animal species were present. Environmental faecal samples were collected from cattle, pigs, sheep/goats, hens and broilers. Samples were concentrated by NaCl flotation and analyzed using FITC-mAb. Partial amplification and sequencing of the 18S rRNA and HSP70 genes was used for species/genotype analysis. Four additional Swedish human *C. meleagridis* cases infected abroad were analyzed for comparison. *Cryptosporidium* oocysts were identified in 16 of 27 samples and in all sampled animal groups except cattle. By morphology, we found *C. meleagridis* in broilers, *C. meleagridis* and *C. galli* in hens, and *C. parvum*-like oocysts in pigs and sheep/goats.
Cryptosporidium meleagridis was identified in the human sample, broiler samples and two hen samples. Sequences were identical in human and bird 18S rRNA and HSP70 fragments. At the

18S rRNA locus, the samples belonged to genotype I. At the HSP70 locus, a new genotype was identified. All cases infected abroad belonged to 18S rRNA genotype I. At the HSP70 locus, two belonged to genotype 6, and two had unique sequences. *Cryptosporidium* pig genotype II was identified in one pig sample. To our knowledge, this is the first report of a human *C. meleagridis* case where the zoonotic source has been identified. We also identified new genotypes at the HSP70 locus.

IS IT GIARDIASIS OR IS IT IRRITABLE BOWEL SYNDROME?

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The parasitic infection with *Giardia lamblia* is common in our area. If not occult, the condition is clinically manifested by nonspecific dyspepsia and bowel movement disorders. The irritable bowel syndrome (IBS) is also common, having a prevalence of 10% in the general population or more. This is a functional disorders, whose symptoms may mimic giardiasis.
Recently, a certain degree of inflammation has been discovered in the intestinal mucosa of IBS patients. A clinical test for the detection of intestinal inflammation is the assessment of calprotectine in feces.
Inflammation is leading to sensitization and thus to the occurrence of digestive symptoms that have otherwise "unexplained" etiology.
We discuss the overlapping of giardiasis and IBS stressing on common pathogenic mechanisms. Both conditions have recurrent symptoms, thus suggesting that giardiasis may sensitize the gut and induce IBS symptoms.

PREVALENCE OF INTESTINAL PARASITIC INFECTIONS AMONG 1-10 YEAR-OLD CHILDREN IN NORTHWEST OF IRAN

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Intestinal parasitic infections are common infections in children throughout the world, especially in developing countries. The outcome of intestinal parasitic infections varies from growth retardation to severe anaemia in children. This study was conducted to estimate the prevalence of intestinal parasitic infections among 1-10 year-old children in Ardabil (a city in northwest of Iran) during past two years (from May 2010 to February 2012). In this descriptive and cross-sectional study, 6900 children were tested for intestinal parasitic infections in central laboratory of Bu-ali hospital in Ardabil. The stool specimens were collected and tested via two methods: 1- Direct method (by providing stained smears of feces specimens and microscopic diagnosis) and 2- Ether-Formalin method. Of 6900 studied children, 537 cases (7.78%) were positive for intestinal parasitic infections, 321 cases (4.65%) were infected by *Entamoeba histolytica*/*dispar* and 190 cases were positive for *Giardia lamblia infection* (2.75%). 4 specimens were positive for *Iodamoeba butschlii*, 4 others positive for *Blastocystis hominis*, 3 cases were diagnosed as *Entamoeba coli*, 4 cases as *Enterobios vermicularis* and only in one specimen we observed proglottids of *Taenia saginata*. We found a significant association between gender of children and *Giardia lamblia* infection (p=0.001).
In accordance with several epidemiologic and molecular studies in Iran, more than 90 percent of diagnosed isolates of *Entamoeba histolytica/dispar* are *E.dispar* and less than 10 percent are *E. histolytica*. Therefore from 4.65% positive casese for *E. histolytica*/*dispar*, 4.18% belonged to *E.dispar* and 0.47% for *E.histolytica*.Hence we concluded that the highest parasitic infection in our study belonged to *Giardia lamblia* (2.75%).
Keywords: Prevalence, Intestinal Parasitic Infections, Children, *Entamoeba histolytica*/*dispar*, *Giardia lamblia*.

TOXOPLASMA GONDII INFECTION IN WILD BOARS FROM NORTH-WEST OF ROMANIA

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Toxoplasmosis is one of the most important zoonotic diseases produced by *Toxoplasma gondii,* a protozoan parasite with worldwide distribution. The meat of wild boars (*Sus scrofa*) can be a source of *T. gondii* infection for humans. The aims of this study were (1) to evaluate the seroprevalence of anti-*T. gondii* antibodies, IgG type, in wild boars serum samples and (2) to estimate the risk of human contamination with *T. gondii* by consumption of undercooked meat. Pairs of samples, blood and heart tissues, from 256 wild boars were collected during the hunting season 2012 in Bihor county, North-West of Romania. The age of animals was between 3 months and 8 years old, and their weight was between 8 and 168 kg. The wild boars serum samples included in this study were collected from cruoric clots. All serum samples were tested by ELISA using ID Screen *Toxoplasmosis* Indirect Multi-species (ID.vet, France). The heart tissues in which serum samples were strongly positive for *T. gondii* were tested by bioassay. The seroprevalence of anti-*T. gondii* antibodies in wild boars was 72.3% (185/256). The seroprevalence obtained in females (72.5%; 103/142) was similar with the seroprevalence obtained in males (71.9%; 82/114). There were tested by bioassay 20 heart samples, which were inoculated in 40 mice (2 mice / tissue sample). At 4 weeks after inoculation, mice were sacrificed and their brain was tested by direct examination. *T. gondii* was observed from mice inoculated with two heart samples. From wild boars heart tissues we obtained two *T. gondii* isolates on mice. The highest seroprevalence obtained in our study showed that wild boars can represent a source of human infection with *T. gondii.*

SY23/3

OPPORTUNISTIC INTESTINAL PARASITES AND MALNUTRITION IN MADAGASCAR: HOW TO DESIGN STUDIES?

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Opportunistic intestinal parasites (cryptosporidia, microsporidia, etc.) were mostly described in Europe after emergence of the HIV outbreak but their prevalence fall down dramatically with effective antiretroviral tritherapies. Some of these parasites could affect immune-competent hosts and are described in travellers coming back from their trip with diarrhoea. In tropical countries prevalences of opportunistic parasites seem to be higher than in Europe, but are not well documented. Moreover, in tropical areas other causes of immune-depression can occur like malnutrition or tuberculosis. In Madagascar chronic malnutrition concerns 50% of children fewer than 5 years. Chronic malnutrition could induce immunodepression and opportunists have thus to be intensively researched in these children during diarrhea. To address this link between malnutrition and opportunists, we started studies on four pathogens: cryptosporidias, microsporidias, *Isospora belli* and *Cyclospora cayetanensis* in Madagascar. However the difficulty is to define a technical procedure usable i) to analyse large set of stools collected in the same time on the field, ii) sensitive, specific and at low cost and iii) which do not require trained personal. Quantitative PCR could be the best solution for epidemiological campaigns as automatic extraction of DNA can be done at the laboratory. However definition of primers and probes must take into

account the intra-species diversity of the gene targeted by the PCR and the large diversity of "exotic" species potentially pathogenic in tropical countries. Another pitfall in definition of PCR is the low number of genes potentially targeting due to the low number of sequences available to do multiple alignment. To define PCR for cryptosporidias we analysed rARN; ribosomal small sub-unit; COWP; actin; GP-60 for more than 60 species and strains available in GenBank. Very few sequences can be used to design pan-primers and these sequences harboured high similarity with human ones or with pathogens like schistosome. Moreover in tropical countries water and vegetables can be a huge source of parasites "in transit", which should not be detected. Overall we choose a three steps procedure for epidemiological studies: Q-PCR / microscopy / sequencing, using ribosomal small subunit sequences. In the same time we setup studies in two hospitals to target under-fed children. A third study was conducted on samples already collected to analyse causes of diarrhea in children leaving in the suburban area of Moramanga (Madagascar). Studies are still in process but associations of cryptosporidias and microsporidias have been already found in several patients.

PREVALENCE OF *TOXOPLASMA GONDII* IN WILD CERVIDS FROM ROMANIA

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Toxoplasma gondii is a parasite with a worldwide distribution that can affect many species of animals, domestic and wild. No studies were done regarding the epidemiology of *Toxoplasma gondii* infection in cervids in Romania and little is known about *T. gondii* in wild animals from our country. The aims of the study were to evaluate the seroprevalence of anti-*T. gondii* antibodies in wild cervids (*Cervus elaphus*) and to estimate the risk of *T. gondii* infection by consumption of meat from this animals. During the hunting season in 2012, there were collected 22 serum samples and heart tissues from wild cervids from North-West of Romania, Bihor county. The age of animals was between 4 months and 6 years old, and their weight was between 23 and 225 kg. Serum samples were evaluated by ELISA technique using a commercial kit, ELISA ID.VET - ID Screen Toxoplasmosis Indirect Multi-Species (ID VET Innovative Diagnostics, France), cut off 1:10. The overall prevalence of *T. gondii* antibodies was 68.2% (15/22). No difference between the prevalence rate of infection among females (9/13; 69.2%) and males (6/9; 66.7%) was observed. Heart tissues of the seropositive cervids were bioassayed in mice (2 mice / sample). After four weeks, the mice were killed and 2 isolates of *T. gondii* were obtained. Epidemiological results indicated a widespread exposure to *T. gondii* among wild cervids in Romania. This is the first evidence of *T. gondii* in cervids in Romania.

GIARDIASIS IN INFANTILE POPULATION FROM CLUJ COUNTY

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There are contradictory reports about the incidence of giardiasis in our country both in studies which appreciate this parameter in general population and those which present the incidence separately for adult or infantile population. The majority of the studies were made either on small group of subjects or following a short period of time. We perform an analytical study in children community (social assistance center) in 2012 and on infantile population of Cluj county and neighboring area on a three year interval (2008-2011), comprising a total of 18486 children. Each child was tested through the microscopic examination of three samples using the standardized O&P examination for intestinal parasites. The overall incidence of giardiasis was 0.41%, representing the major intestinal parasite (25.5% of all positive cases for parasites). In this large group of population we did not find statistically significant association between the gender or age group and giardiasis. However the majority of cases with giardiasis belong to small school age

children (7-14 years old) and were diagnosed during spring and summer months. In children from social assistance center we found a higher incidence of giardiasis (9.27 %) with 8.25 % being boys (statistic significant $p = 0.039$); all patients belong to school age group (7-18 years old). Half of the cases of giardiasis were associated with the presence of other protozoa and helminthes, the association with *Blastocystis* spp. being statistically significant ($p = 0.0028$). We did not find a statistically significant association between giardiasis and growth impairment in the subjects of our study. In both groups of children (disadvantage children community from social assistance center and general infantile population) the incidence of giardiasis was higher in school age boys but when appreciated in larger groups this association did not have statistic significance. The association between the presence of *Giardia duodenalis* and *Blastocystis* spp. had no statistic significance when appreciated in the general population of children.

SY21/1

UPSTREAM-DOWNSTREAM GRADIENT IN INFECTION LEVELS BY FISH PARASITES: A COMMON RIVER PATTERN?

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Physical habitat structure can influence the distribution and abundance of organisms. In rivers, a common process originating from the unidirectional water flow, stream drift, favours the displacement and downstream dispersion of invertebrates. This process could also generate a gradient in infection levels, leading to increasing numbers of parasites per host as one moves downstream from the river headwaters. We tested this hypothesis using four trematode species infecting the fish *Gobiomorphus breviceps* in the Manuherikia River (New Zealand). We performed generalized linear models of the abundance of each trematode as a function of distance from the river junction and fish size. We found support for the existence of a longitudinal gradient in trematode abundance along the river with an increasing upstream-to-downstream continuum, which is in agreement with riverine ecological theories in particular with the River Continuum Concept. This gradient applied to three out of the four trematode species studied, suggesting that it might be a common pattern in river populations. The exception was an avian trematode, the only species with an allogenic life cycle, for which host dispersal abilities may compensate downstream drift. Thus, a major process like drift in lotic systems, that influences the dynamics and distribution of invertebrate hosts, can also affect trematodes. Host properties like habitat preference, as well as parasite traits, particularly those related to transmission and the number of stages released in the water column, can interact with river conditions like flow rate, substratum type and biotic factors like primary productivity to influence the strength of the observed pattern or our ability to detect it. Baseline knowledge of infection patterns in rivers systems is needed in order to produce more accurate modelling of disease incidence and spread in freshwater ecosystems.

PARASITES OF FISH FROM LAKES NAIVASHA AND TURKANA, RIFT VALLEY, KENYA

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Lake Naivasha lies in the Kenyan Rift Valley between 00°45'S and 36°20'E and covers about 160km^2 of a closed basin at an altitude of 1890 m above sea level. It is the only freshwater lake in the Rift Valley without an outlet and is strongly influenced by humans using its water for the

surrounding flower farms and its fish as an important protein source. All the 7 fish species present in this lake were introduced. Since the year 2004, the common carp, *Cyprinus carpio* has been forming above 85% of the catch with up to 90t per year. On the other hand, Lake Turkana lying between 20° – 27° N and 40° N, with an area of 7,560km^2, it is the world's largest alkaline lake. It supports a rich lacustrine wildlife and is the second most important lake in its contribution to fisheries in Kenya after Lake Victoria. The lake has over 48 species of fish although only 12 are of economic importance. Several studies on parasites of fish from Lake Naivasha have been published so far, but to the best of our knowledge no reports of ecto-parasites exist, nor has the parasitic community of *C. carpio* from this lake been studied. For Lake Turkana, little parasitological work has been done mainly on description of new species of nematodes. Therefore it seemed of high interest to analyze the ecto- and endo-parasitic fauna of *C. carpio* and to include other fish species from Lake Naivasha in order to contribute to the existing body of knowledge. Between the period February and August 2011, 329 fish belonging to the species *C. carpio* (n=145), *Barbus paludinosus* (n=67), *Oreochromis leucostictus* (n=56) and *Tilapia zillii* (n=18) were collected from Lake Naivasha, and one species, namely *Hydrocynus forskahlii* (n=43) from L. Turkana and examined for protozoan and metazoan ecto- and endoparasites. For Lake Naivasha, the following protozoan parasites were recovered in all the fish but in low intensities: *Trichodina* sp., *Trichodinella* sp., and *Tetrahymena* sp. The most prevalent metazoan parasites from *C. carpio* were *Dactylogyrus minutus* (99.3%) and *D. extensus* (25.5%) and a yet to be identified monogenean (<1%) and *Tylodelphys* sp. (54.5%); from *O. leucostictus*, *Cichlidogyrus* spp. (91.1%) and *Tylodelphys* sp. (66.1%); from *T. zillii*, *Tylodelphys* sp. (83.3%) and *Cichlidogyrus* sp. (55.6%). For *B. paludinosus*, *Dactylogyrus* sp. (83.6%), metacestodes of *Amirthalingamia macracantha* (62.7%) and *Contracaecum* spp. (62.7%). From *H. forskahlii, Contracaecum* sp. (83.7%) and one yet unidentified dactylogyroid monogenean (79.1%). This study reports protozoans, monogeneans and Tylodelphys spp. among other parasites for the first time from Lake Naivasha.

PARASITE FAUNA OF COMMON CARP, *CYPRINUS CARPIO* L. 1758 IN A NATURAL CONSERVATION AREA IN SAMSUN, TURKEY AND ITS RELATION WITH HOST SIZE AND SEASON

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The common carp, *Cyprinus carpio* is a widespread freshwater fish of eutrophic waters in lakes and large rivers in Europe and Asia. The present study aimed to determine the parasitic fauna of common carp collected from Lower Kızılırmak Delta, a natural conservation area, in Samsun, Turkey. The investigation was conducted in the period from December 2011 to November 2012. A total of 156 fish specimens were examined for their ecto- and endoparasites. Fish were divided into 3 different size classes to determine any possible differences in infection values. Standard parasitological investigation methods were used and standard indices of infection (prevalence (%) and mean intensity MI) were applied. Fifteen different parasites species were identified; one species of ciliophora, *Trichodina acuta* (Lom, 1961); three species of monogenea, *Gyrodactylus cyprini* (Diarova, 1964), *Dactylogyrus extensus (*Mueller & Van Cleave, 1932), *D. chalcalburni* (Dogiel & Bychowsky, 1934); five species of digenea; *Ascocotyle* sp., *Petasiger* sp., *Diplostomum spathaceum* (Rudolphi, 1819), *Tylodelphys clavata* (Nordmann, 1832), *Tetracotyle* sp.; two species of cestoda; *Bothriocephalus acheilognathi* (Yamaguti, 1934) and *Caryophyllaeus laticeps* (Pallas, 1781); two species of nematoda; *Spiroxys contortus* (Rudolphi, 1819), *Contraceacum* sp. and two species of arthropoda; *Argulus foliaceus* and *Ergasilus sieboldi* (Nordmann, 1832). The existence of parasitic fauna in relation to different size classes of common carp and seasonal occurrences were also determined. The overall infection prevalence and mean intensity levels were 94.23% and 28.42 ± 2.77 parasites per infested fish, respectively. *Dactylogyrus* spp. *(D. extensus* and *D. chalcalburni)* were detected as dominant with their highest prevalence (81.21%) and mean

intensity (21.02 ± 2.33 per infected fish) values. Fish size had a statistically significant effect on parasite distribution (p<0.05) and a gradual increase in infection values was determined as the size of fish increased. Statistically significant differences in infection values according to seasons were also observed (p<0.05) and infection prevalence stayed above 90% in all seasons, winter having the highest level of infection. This research study on the parasite fauna of a commercially important fish species yielded valuable information in one of the most significant natural conservation area in Turkey.
Keywords: common carp, ectoparasite, endoparasite, Kızılırmak Delta, Turkey.

PARASITE FAUNA OF THE BLACK SEA WHITING, *MERLANGIUS MERLANGUS* L. 1758 AND ITS DYNAMICS IN RELATION WITH SOME HOST FACTORS

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The Black Sea whiting, *Merlangius merlangus*, is a commercially important food fish in the Black Sea, especially in Turkish region. In this research study, a total of 217 fish specimens caught in the Black Sea coasts in Sinop, Turkey and 181 fish specimens off Balaklava, Ukraine were investigated for their parasite fauna during 2011-2012. Fish were divided into 3 different length classes and their sex determined. Standard parasitological investigation methods were used and standard indices of infection (prevalence P%; mean intensity MI and abundance A) were applied. When calculating the mean intensity of infection, myxozoan and coccidian species were recorded in gradual definition. Eight parasite species were identified; they were *Trichodina domerguei* (Wallengren, 1897), *Eimeria merlangi (*Zaika, 1966), *Ceratomyxa merlangi* (Zaika, 1966), *Myxidium gadi* (Geogevitsch, 1916), *Gyrodactylus alviga (*Gaevskaya & Dimitrieva, 1967)*, Grillotia erinaceus* (van Beneden, 1858), *Prodistomum polonii* (Molin, 1859) and *Hysterothylacium aduncum* (Rudolphi, 1802). All the parasites mentioned were registered as near Sinop as off Balaklava except for *P. polonii* – this digenean was registered in the North part of the Black Sea only and was found for the first time from *M. merlangus*; Black Sea whiting believed to be an accidental definitive host for *P. polonii* (P=0.8%, A=0.006). *Hysterothylacium aduncum* was the core species among all with its highest P (91.2%) and A (41.79±10.03) value near Turkish coasts and P=40.5%, A= 1.99±0.52 in fishes from the north part of the sea. *Ceratomyxa merlangi* and *Myxidium gadi* were found either single or mixed infections in the gall bladder both in Sinop and Balaklava. P (%) for mixed infection was 11.98%, however, it was 20.74% for *Ceratomyxa merlangi* and 17.97% for *Myxidium gadi* in Turkish samples. On the other hand, prevalence (%) for mixed infection was 12.15%, however, it was 6.08% for *Ceratomyxa merlangi* and 27.62% for *Myxidium gadi* in Ukrainian samples. A gradual increase in infection prevalence was observed as the length of fish increased. Similar pattern was recorded for MI values for some parasite species, though not statistically significant (p>0.05). P (%) and MI values for each parasite species in relation to fish length were also determined. While male fish had higher infection prevalence values for most of parasite species than females in Ukraine samples, however, it was the reverse in Turkish samples. This research study financially supported by TÜBİTAK in Turkey and NASU in Ukraine yielded valuable information about the parasite fauna and their distribution on a commercial fish species at the Northern and Southern parts of the Black Sea.
Keywords: whiting, *Merlangius merlangus,* parasites, new records, Black Sea.

SY21/2

THE *GYRODACTYLUS* SPECIES COMPLEX INFECTING HOLARCTIC *SALVELINUS* SPP.

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We present an extensive review on gyrodactylids infecting holarctic *Salvelinus* spp., and utilize morphometric and molecular tools to study interrelationships between the Palearctic Arctic charr-complex infecting species *Gyrodactylus salvelini* and *G. birmani*, and the Nearctic species *G. salmonis*. Thus, this study includes all *Gyrodactylus* species currently described from *Salvelinus* except *Gyrodactylus bohemicus* Ergens, 1992 from *Salvelinus fontinalis* and *Oncorhynchus mykiss* (type host) from a trout farm in Kaplice, South Bohemia, Czech Republic. Both host species are introduced to Europe and unlikely the natural hosts of *G. bohemicus*. Thirty morphometric characters were assessed for *G. birmani* from Dolly Varden (*S. malma*) sampled from the type locality Azabachie Lake, Kamchatka, Russia, as well as for *Gyrodactylus* sp. from the lake Tyrifjorden, Norway, and the type specimens of the recently described *G. salvelini*, both collected from Arctic charr (*S. alpinus*). There was a substantial range overlap in many morphometric characters, but Principal component analysis (PCA) readily distinguished between the three populations. The ribosomal internal transcribed spacer (ITS) regions and the mitochondrial cytochrome oxidase I (COI) gene were used for phylogenetic analyses of the Arctic charr complex infecting species, *G. salmonis* from brook trout (*S. fontinalis*) and *G. lavareti*, *G. salaris*, *G. thymalli* infecting European salmonids. There was very little variation in the ITS sequences across the three charr infecting species indicating conspecifity. In contrast distance- and likelihood based phylogenetic inference using COI sequences revealed well-supported clades consistent with host species. *Gyrodactylus* populations infecting Arctic charr (i.e., *G. salvelini*) formed a basal sister group (average K2P group distance 0.028) to the more recently derived *G. birmani* on Dolly Varden and *G. salmonis* on brook trout (average K2P group distance 0.017). The currently available host-, morphological- and molecular information does not allow rejection of the hypothesis that the three species *G. salvelini*, *G. birmani* and *G. salmonis* are distinct. Assessing the phylogenetic findings in the light of the widely accepted host phylogeny with *S. alpinus* and *S. malma* being relatively young sister species within the *S. alpinus* species complex while *S. fontinalis* is a basal member of the genus, recent host switch events and subsequent allopatric speciation as the driving evolutionary forces in this *Gyrodactylus* species complex is most likely. However, extended geographical sampling, especially from areas where the host species occur sympatrically, and additional single copy nuclear markers will be needed to infer a more detailed phylogeographic history of this complex of charr-infecting gyrodactylids.

MUSEOMICS FOR ECTOPARASITES RECOVERED FROM HISTORICAL FISH COLLECTIONS – LESSONS FROM *GYRODACTYLUS*

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Gyrodactylus v. Nordmann, 1832 (Platyhelminthes; Monogenea) is a genus of viviparous ectoparasites infecting teleost fish species throughout the world. The ~450 described species are expected to only represent about 2% of the worldwide diversity. *Gyrodactylus salaris* is known as a major pathogen of Atlantic salmon in Norway which, apart from its economic importance, has imposed significant ecological burdens upon freshwater ecosystems. *G. salaris* may have evolved by a host shift from the related *G. thymalli* on grayling. Despite extensive research neither morphological nor molecular analyses have yet identified the origin of the host shift. To reconcile the host patterns with the complex *Gyrodactylus* phylogenies and to disentangle host-switches from co-evolutionary events is an ambitious task, particularly as human impacts caused regional

extinction of former abundant freshwater fish species. The impact of stocking on autochtonous fish populations is also an important issue in conservation biology. Historical changes in fish diversity are well documented in ichtyological collections, but so far very few attempts have been made to explore the ectoparasites unwittingly collected together with their hosts. We apply museomics approaches to fish ectoparasite diversity and we intend to add a temporal dimension to the understanding of the current distribution of *Gyrodactylus* in European watercourses. Until now gyrodactylids could be identified from historical fish material from the NHMs Vienna (Austria), Oslo (Norway), Paris (France) and Berlin (Germany). The research visits to the latter two collections were funded by the SYNTHESYS program. Most of the recovered parasites still allow for morphological species identification, e.g., *Gyrodactylus* specimens from salmon collected in 1876 and grayling in 1880 were identified as *G*. *derjavinoides* and *G*. *thymalli*, respectively. Furthermore, we have successfully amplified and sequenced the intergenic spacers of the nuclear ribosomal gene cluster and the mitochondrial cytochrome oxidase I gene from this material. Further gyrodactylids, most interestingly from *Salmo salar* e.g., collected in 1884 in Norway and 1877 in France, are soon to be identified. Trials in order to identify ideal protocols for DNA extraction and amplification from this difficult material are in progress.

INFESTATION OF GOBIID FISHES BY MONOGENEANS IN THE VISTULA RIVER BASIN, POLAND

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Four Ponto-Caspian gobiid fishes (Actinopterygii: Gobiidae) (monkey goby *Neogobius fluviatilis*, racer goby *N. gymnotrachelus*, round goby *N*. *melanostomus*, and western tubenose goby *Proterorhinus semilunaris*) were inspected for invasion by Monogenea in the Vistula river basin, Poland from June to July 2011. The aim of the present study was to compare infection of Monogenea from all available hosts. Monogeneans were found under the stereomicroscope, counted and mountered in glycerin-jelly or at 96% ethanol. Satistical data analysis was held in Quantitative Parasitology - version 3.0 (Rozsa et al., 2000). Gyrodactylus spp. was found on *N*. *fluviatilis* and *N*. *gymnotrachelus*. *N*. *fluviatilis*: Total number of hosts - 102. Prevalence 6.9%, Mean Intensity 2, 0; Median Intensity 1, 0; Var/mean ratio 4, 05 (calculated including uninfected hosts as well). Exact confidence limits for the population prevalence: 2.8 to 13.6% (95% confidence limits). Infestation of *N*. *fluviatilis* by gyrodactylids can be simulated by negative binomial distribution. The Index of Discrepancy (D) = 0.950, Chi square = 1.8997. Observed and expected frequencies do not differ significantly (at p=0.05), thus there is no statistical evidence to reject the null hypothesis (fitting the negative binomial distribution). Exponent of the negative binomial k = 0.059. This type of distribution indicates a stable nature of the relationships in the parasite-host system. *N*. *gymnotrachelus*: Total number of hosts - 136. Prevalence 16.9%, Mean Intensity 6.35; Median Intensity 1.0; Var/mean ratio 31.82. Exact confidence limits for the population prevalence 11 to 24% (95% confidence limits). D=0.944. Chi square = 18.9461. Observed and expected frequencies differ significantly (at p=0.05) thus the null hypothesis is rejected and k cannot be interpreted. (A similarly aggregated negative binomial distribution has k=0.062.).

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PARASITES OF NON-NATIVE GOBIIDS FISH IN THE WŁOCŁAWEK RESERVOIR ON THE LOWER VISTULA RIVER: FIRST STUDY IN POLAND

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Parasitological study of Ponto-Caspian gobies including monkey goby *Neogobius fluviatilis*, racer goby *Babka gymnotrachelus* and tubenose goby *Proterorhinus semilunaris* carried out over four years (from May 2006 to July 2010) in the Włocławek Reservoir on the lower Vistula River in Poland is presented here. Those fish species represent one of the most impressive invasions of European inland waters, connected with the spontaneous, east-to-west intracontinental movement observed in the last two decades. Parasite community of seasonally caught individuals (318 in total) consisted of 24 taxa. Typical for racer goby were species like: *Trichodinea domerguei*, *Diplostomum pseudospathaceum*, *Gyrodactylus proterorhini* and glochidia of unionids (three different species considered together). The list of parasites typical for monkey goby, compared with the racer goby, should be supplemented with two further species like: *Tylodelphys clavata* (met.) and *Eimeria* sp., while the glochidia detected rarely in this fish-host should be omitted. *Holostephanus* spp., *Apathemon gracilis*, *Diplostomum gobiorum* and glochidia predominated in the parasite community of tubenose goby. Contrary to species of the genera *Neogobius* the *Proterorhinus semilunaris* was rarely infected with *T. domerguei* and *G. proterorhini*. Long-term observations suggest three important determinants which affect the parasite community of Ponto-Caspian gobies in the studied area: i) habitat preferences of the fish species (glochidia), ii) Coexistence with a new, closely related invader (*A. gracilis*), and iii) Simultaneous growth of populations of different hosts, involved in the parasite life cycle (*Eustrongylides* spp.).

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THE OCCURRENCE AND PARASITIZATION OF THE INVASIVE PONTO-CASPIAN GOBIIDS IN THE VISTULA RIVER BASIN, POLAND

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Four non-indigenous Ponto-Caspian gobiid fishes (the monkey goby *Neogobius fluviatilis*, the racer goby *N. gymnotrachelus*, the round goby *N. melanostomus*, and the western tubenose goby *Proterorhinus semilunaris*) inhabiting the Vistula River basin, Poland, were examined for parasites. The field studies were carried in the Bug River, the Bugo-Narew region (the Zegrze Reservoir and the lower Narew River), the Włocławek Reservoir, Lower Vistula River, and the Vistula Delta. The racer goby and the monkey goby occurred in all the inspected localities, while the western tubenose goby was not registered in the Bug River and the Bugo-Narew, and the round goby occurred only in the Vistula Delta. 18 and 17 parasite species occurred in the racer goby and the monkey goby, respectively; the parasite fauna of the tubenose goby consists of 10 parasite species, the round goby consists of six species. For the monkey goby the richest parasite fauna was found in the Bugo-Narew (twelve species), but the poorest in the Vistula Delta (five species). For the racer goby the richest parasite diversity was recorded in the Bugo-Narew (thirteen species), but it was poorest in the Vistula Delta (three species). The maximum diversity of parasites infecting tubenose goby was registered in the Włocławek Reservoir (nine species), but it was reduced to one species in the Vistula Delta. Generally, the parasite diversity of Ponto-Caspian

gobies inhabiting the Vistula Delta was reduced in comparison to the other investigated localities. According to the Sørensen Index, the highest similarity of the monkey goby parasite fauna was between the Bugo-Narew and the Lower Vistula (76. 2%) and between the Bugo-Narew and the Włocławek Reservoir (70%), but the lowest between the Bug River and the Włocławek Reservoir (16. 6%) and no similarity between the Bug River and the Vistula Delta. The highest similarity of the racer goby parasite fauna was between the Bugo-Narew and the Włocławek Reservoir (78. 3%) and between the Lower Vistula and Vistula Delta (75%), but the lowest between the Bug River and the Włocławek Reservoir (23. 5%). So, the similarity between the Narew and the Włocławek Reservoir was rather high in both gobiids. The similarity between the parasite fauna of the tubenose goby in different regions was low (20-40%).

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SY01/1

FATTY ACID-COA LIGASE (ACL) AS A NOVEL DRUG TARGET IN *GIARDIA DUODENALIS*

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Based on the genome sequences, *Giardia* lacks the capacity to synthesize fatty acids de novo. However, it possesses at least 5 fatty acid-CoA ligases (ACLs) that are responsible to activate fatty acids to form fatty acyl-CoAs. Our major goal is to characterize the biochemical features of *Giardia* ACLs and explore them as novel drug targets. We have cloned and expressed two *Giardia* ACLs (GiACL1 and GiACL2) as recombinant proteins for functional analysis.
Both GiACL1 and GiACL2 could use C16 palmitic acid but GiACL1 has relative low activity. The GiACL2 uses C16 palmitic acid with Km=9.4μM and Vmax=2.7μmol/min/mg. Its activity could be inhibited by the inhibitor triacsin C (IC 50 =2.5μM). These ligases are able to use medium to long chain fatty acid. The 5 *Giardia* ACL genes have different expression profiles in the trophozoites and cysts, and during encystation and excystation, suggesting that they may play different roles in the parasite. Our ongoing drug assay show that ACL inhibitors could inhibit the growth of *Giardia* and development, which confirms that ACLs may truly serve as drug targets in the parasite.

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PREVALENCE OF GIARDIOSIS IN CHILDREN SOCIETIES AND RISK FACTORS

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Giardiasis is an intestinal parasitosis (parasite zoonozis) that affects humans and other vertebrates, produced by the flagellated protozoa *Giardia* spp. In the current document, the objective has been to analyze the giardiasis spreading through across children societies from

Oradea and evaluation of some intrinsic risk factors (age, sex) and extrinsic (environment, drinking water, toilets) on developmental stage. The incidence of giardiasis has been observed on children from 2 kindergartens and one Placement Centre for Children with Handicaps from Oradea, Bihor County. A total number of 116 fecal probes have been taken. The copro-parasitic test has been made with Willis and modified Blagg method within the Faculty of Veterinary Medicine of Cluj-Napoca and the laboratory from the Infectious Diseases Clinic of Oradea. Prevalence of the *Giardia* spp. infection in the 3 mentioned institutions has been 30.33%. From the total of studied children, the highest prevalence of *Giardia* spp. parasite has been recorded in the G2 kindergarten, namely 29 cases (58%). Infection with *Giardia* spp. parasite has been higher on male cases than on female cases (32.73% versus 31.15%) but without any significant differences. From the current study it has been observed that the strongest risk factors for *Giardia* spp. infection on children are: bottled water (RR=2.062), presence of some allergic manifestations and previous history of *Giardia* spp. infection (RR=1.824). Existence of personal previous pathologic history also represents a risk factor for the *Giardia* spp. infection (RR=1.499) and association of other diseases increases this risk (RR=1.683). Age below 5 and presence of pets represent the lowest risk (RR=1.234, respectively RR=1.179).

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SY02.P.01

HUMORAL RESPONSE TO THE C-TERMINAL CONSERVED REGION OF THE MUCIN-ASSOCIATED SURFACE PROTEIN (MASP) FAMILY OF *TRYPANOSOMA CRUZI*

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Trypanosoma cruzi is the etiological agent of Chagas' disease, or American trypanosomiasis, a neglected tropical disease that is endemic to South America but has been spreading throughout the world over recent years due to increasingly extensive human migration. The MASP multigene family is specific to *Trypanosoma cruzi* and accounts for approximately 6% of the parasite's genome. Its proteins bind to the parasite membrane at GPI anchor sites and are highly expressed during its infective trypomastigote phase. The N- and C-terminal regions of these proteins are much conserved. We have studied the humoral response to the conserved C-terminal region and via a comparative sequence analysis of this region have found a high degree of conservation, both amino-acid and physico-chemical, in phylogenetically distant strains such as PAN4 (DTUI) and CL-Brener (DTUVI). By mapping the avidity of overlapping peptides of this region using a pool of human positive sera, we have been able to demonstrate the existence of a positive response throughout the entire region (30 a.a.), with peptide C5 (positions 13 to 28) showing the greatest degree of avidity. These data were collated with sera from patients suffering from chronic Chagas' disease in Chile and Panama, in which considerable differences in both the sensitivity and specificity of the response were observed. The kinetics of IgM and IgG antibodies to the MASP C-terminal region in response to infection of Balb/c mice with *T. cruzi* showed a rapid, high response for IgM but an insufficient switching to the IgG isotype. These results confirm the existence of a humoral response against the C-terminal region, and suggest that these types of protein may well favor the maturing of B cells in response to infection with Chagas' disease.

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SY02.P.02

DISTINCTIVE MODULATION OF CYTOKINE PRODUCTION IN ORAL AND INTRAPERITONEAL *TRYPANOSOMA CRUZI* INFECTION TRIGGERED BY CL AND Y STRAINS

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Several studies have demonstrated a high capacity of infection by metacyclic trypomastigotes with majority vector prevalence in some regions. Oral infection has caused frequent outbreaks of Chagas' disease arising from the ingestion of contaminated food. However little to nothing is known about the immunopathology comparing these two different routes of infection with strains of high (Y) and low virulence (CL). The aim of this study was to assess of concentrations of IFN-γ and IL-10 produced in Wistar rats infected orally and intraperitoneally with different strains of *Trypanosoma cruzi*, Y and CL. Wistar rats were intraperitoneally infected (i.p.) ($1x10^5$; *n*=10) and orally infected (i.o.) ($8x10^5$; *n*=10) with Y and CL strains of *T. cruzi*. Metacyclic trypomastigotes infective forms were used. Cytokines were measured in the serum of these animals at the peak of the infection for each strain. For determination of IFN-γ and IL-10, the R&D Systems kit (Minneapolis,MN, USA) was used. All samples were analyzed separately twice. The i.p infection with the Y strain showed a higher concentration of INF-γ when compared with orally infected

groups (p<0.01). On the other hand the animals orally infected with CL strain showed an increase of IFN-γ compared to the group orally infected with Y strain (p<0.05). The infected intraperitoneally groups with the Y strain triggered higher concentrations of IL-10 (p<0.01) and animals infected orally with the Y strain showed a significant increase of IL-10 compared to oral infection with strain CL (p<0.05). In intraperitoneal infection Th-1 inmune response induced by Y strain was significantly pronounced when comparing with CL strain. The Th-1 immune response in oral infection by CL strain was significantly accentuated when comparing with Y strain.

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SY02.P.03

ANTI-INFLAMMATORY PROTECTIVE ACTIONS OF MELATONIN AGAINST HEART DAMAGE DURING THE CHRONIC PHASE OF CHAGAS' DISEASE

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The inflammatory answer caused by the infection with *T. cruzi* plays an important role for the host, with respect to both resistances against the infection as well as the evolution of the Chagas' disease chronic phase. The plasmatic levels of the biochemical marker creatine kinase-MB (CK-MB) allow measuring the severeness of the lesions of the myocardial muscle. In this context, the transformation factor of the growth β (TGF-β) can be considered as an important regulator of the inflammation, controlling the production of the dose rates of Nitric oxide (NO) and preventing further pathologies that are mediated by an exacerbated immunological answer. Melatonin is a neuro-hormone, derived from the aminoacid tryptophan which is being produced mainly by the gland Pineal. It (melatonin) displays immunomodulatory antioxidant and anti-inflammatory properties. We evaluated the effects of Melatonin on the chronic cardiac inflammatory process of the Chagas' disease. This was done by analyzing the levels of CK-MB, TGF- β and NO of Wistar rats infected by $3x10^5$ forms of the Y strain *T. cruzi*. We worked in groups consisting of 5 male Wistar rats each, with a weight of 250g each individual. They were divided as follows: Not infected control (NIC), Not infected and treated with Melatonin (NITM), infected control (IC), infected and treated with Melatonin (ITM). After a span of 60 day post-infection, the animals were treated orally with a solution containing Melatonin dissolved in polyethylene glycol 400 and distilled water 1:1. The same experiments were undertaken 90, 120 and 180 days after infection. Then the rats were decapitated after being anesthetized. We quantified the NO produced by cardiomyocytes using the Griess reaction. The concentration of TGF- β and CK-MB was determined examining cardiac tissue samples and serum of the animals, using specific kits. The animals treated with melatonin showed a significant reduction in the concentration of inflammatory mediators and plasmatic levels of CK-MB - p<0.05. Considering the reduction of the inflammatory process and cardiac damage, the above results suggest a protective effect of Melatonin.

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SP03.P.01

LIPIDOMIC OF *LEISHMANIA DONOVANI* AND *LEISHMANIA INFANTUM*: FROM PROMASTIGOTE TO AMASTIGOTE

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Leishmaniasis is a disease caused by a number of species of protozoa of the genus Leishmania. There are four major clinical types of this infection: cutaneous, diffuse cutaneous, mucocutaneous and visceral leishmaniasis. *Leishmania donovani* and *Leishmania infantum* are the causative agents of visceral leishmaniasis (VL) in humans and canine leishmaniasis in dogs. The disease, affecting both children and adults, typically presents fever, hepatosplenomegaly and pancytopenia. Of all forms of leishmaniasis, VL is the most severe one, with 500,000 new cases and approximately 50,000 deaths annually.
The *Leishmania* life cycle is divided into two phases, each of them involving a different stage: the promastigote in the insect vector and the amastigote in the vertebrate's macrophages. The promastigotes inoculated during the blood meal of the hematophagous sand fly are phagocyted by endocytosis and undergo a transformation into the amastigote stage within a parasitophorous vacuole of phagolysosomal origin.
We are very interested in the lipids and their implication in the infestation with *L. donovani* and *L. infantum*. Our goal is to analyze the variations of different classes of lipids that the parasite needs at the time of the passage of the form promastigote to the form amastigote. We quantified the phospholipides, the triglycérides, the cholesterol esters, the free fatty acids and the cholesterol, in the promastigotes and amastigotes forms.
The promastigotes forms are cultivated axenically and obtained massively for the different analyses. The amastigotes forms are extracted from macrophages after their infestation. For that, we experimentally infest macrophages J774 and we extract the amastigotes after cells breaking by passage in syringe Hamilton (diameter 22 G) and we purify them by a series of centrifugations and filtrations.
Lipids Extraction was done using the method of Bligh and Dyer, Separation of the lipides by chromatography on thin layer. The samples are analyzed by gaseous chromatography, and derivation of the cholesterol was analyzed by mass spectrophotometry.
Our results showed that transformation of the promastigote into the amastigote stage is correlated with enrichment in phospholipids, free fatty acids and cholesterol. Triglycérides and cholesterol esters decrease in the amastigotes forms. The cholesterol is the more solicited by the parasite during his enter into the host cell. We suggest that parasite transform ergosterol into cholesterol and use it for the formation of the parasitophorous vacuole.
We intend to further investigate the role of these lipids in the infectivity of *Leishmania* and their potential use as therapeutic targets.

SY03.P.02

COMPARISON OF THREE ANTIGEN-BASED ELISAS IN THE DIAGNOSIS OF MEDITERRANEAN VISCERAL LEISHMANIASIS

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Current methods for diagnosis of visceral leishmaniasis (VL) are based on parasitological examination of bone marrow aspirates, PCR assays and specific IgG detection. Enzyme-linked immunosorbent assay (ELISA) is one of the most used techniques in VL serological diagnosis. But, the biggest challenge remains the use of antigens with high sensitivities and specificities. The aim

of this study was to compare ELISA's performances in Mediterranean VL diagnosis, using 3 different antigens: Crude *Leishmania* Histone (CLH), recombinant K39 antigen (rK39) and Soluble *Leishmania* antigen (SLA).
One hundred and twelve sera, obtained from 42 confirmed VL Tunisian patients and 70 control subjects, were tested by the 3 ELISAs. CLH antigen was obtained following a standard approach of histone acid extraction, rK39 was kindly provided by the Infectious Disease Research Institute, Seattle, WA and SLA was prepared as described elsewhere.
Statistical analysis was performed by the MedCalc Statistical software (version 11.4.4.0). The Receiver Operating Characteristic (ROC) curves were used to analyze the diagnostic performances of each test in discriminating patients with VL from those without and to assess the sensitivities and specificities of all diagnostic assays. Analysis of the areas under the ROC curves (AUC) allowed the comparison of performance characteristics of one test with those of another.
CLH and rk39-based ELISA showed an excellent ability to discriminate between VL cases and healthy controls (sensitivities of 97.6 % and specificities of 100 and 97.1% respectively). SLA-based ELISA, however, was less accurate (sensitivity and specificity of 85.7 and 90% respectively).On the other hand, ROC curves analysis demonstrated that there was no statistical difference between AUCs of CLH-based ELISA and rK39-based ELISA ($p= 0.6$). However, there was a significant difference between AUCs of CLH-based ELISA and SLA-based ELISA ($p<0.05$). These results suggest that CLH-based ELISA has a similar performance in discriminating VL cases from healthy controls compared with rK39-based ELISA and a better one compared with SLA-based ELISA, making of CLH a promising antigen for clinical use.

SY03.P.03

CLINICAL CRITERIA AND PCR-RFLP IN THE IDENTIFICATION OF TUNISIAN CUTANEAOUS LEISHMANIASIS FORMS AND *LEISHMANIA* SPECIES INVOLVED

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Three cutaneous leishmaniasis (CL) forms are endemic in Tunisia: sporadic CL (SCL) due to *Leishmania* (*L.*) *infantum*, zoonotic CL (ZCL) due to *L. major* and chronic CL (CCL) due to *L. tropica.* Recent spreads of the geographical distribution of these forms have induced the coexistence of more than one *Leishmania* species in some foci complicating the cases characterization. The identification of the causative species is useful to complete the epidemiological data and to manage the patients.
The aim of this study was to establish some clinical criteria associated to the CL forms and to compare PCR-RFLP and iso-enzyme analysis, the reference test, in identifying *Leishmania* strains.
One hundred and twelve CL biologically confirmed cases were involved in the study. Data concerning the number, the location, the morphologic aspect and the month corresponding to the outbreak of the lesions were noted for each patient. Identification of *Leishmania* species was performed by both iso-enzymatic typing (for positive cultures) and RFLP after HaeIII enzyme amplicon digestion.
The iso-enzymatic analysis (48 isolates) and the PCR-RFLP digestion profiles (97 strains amplicons) allowed the identification of the 3 *Leishmania* species endemic in Tunisia: 72 *L. major* (64.3% of ZCL cases), 26 *L. infantum* (23.2% of SCL) and 14 *L. tropica* (12.5% of CCL). Thirty three isolates were characterized by both techniques and results were concordant in all cases. Species identification also correlated with the classical geographical distribution of CL forms in Tunisia.

Sporadic CL lesions seemed to break out later than those of ZCL (53.8% of cases appeared from December vs 23.6%, p<0,001). Zoonotic CL lesions were often multiple (75%) and limb-situated (84.7%, p<0,001) whereas those of SCL were single (92.3%, p<0,001) and face-situated (84.6%, p<0,001). CCL lesions were also single (78.6%) and face-situated (71.4%). The classical ulcerous presentation with scabs was mainly observed in ZCL cases (69.4%) and the erythematous presentation was more described in SCL cases (75%, p<0,001).
In conclusion, the number, the location, the morphological aspect and the lesions outbreak month could be considered as interesting criteria that help to differentiate between the 3 noso-geographical CL forms prevailing in Tunisia. PCR-RFLP assay, directly performed on skin samples, appears as an interesting alternative to iso-enzyme analysis for the identification of *Leishmania* strains involved.

SY03.P.04

IN VITRO LEISHMANICIDAL ACTIVITY OF A NEW BENZIMIDAZOLE DERIVATIVE COMPLEXED WITH METHYL-BETA-CYCLODEXTRIN

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The biological activity of 5,6-dichloro-2-(trifluoromethyl)-1H-benzimidazole (G2), an antiprotozoan agent with poor aqueous solubility, alone and complexed with methyl-beta-cyclodextrin (G2/MβCD) were compared in 2 *in vitro* model systems: promastigotes and intracellular amastigotes of 4 *Leishmania* species: *Leishmania amazonensis*, *Leishmania braziliensis*, *Leishmania guyanensis* and *Leishmania infantum.* Previous studies have shown that with methyl-beta-cyclodextrin a typical host-guest system was obtained with enhanced water solubility. Here, when tested against promastigotes the G2/MβCD complex was significantly less active than the uncoupled compound after 24 hr contact (IC_{50} ranging from 2.86 to 7.22μM for G2 against *L. guyanensis* and *L. infantum,* respectively and from 34.58 to 47.41 μM for G2/MβCD against *L. guyanensis* and *L. amazonensis*, respectively) although this difference was markedly decreased following 48 hr incubation (IC_{50} from 14.82.to 19.25 μM for G2/MβCD, respectively). For both formulations the antiparasitic activity decreased when they were tested against intracellular amastigotes with regard to promastigotes (IC_{50} 15.79 μM and 59.34 μM for G2 and G2/MβCD, respectively against *L. infantum* and 9.00 μM and 72.04 μM, respectively against *L. amazonensis*). However by complexing in MβCD the cytotoxicity of G2 was significantly decreased (CC_{50} rose from 43.56 μM to 323.42 μM) thus improving safety against each of the 4 *Leishmania* species). MβCD alone had no any biological activity. Complementary confocal studies confirmed β-tubulin as the main target for G2 and for its MβCD complex.
These results lead as to conclude that although increased solubility does not improve G2 parasiticidal activity its pre-formulation as MβCD complexes may be suitable for improvement of pharmacological safety of this benzimidazole compound.

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SY03.P.05

LYMPHOPROLIFERATIVE RESPONSE, PRE AND POST-INFECTION, IN BALB/C MICE IMMUNIZED WITH THE RECOMBINANT CHIMERIC PROTEIN L25A-HSP70M2 OF *LEISHMANIA BRAZILIENSIS*

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The aim of this study was the analysis of the lymphoproliferative response of BALB/c mice immunized with the recombinant chimeric protein, consisting of the N-terminus of the protein L25 (L25a, 1-68aa) and amino domain fragment of HSP70 of *L. braziliensis* (HSP70M2, 240-357aa), named L25a-HSP70M2 and cloned into the expression vector pQE30 (Qiagen). The protein expressions were performed in *E. coli* Topp3 and purified in native conditions. Three groups of 10 mice/group were immunized 3 times: a group with 5 μg/dose, another with 20 μg/dose and a control group with PBS, subcutaneously without adjuvants. We performed the study of the lymphoproliferative response at different times of the assay against each protein (L25a, HSP70M2 and L25a-HSP70M2) through stimulation of splenocytes from BALB/c mice immunized, before and after infection with 10^3 metacyclic promastigotes of *L. amazonensis*. The results showed that the lymphoproliferative response before the infection were higher in the groups immunized with 5 and 20 μg/dose of L25a-HSP70M2, with stimulation index (SI) at 2-4 respectively. At 4 and 6 months post-infection (mpi), the SI of mice group immunized with 5 and 20 μg/dose and infected, were similar than the uninfected group. At 6 mpi we observed an increase in the SI of splenocytes from the group immunized with 20 μg/dose and infected, against the same group of mice uninfected.

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SY03.P.06

CYTOKINE PROFILES, HISTOPATHOLOGY AND PARASITE LOAD OF EXPERIMENTAL CANINE LEISHMANIASIS

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Leishmaniasis, caused by *Leishmania infantum*, is an endemic zoonosis in the Mediterranean basin and South America. Dogs are the main hosts for these parasites. Clinical presentation of canine leishmaniasis (CanL) depends in most part on the host's immune responses. Some data suggest that asymptomatic dogs develop Th1 profile whilst symptomatics present Th2 profile. Although it is known to be a severe systemic disease, studies based upon histopathological analyses of distinct host compartments of animals infected and their relationship with cytokine and parasite load are unfrequent. The aim of this work was to evaluate the presence and quantification of parasites, expression of IFN-γ, TGF-β, TNF-α, IL-10, IL-4, iNOS mRNA and histopathological alterations in the different organs/tissues in twelve experimental infected dogs with *L. infantum.*
Parasites were detected in the spleen (SP), liver (LV) and skin (SK) of all twelve dogs, in bone marrow (BM), lymph node (LN) aspirates and buffy coats of eleven, being spleen the most parasitized organ by qPCR. In general, the higher levels of cytokine expression were observed in BM and LN, namely IL10; TGF-β was the most expressed in BM and blood while TNF-α, IFN-γ,

iNOS were detected in different tissues in low number of animals. Both Th1 and Treg cytokine responses occur during CanL without clear dominance in any direction. At necropsy, splenomegaly was the only macroscopic alteration observed in twelve infected dogs. Eight spleen samples showed large numbers of confluent granulomas, mainly formed by epithelioid type cells, replacing the red pulp. Liver was the third more parasitized organ and samples of all animals showed marked periportal infiltration with mononuclear cells. A high number of small granulomas were identified randomly dispersed in the parenchyma. Half of the mesenteric lymph nodes presented hyperplasia of the cortical follicles and discrete edema of the medular region. The histopathology of the skin showed focal perifollicular dermatitis and, occasionally, sebaceous adenitis. An intermediate parasite load was also observed in this tissue and no cytokine expression was detected.
From this work it can be concluded that both Th1 and Treg cytokine responses occur during CanL without clear dominance in any direction. Furthermore, LN and BM can be considered as suitable clinical samples to detect the presence of *Leishmania* parasites and cellular immune response to infection. The identification and characterization of pathological changes and immune responses associated with disease progression would be useful for the development of new diagnostics, drugs and vaccines.

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SY03.P.07

IN VITRO BIOLOGICAL BEHAVIOR OF *LEISHMANIA INFANTUM*, *L. MAJOR* AND *L. INFANTUM/L. MAJOR* HYBRIDS FROM MEDITERRANEAN BASIN

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Leishmaniasis is one of the most prevalent neglected diseases in the world and visceral leishmaniasis (VL) is the most severe clinical form and fatal if untreated. Dogs are the major reservoir hosts of *L. infantum* parasites in the Mediterranean region, and responsible for transmission to humans. *L. major* and *L. tropica* are the main aetiological agents of cutaneous leishmaniasis (CL) in the Old World. Although *Leishmania* are considered parasites which have a clonal structure, recombination between different species has been detected, as shown by the existence of natural hybrids between closely related species from the New and Old World. In 2006, hybrids from two genetically distant species, *L. infantum/L. major*, were identified for the first time from human cases in Portugal. In addition, increased transmission potential of one of these hybrids was previously demonstrated in a colonized *L. major* vector (*Phlebotomus papatasi*). These findings raise questions concerning the importance in pathogenicity, diagnosis, therapeutics and epidemiology of hybrid strains.
The aim of the present study was to evaluate biological behaviour, by *in vitro* growth kinetics and inhibitory effect in the presence of the reactive oxygen species (hydrogen peroxide, H_2O_2), of *L. infantum/L. major* hybrids isolated in Portugal in comparison with *L. infantum* and *L. major* strains.
The results showed that hybrid strains present high parasite densities in comparison with the putative parental strains. In addition, IC50 of hybrids was superior than their parental strains, when submit to H_2O_2 presence. These results suggest that hybrids seem to have greater resilience to adverse conditions such as the toxic effect of reactive oxygen species.
Other studies are in progress in order to contribute to the knowledge of novel phenotypic traits of hybrids and their selective advantages.

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SY03.P.08

IN VIVO EVALUATION OF THE LEISHMANICIDAL ACTIVITY OF URSOLIC ACID FROM *ERICA* SPP.

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There is a greater interest in new drug developments from traditionally used medicinal plants which offers a large diversity in structures and bioactivity.
The genus *Erica* (Ericaceae) is represented by more than 700 species in the world, and mainly found in the South Africa, furthermore Mediterranean and West Europe. Ursolic acid previously isolated from several *Erica* spp was evaluated *in vitro* on *Leishmania* promastigotes. In the present study we evaluated its efficacy in a hamster model for chronic visceral and cutaneous leshmaniasis.
Extraction of leaves and flowers from *Erica* was done using solvents as dichloromethane, ethyl acetate, butanol and methanol. Ursolic acid, the main compound, was isolated by column chromatography on silica gel with hexane-dichloromethane-methanol.
In the chronic visceral model of leishmaniasis, each hamster was infected with $5x10^6$ promastigotes of *L. infantum* given by the intracardiac route. Treatment started on day seventy-five post-infection and lasted for seven continuous days. The animals were dosed once daily with ursolic acid at 5 mg/kg administered by the intraperitoneal route in 0.1 ml final volume of saline solution. Parasite burden was estimated by the limit dilution assay in target organs (spleen and liver).
In the chronic cutaneous model, 1×10^7 *L. amazonensis* promastigotes were injected subcutaneously in the footpad of hamsters. From week 5^{th} up to week 9^{th} post-infection each animal was topically treated once daily with a cream wearing 0.2% ursolic acid. The lesion size was measured every week for 15 weeks.
In the chronic visceral model treatment reached 42% and 79% reduction of parasite burden in spleen and liver respectively with regard to untreated controls.
In the chronic cutaneous model the topical administration of the ursolic acid based cream produced a significant decrease in lesion size (15%), achieved during the treatment period. Following treatment this curative effect was amplified up to 25% decrease in lesion size with regard to control.
These findings suggest that ursolic acid may constitute a promising candidate as a lead compound in the development of new drugs against both cutaneous and visceral leishmaniasis.

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SY03.P.09

ASPARAGOPSIS TAXIFORMIS: A NOVEL ANTI-*LEISHMANIA* THERAPY?

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Marine macroalgae produces a wide variety of remarkable natural compounds, usually referred as bioactive metabolites. *Asparagopsis taxiformis* is a red alga from the Strait of Messina (Italy) and in the Mediterranean Sea that produces chemicals that have potent biological effects. Numerous natural products, including halogenated compounds, methanes, ketones, acetates and acrylated were described as produced by the genus *Asparagopsis*. Natural compounds revealed antiprotozoal activity against *Leishmania*, parasite which cause a variety of diseases, known as Leishmaniasis. Leishmaniasis is a vector-borne disease caused by obligate intramacrophage protozoan parasite of the genus Leishmania and its incidence is increasing in non-endemic areas due to changing patterns of international travel and to population migration; it is a disease with a worldwide distribution, especially in many tropical and sub-tropical countries, affecting both humans and animals. The aim of this study was to analyze the toxicity of algal compounds against *in vitro Leishmania infantum* cultivation in a novel RPMY-PY medium. The authors role out a number of experiments demonstrating the toxicity of some chemical extract from the *Asparagopsis taxiformis*. $1x10^6$ *Leishmania infantum* promastigotes were plated into 25 cm^2 flasks containing medium supplementing with FCS (10%) and treated with scalar concentration of compounds. The percentage of apoptotic Leishmania was determinate by morphological examination using a fluorescence microscope after ethidium bromide and acridine orange staining. Among compound analyzed, Pentadecane, Heptadecanoic Acid and the synergy between Linoleic Acid and Linolenic Acid have showed an interesting activity against promastigotes in vitro cultivation, revealing such algae as a great source of natural antiprotozoal products.

SY03.P.10

ANALYSIS OF HOMOLOGY BETWEEN THE ONLY KNOWN NUCLEOSIDE TRANSPORTER FROM *LEISHMANIA BRAZILIENSIS* AND OTHER HIGHER EUKARYOTES

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The genus *Leishmania*, among others, belongs to the family Tripanosomatidae. This parasite is transmitted by the diptera females of the genus *Phlebotomus* and *Lutzomyia*. These protozoa are auxotrophic for purines, and rely for their purine supply on salvage from the host environment. The nucleosides are uptake using integral membrane proteins, named nucleosides transporters.
The aim of this study was to analyze the sequence homology between the only known nucleoside transporter from *L. braziliensis* with other other higher eukaryotes (mouse and human), using different bioinformatic tools like Blast, TMHMM, ESPript 2.2, MEGA5.
The results showed that the nucleoside transporter from *L. braziliensis* belongs to the family of equilibrative transporters (ENT type). The highest identity was observed between mouse and human transporters (73-88%), while not exceeded 21% between *L. braziliensis* and higher eukaryotes. Most of changes in the nucleotide sequence are transitions with regard to transporters of higher eukaryotes, which allow to generate changes in the amino acid sequence.We only found the amino acid Y (tyrosine) which coincides in the same position of the first external loop to align all sequences with ClustalW2. In the other sequences, of the outer and inner loops, there is no match.

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SY03.P.11

NUCLEOSIDE TRANSPORTER FROM *LEISHMANIA BRAZILIENSIS*. COMPARATIVE ANALYSIS OF SEQUENCES BETWEEN THIS PROTOZOA AND OTHER TRYPANOSMATIDS

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Parasitic protozoa of the genus *Leishmania* are the etiological agents of leishmaniasis, a disease that affects an estimated 12 million peoples worldwide. This parasite is transmitted by the diptera females of the genus *Phlebotomus* and *Lutzomyia*. A nucleoside transporter is an indispensable protein for the trypanosomatids, because they are auxotrophs for purines. That is, probably, the most striking metabolic discrepancy between these parasites and their hosts. Whereas most mammalian cells synthesize purines *de novo*, all parasitic protozoa studied to date are unable to synthesize purines.
The aim of this study was to analyze the sequence homology between the only known nucleoside transporter from *L. braziliensis* with other other trypanosomatids, using different bioinformatic tools like Blast, TMHMM, ESPript 2.2, MEGA.
The results showed that the nucleoside transporter from *L. braziliensis* belongs to the family of equilibrative transporters (ENT type). The highest identity was observed between *L. major* and *L. infantum* (92%), while not exceeded 28% between *L. braziliensis* and other trypanosomatids. Most of changes in the nucleotide sequence are transversions with regard to transporters of trypanosomatids, which allows to generate changes in the amino acid sequence. We only found the amino acid Y (tyrosine) which coincides in the same position of the first external loop to align all sequences with ClustalW2. In the other sequences, of the outer and inner loops, there is no match.

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SY03.P.12

POLIMERASE CHAIN REACTION AND REAL TIME PCR FOR DIAGNOSIS OF INFECTION IN DOGS BY *LEISHMANIA INFANTUM* USING DIFFERENT BIOLOGICAL SAMPLES

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Visceral leishmaniasis (VL), also called to calazar, is an illness of zoonotic character that has great importance for the Public Health, for presenting ample world-wide distribution. In the last years VL comes suffering a process from urbanization, mainly in the important urban centers of Brazil. The VL cause serious and systemic disease, of difficult diagnosis, however, serological tests has been used in the diagnosis of the infection. Dogs are the main reservoir of *Leishmania infantum* parasites, and they play a central role in the transmission cycle to humans by vector phlebotomine sand flies. The importance of dogs as reservoir for *L. infantum* in the urban environment has stimulated numerous studies on the assessment of diagnostic techniques. At least two serological tests have been applied for antibody detection of CVL diagnosis in Brazil, including Immunofluorescent antibody test (IFAT) and enzyme-linked immunoabsorbent assay (ELISA). Molecular diagnosis methods have become prominent for this purpose. The aim of the present study was to determine the performance of polymerase chain reaction (PCR) and real-time PCR (qPCR) in the diagnosis of CVL using different biological samples. For such, thirty five dogs (positive IFAT ≥40) from an endemic area for CVL were used, with bone marrow aspirate and lymph node and spleen fragments used for the molecular diagnosis (PCR and qPCR). In the present study, qPCR was able to detect a greater number of positive animals in comparison to PCR. No significant differences between biological samples were found in the detection of *L. infantum* DNA using PCR or qPCR.

SY03.P.13

QUANTIFICATION OF *LEISHMANIA INFANTUM* DNA IN SKIN OF NATURALLY INFECTED DOGS USING REAL-TIME POLYMERASE CHAIN REACTION

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Visceral Leishmaniasis (VL) is a major public health problem particularly in poverty countries. In Brazil, VL is present all over the country with high prevalence ranges, particularly in northeast region. Dogs are the main reservoir of *Leishmania infantum* parasites in Brazil, and they play a central role in the transmission cycle to humans by phlebotomine sand flies. Depending on the immune response of animals infected with *L. infantum*, only a portion of infected dogs develop clinical disease and other remains as asymptomatic dogs. Thus, the parasites can spread from the inoculation point to different organs and tissues with different levels of lesions. Symptomatic animals can be show not only weight loss, generalized lymphadenopathy, anorexia, but also the cutaneous lesions characterized by inflammatory process usually accompanied by macrophages and lymphocytes cells. Considering that many infected animals remain as a reservoir with no clinical signs, but with a large parasitism in skin, the aim of the present study was to quantify the parasite load in the skin of dogs naturally infected by *L. infantum* and relate the findings to clinical status. Skin samples from 15 dogs (bone marrow positive) were utilized in the present study. Parasites were quantified by absolute counts. The mean number of parasites was 0.23/mL in the asymptomatic group, 16,518.04/mL in the oligosymptomatic group and 1,326,391.2/mL in the polysymptomatic group. The results demonstrate that clinical signs of infection become more evident as the parasite load increases, as polysymptomatic animals exhibit a greater parasite density on the skin in comparison to oligosymptomatic and asymptomatic animals.

SY03.P.14

CANINE LEISHMANIASIS TREATMENT IN Portugal

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Zoonotic leishmaniasis (ZL) caused by *L. infantum* is endemic in Portugal. Dogs are the major domestic host for these parasites, and the main reservoir for human visceral infection. Parasites are transmitted among mammals by phlebotomine sand flies, being *Phlebotomus perniciosus* and *P. ariasi* the proven vectors in Portugal.
Early detection of infected dogs, their close surveillance and treatment are essential control strategies to avoid the spread of canine infection and consequently human ZL by blocking parasite transmission to sand flies.
In order to assess which drugs and drug regimens were used in the treatment of canine leishmaniasis (CanL) a veterinary practitioner's questionnaire survey was carried out in all districts (18) of mainland Portugal.
Only 68 of the 364 questionnaires sent to Veterinary Centres were completed and returned (reply rate of 19%). The average number of new CanL cases diagnosed per veterinarian clinic ranged from zero to one hundred new cases. From the 68 veterinaries, 91 therapeutic protocols were identified with 38 regimens: for the antimony N-methylglucamine, 31 for miltefosine, 16 for allopurinol, 5 for aminosidine and 1 for levamisole. These results demonstrate the absence of a standardized CanL treatment in Portugal. In none of the questionnaires it was mentioned the

concomitant use of insecticides or repelents to block sand fly transmission and to avoid re-infections.
Taking into account that none of the drugs allow parasitological cure, the use of combined therapy together with the concomitant application of insecticides and/or repellents to reduce the risk of reinfection and transmission must be the control measure. This strategy, not only avoid or reduce relapses but also to avoid the potential emergence and spread of parasite resistance to the few available drugs for human and canine leishmaniasis treatment.

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SY03.P.15
FELINE LEISHMANIASIS IN PORTUGAL

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Zoonotic leishmaniasis caused by *Leishmania infantum* is a serious veterinary and public health problem in the Mediterranean basin and Latin America. Dogs are the main hosts and the main domestic/peridomestic reservoir hosts for human visceral infection. Among reports on newly identified mammalian hosts recurrently found infected with *L. infantum*, those regarding domestic cats deserve attention for the potential implications to the public health. It has been shown that these animals co-habiting with humans can be infected without developing disease, harbour parasites in peripheral blood and skin and are able to transmit parasites to competent vectors. This work reviews feline leishmaniasis and *L. infantum* infection in cats from Portugal, an endemic country of zoonotic leishmaniasis. Serological surveys have been carried out in Lisbon and Trás-os-Montes e Alto-Douro, two endemic regions of zoonotic leishmaniasis showing a seroprevalence between 0 and 20% in screened animals. *L. infantum* infection was also evaluated by PCR on peripheral blood of cats from North and Centre of Portugal as well as from Lisbon Region. The percentage of Leishmania DNA detection ranged between 0.3 and 30.4%. From data obtained it can be concluded that domestic cats from endemic areas of leishmaniasis in Portugal are frequently in contact with *L. infantum*. However, from an epidemiological and control perspective, it is crucial to evaluate the proportion of transmission in endemic areas attributable to cats in order to clarify the role of these animals in sustaining and spreading Leishmania infection.

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SY03.P.16

THE POTENTIAL USE OF APATHOGENIC TRYPANOSMATIDS AS ALTERNATIVE SOURCE OF ANTIGEN FOR SEROLOGICAL SCREENING OF LEISHMANIASIS IN DOGS

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Leishmaniasis is an anthropozoonotic disease distributed in many areas of the world, caused by many members of *Leishmania* genus. In Europe it is caused with two endemic species, *Leishmania infantum* and *Leishmania tropica*. They belong to the genus *Leishmania*, family Trypanosomatidae, a family of monoxenous parasites of insects as genera *Leptomonas*, *Crithidia*, *Blastocrithidia*, *Herpetomonas*, heteroxenous parasites of insects and plants as genus *Phytomonas* and heteroxenous parasites of insect and vertebrates as genera Trypanosoma and Endotrypanum. Two genera, *Leptomonas* and *Phytomonas*, are apathogenic for vertebrates. In their original hosts and in *in vitro* culture, they occur in the same promastigote form as Leishmania parasites in its insect host (sand flies) and in *in vitro* culture. Sources of antigen for serological tests in routine diagnostics of leishmaniasis are usually in vitro cultivated *Leishmania* promastigotes, which are known to be infective for laboratory workers under certain conditions. Because of its infectivity for laboratory workers, its same form of growth in in vitro cultures as apathogenic genera and its belonging to the same family with apathogenic trypanosomatids, the aim of this study was to define the potential use of *in vitro* cultivated harmless promastigote forms of *Leptomonas seymouri* and *Phytomonas serpens* as an alternative source of antigen in the routine animal serology. Three hundred dog sera were tested with indirect immunofluorescence assay and obtained results compared with the ordinary *L. infantum* promastigote antigen. The crossreactivity level was the same when compared with the ordinary *Leishmania* promastigote antigen. Obtained results point to the possibility of using harmless promastigote antigen of *L. seymouri* and *P. serpens* as a alternative source of antigen for the large scale serological screening of leishmaniasis in dogs.

SY03.P.17

EVALUATION OF IN VITRO ANTI-LEISHMANIAL ACTIVITY OF *PERGULARIA TOMENTOSA L. (ASCLEPIDACEAE)* FROM BUSHEHR PLAINS, SOUTHWEST IRAN

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Leishmania is a trypanosomatid protozoan which is transmitted by the female Phlebotomus sand flies and is prevalent in 88 countries of four continents. The current therapy against leishmaniasis is unsatisfactory and the recommended drugs exhibit the thrapeutic failure and side effects. Over the past two decades some studies have been conducted about wild plants, because, they contain chemical substances that produce definite physiological actions on the human. *Pergularia tomentosa* is a climbing to semi erect perennial herb. It was reported to have molluscidal, hypoglycaemic, anti-kaposi's sarcoma and anti-dermatophyte effects.
Leishmania major promastigotes were cultivated in RPMI 1640. Parasites from logarithmic phase were collected and resuspended in fresh RPMI 1640 medium to a final concentration of 4×10^6 cells/ml. *Pergolaria tomentosa* L. (Asclepiadaceae) was collected from its natural habitat. Different parts of the plant after being washed were open air-dried in the shade then pulverized. Cold and hot water and ethanol-waer crude extracts were prepared. From the each stock solution, serial dilutions were made with phosphate buffered saline (PBS) and 100 µl of each prepared concentration was added to each well of micro plate and their activities against Leishmania were evaluated by using the MTT assay test.

Different Pergularia extracts including, hot and cold water, thanol-water and control (Amphotericin B) showed different effects against Leshmania. Cold water extract with 25, 50, 100, 200 and 400 µg/ml showed 30%, 38%, 56%,68%, and 78% cytotoxicity respectively, these above mentioned concentrations for hot water extract showed 35%, 50%, 68%, 75% and 85% cytotoxicity and for ethanol-water extract showed 40%, 63%, 80%, 88% and 98% cytotoxicity respectively.
Amphotericin B with above mentioned concentrations showed 52%, 95%, 100%, 100% and 100% cytotoxicity respectively.
Comparison of cytotoxicity of various Pergularia extracts with each other and with control (Amphotericin B), against *Leishmania* promastigotes, showed significant differences among these groups. Data analysis showed that ethanol-water, cold and hot water extracts of Pergularia with 25, 50, 100, 200 and 400 µg/ml have the strongest, weakest and the moderate effects against *Leishmania* respectively. Among different concentrations of Pergularia extracts ethanol-water extract was more effective than others but was weaker than control, so that the ethanol-water extract with 100, 200 and 400 µg/ml showed 80%, 88% and 98% cytotoxicity against *Leishmania* respectively. We think this study is encouraging and gives good promise, especially Pergularia extracts made with organic solvents use for in vivo study.

SY03.P.18
LEISHMANIASIS IN MOROCCO: TEN YEARS OF CASES REPORTED

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Morocco lies in the Mediterranean region where leishmaniasis is prevalent. The latter, a vector borne disease, affects 2 million people annually in more than 100 countries whose populations are at risk for the disease inflicts high economic costs (Desjeux, 2004; Ashford, 2000). In Morocco, Cutaneous leishmaniasis (CL) are caused by three clinically important *Leishmania* species (*L*. *major*, *L*. *tropica* and *L*. *infantum*), a flagellate protozoa of the Family of Trypanosomatidae (Guessous-Idrissi et al., 1997; Ashford, 2000; Rhajaoui et al., 2004, Boussaa et al., 2005). The Northern coastal regions of Morocco are endemic for human and canine visceral leishmaniasis (VL). As in other VL endemic regions surrounding the Mediterranean Sea, this disease is caused by *L*. *infantum* (Ashford, 2000). In Morocco, the only previous human CL case caused by *L*. *infantum* was reported in 1996, within an active focus of VL (Rioux et al., 1986; 1996). CL and VL overlap in many provinces of central Morocco. In fact, anthroponotic foci of *L*. *tropica* CL are found in the cities of "Fez" and "Taza", not far from existing VL foci including the city of "Sidi Kacem" (Guessous-Idrissi et al., 1997; Chiheb et al., 1999; Rhajaoui et al., 2004). Furthermore, several cases of canine VL caused by *L*. *tropica* have been reported in regions where canine VL is caused by *L*. *infantum* (Rhajaoui et al., 2007). In this work, we show the evolution of leishmaniasis numbers of cases in different provinces of Morocco during the ten past years (2001-2010). We'll discuss the relationship between the 3 clinical leishmaniasis forms and the areas where they have been respectively reported to. The Moroccan Ministry of Health (MMH) has reported in this decade, 24.120 CL cases caused by L. major, 14.372 CL cases caused by *L*. *tropica*, and 1374 VL cases due to *L*. *infantum*. In 10 years, the number of cases of leishmaniasis has increased from 2143 cases (in 2001) to 8846 (in 2010), with a growth rate of over 75%.
Keywords: Morocco, Cutaneous and visceral leishmaniasis, decade, 8846 cases, epidemiology, growth rate.

SY03.P.19

HISTOPATHOLOGICAL CHANGES IN LIVER AND SPLEEN OF CANINE VISCERAL LEISHMANIASIS DUE TO *LEISHMANIA INFANTUM*

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Visceral leishmaniasis in dogs is an excellent model for studying visceral leishmaniasis in humans. The current study was designed to study the histopathological changes in liver and spleen of dogs with visceral leishmaniasis, due to *Leishmania infantum*.
In this experimental study, liver and spleen samples form ten dogs with visceral leishmaniasis were compared with normal liver and spleen samples of ten dogs from non-endemic area. Liver and spleen tissue samples were stained by hematoxylin and eosin and also by Giemsa methods after tissue processing. Finally, using optical microscope, samples were carefully analyzed for finding any histopathological changes.
In spleen of infected dogs', in four cases (40%) severe infiltration of plasma cells, in five cases (50%) presence of megakaryocytes indicating extramedullary hematopoiesis, in one case (10%) inflammatory granulomatous reaction, in three cases (3%) atrophy of lymphoid follicles and loss of germinal centers, in four cases (40%) lymphoid follicles hypertrophy and also in one case (10%), the presence of Leishman bodies in large scale were observed. Liver tissue sections showed no significant changes.
Visceral leishmaniasis can cause histopathological changes in spleen and among them the changes in the size and number of lymphoid follicles, chronic inflammation, granuloma formation and presence of Leishman bodies in spleen macrophages could be mentioned.

SY03.P.20

GENOTYPES OF LEISHMANIA SPP. IMPORTED TO POLAND

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Leishmaniasis is a parasitic disease occurring in 88 tropical and subtropical countries except for Australia and Oceania. The infection is caused by several protozoan species of the genus *Leishmania* and it is characterized by a wide variety of clinical forms from cutaneous, mucocutaneous to visceral. The disease concerns 12 millions of people, and each year about 500 000 new cases have been reported. The global warming and more and more people traveling for tourist destination results in increasing number of cases of this exotic disease "imported" to European countries, including Poland. Each of the basic diagnostic methods of leishmaniasis: the microscopic examination of material from patient, the cultivation of parasites *in vitro* or *in vivo* as well as serological examination, is burdened with some defects, i.e. insufficient sensitivity and time-consuming. Moreover, they do not allow specific identification of *Leishmania* that is of prime importance because the therapeutic response is species, and perhaps even strain specific. Isoenzymatic profile or monoclonal antibodies can be used in order to identify the species. However, these methods need prior multiplication of the parasite by *in vitro* cultivation. Molecular methods: DNA amplification and direct sequencing of the obtained product allows rapid detection and identification of *Leishmania* species. The aim of work was to determine genotypes of *Leishmania* imported to Poland by 6 Polish citizens returning from tropical and subtropical

countries. Three of patients had visceral, and the others had cutaneous form of the disease. The amplification and direct sequencing of the fragment of ITS2 rDNA was performed with clinical samples (bone marrow and skin ulcers, respectively) obtained from these patients. Obtained sequences were compared with those from GenBank. The following species of *Leishmania* were detected: *L. donovani* and *L. chagasi* from patients with visceral form, and *L. major* from patients with cutaneous form of the disease. Genotypes of parasites within species differed in dependence on their geographic origin. The amplification and direct sequencing of fragment of ITS rDNA allows determination of species and genotype of *Leishmania*. Consequently, it can help in choice of the best therapeutic method. Moreover, it is useful in epidemiological studies on geographical distribution of *Leishmania* spp.

SY03.P.21

APOPTOTIC ACTIVITY OF SOME NATURAL AND SYNTHETIC STILBENE AND TERPHENYL COMPOUNDS AGAINST *LEISHMANIA INFANTUM* PROMASTIGOTES

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Leishmaniasis is globally widespread parasitic disease caused by several protozoan parasites of the *Leishmania* genus. *Leishmania* species is able to undergo programmed cell death or apoptosis, as well as mammalian cells. Stilbenes-based compounds are widely represented in nature, and have become of particular interest to chemists and biologists because of their wide range of biological effects including chemopreventive, antitumor, antioxidant, antimicrobial, anti-inflammatory and antihistaminic activities. Recently it was demonstrated in vitro the leishmanicidal effect of some stilbene derivatives (2-hydroxystilbene, combretastatin and heteroanalogous) and terphenyls. In this study we evaluated the anti-leishmanial activity of a pool of stilbene and terphenyls derivatives which had shown high apoptotic efficacy against neoplastic cells.
$1x10^6$ *Leishmania infantum* promastigotes were plated into 16 mm diameter wells containing RPMI-PY medium, a new medium, supplemented with FCS (10%) and 1% glutamine and treated with scalar concentration of 23 different artificial compounds. After 48 hours of incubation at 27°C leishmanias were harvested and their number was determined by using a counter. The percentage of apoptotic leishmanias was determined by morphological examination using a fluorencence microscope after ethidium bromide and acridine orange staining. Morphological alterations including cell shrinkage, an aflagellated ovoid shape and chromatin condensation were suggestive of apoptosis. Apoptotic activity of synthetic compounds was compared to that of pterostilbene (3, 5-dimethoxy resveratrol), a natural stilbene compound.
Among compounds tested; ST18, TR3 and TR4 showed an interesting anti-leishmanial activity.
Our preliminary results suggest that some stilbene derivatives and terphenyls are highly effective at inducing apoptosis in *Leishmania infantum* promastigotes, thus they could represent potential anti-leishmanial agent that merit further pharmacological investigation.

SY04.P.01

ELIMINATION OF MALARIA IN THE RUSSIAN FEDERATION

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In the early 90s of last century in Russia there was a sharp increase in the incidence of malaria. This was due to the fact that in neighboring countries - Uzbekistan, Tajikistan, Armenia, Azerbaijan antimalarial measures are not carried out. From there, in Russia there was migration of gastarbaiters, some of whom were suffering from malaria, and so malarial mosquitoes flew. The

incidence of malaria in Russia in some years reached more than 1,000 cases, of which 600 were local.
Over the past 20 years have been carried out comprehensive control measures of malarial mosquitoes, detection and medical treatment of malaria patients. In Russia has registered a three-day and tropical malaria, are agents of *Plasmodium vivax* and *P. falciparum*.
In the towns of Russia reported 14 species of mosquitoes genus *Anopheles*, of which are dominant *Anopheles messeae* and *An*. *maculipennis*. Every summer in cities spend antilarvicidal events. Reservoirs, which is the development of larvae of *Anopheles* mosquito reated biological insecticides on the basis of *Bacillus thuringiensis*. The residual effect of these agents is in the temperate climate of 15-25 days, so the process is repeated 4-6 times in a season. The control of adult *Anopheles* mosquitoes conducted during the summer of children's health camps, health centers, etc. Insecticides from the group of organophosphorus compounds and pyrethroids treated rooms, as well as the vegetation around these institutions.
Over the past five years, the number of malaria cases in Russia has fallen to less than 100 per year, while in Moscow, malaria is not marked by the last two years.

SY04.P.02

GENETIC CHARACTERISTICS OF *PLASMODIUM VIVAX* APICAL MEMBRANE ANTIGEN-1 GENE IN ISOLATES FROM IRAN

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Plasmodium vivax is a widely distributed human malaria parasite, prevalent in South America, Asia and Oceania, and the 70—80 million cases currently recorded annually are of global public health importance. In Iran *Plasmodium vivax* is responsible for approximately 90% of cases of the disease reported per year. Despite *P. vivax* malaria is non- lethal infection but its prolonged and recurrent infection can have major deleterious effects on personal well-being, growth and on the economic performance at the individual, family, community and national levels. As the burden of disease, the need for an effective vaccine has also assumed greater importance and the apical membrane antigen -1 is one of the most promising malaria vaccine candidates. Apical membrane antigen 1 is a type 1 integral membrane protein present in all species malaria parasite. Genetic diversity of AMA-1 among Plasmodium field isolates and presence of variant forms in different geographic areas presents a complexity in successful malaria vaccine design. Therefore, studies of the population diversity of the malaria parasites have practical significance for the understanding of epidemic status and for vaccine development. In this study we obtained 52 *Plasmodium vivax* isolates from south-eastern part of Iran and then analyzed for this gene.1000 bp of this gene was sequenced and 880 bp covering domains I and II was considered for analysis. Non synonymous mutations were found frequently at domain I compared to domain II (18 at domain I and 12 at domain II) where almost all of them were dimorphic (29 dimorphic and I tetramorphic).The difference between synonymous and non- synonymous mutations for both domain I and II as well as entire 880bp were negative which suggests the role of purifying (negative) selection in this molecule. The result of this study showed useful information about the nature of *Plasmodium vivax* parasite that circulates in the southeast of the Iran and also useful information for vaccine development based on apical membrane antigen protein.
Keywords: *Plasmodium vivax*, ama-1, genetic diversity, Iran.

SY04.P.03

IMPORTANCE OF SOCIO-CULTURAL CONTEXT IN REDUCING MALARIA INFECTION

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In the era of malaria, there is widespread interest and concern about the cultural, ecological and political factors that are directly related to the increased prevalence of infectious disease.
new challenges to health and social-service providers is that in malaria control and prevention programme not enough attention is paid to understanding the local socio-cultural context prior to programme implementation. The aim of this study is to discover how people in rural sarbaz district understand and explain malaria, the cultural construction of disease and how these factors influence health seeking behavior.
The southeastern Iran has 18 districts. Based upon feasibility within existing resources, sarbaz districts were randomly selected. Sarbaz is predominantly rural. Transmission of malaria is moderately high and occurs throughout the year with peaks during the rains from May- October.
All public facilities in sarbaz district that provide services were included.
Interviews were conducted with 20 professionals using a semi-structured interview for health workers and physicians who provide care for patient with fever in sarbaz , in order to identify the best practices for control of malaria. Approximately half of the providers self-identified as care providers. Nine of those interviewed were physicians and 11 were health workers. A content analysis approach was used to identify themes. Ethical approval for this study was granted by the ethics committee of Tehran University of Medical Sciences. Written informed consent was sought from all participants before the start of all interviews (structured, or semi-structured).
Findings indicate that preventing infectious disease promoted through c lose communication between health workers, Physicians and health providers with people in communities. It also addressing system level concerns, including helping patients' families find health insurance and other social services, as well as having a separate clinic for ethnic groups.
In addition to improving health system factors, gaining the confidence and support of health workers that provide services and deliver malaria interventions to people based on cultural adaptive strategies would be critical for successful implementation. It also helps implications for program development, social service and public health practice.

SY04.P.04

ACUTE RENAL FAILURE DURING SEVERE MALARIA: A CASE REPORT

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Malaria represents a medical emergency because it may rapidly progress to complications which involve the nervous, respiratory, renal, and/or hematopoietic systems. Acute Renal failure (ARF) is a rare complication of severe *Plasmodium falciparum* malaria in non immune adults and a major contributor to mortality in these patients.
We report a case of severe malaria featuring ARF that occurred in a 55 year-old Tunisian man who has been in Burkina Faso for the last two years without chemoprophylaxis. The patient developed symptoms 20 days after his return. Clinical features included fever accompanied by discomfort, myalgia and dry cough. On admission to hospital, physical signs included fever (39°C), tachycardia, a decrease in consciousness, oligo-anuria and hypotension blood pressure of 90/65 mmHg. Laboratory findings revealed anemia (haemoglobin of 10.8g/dl). The patient was

hyponatraemic and had elevated creatinemia of 267µmol/l. A thick and thin blood films were requested, revealing *Plasmodium falciparum* with a parasitaemia of 8%. Patient was successfully treated in the intensive care unit by quinine perfusion. The outcome was favorable with recovery of renal function.
Severe malaria is defined by the combination of asexual *Plasmodium falciparum* forms in blood and one or more clinical or biological severity criteria. ARF is a criterion of malaria severity as defined by WHO. The main physiopathology mechanisms, underlying tubular necrosis, are obstruction of capillaries and post-capillary venules by parasitized erythrocytes and activation of monocytes that release cytokines such as tumor necrosis factor. Renal replacement therapy should be initiated early and for early diagnosis, it is paramount to consider malaria in every febrile patient with a history of travel in an area endemic for malaria. Prognosis is depending on early diagnosis and prompt treatment but the best way to reduce mortality is to improve prevention by the use of chemoprophylactic drugs.

SY05.P.01

SEROPREVALENCE OF TOXOPLASMOSIS AMONG ARTHRITIS RHEUMATOID PATIENTS

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Arthritis Rheumatoid is a chronic inflammatory disease that leads to immune complex-mediated damage against self-antigens and deposition in the joints. Arthritis Rheumatoid is a autoimmune systemic disease and Researches indicated the 1% of the people are infected with this kind of disease. Infectious diseases among rheumatoid arthritis patients have a high prevalence in compare with the other people. Toxoplasmosis is an Opportunistic infections disease with Global outbreak among immune suppression and immune deficient patients. The aim of this study was to determine the prevalence of specific *Toxoplasma* antibodies among patients with rheumatoid arthritis and control group.
This study was established in a Case-control pattern on 110 serum samples including 55 of rheumatoid arthritis patients and 55 of healthy volunteer. The level of *Toxoplasma* antibodies was measured among affected patients with Arthritis Rheumatoid and control group. ELISA kit was used for measuring of IgG&IgM antibody. The collected results were analyzed by SPSS, version 18.0 software.
Based on findings through this study the rate of toxoplasmosis prevalence among Arthritis Rheumatoid patients was higher in comparison with control groups. The rate of *Toxoplasma* IgG antibody seropositivity among Arthritis Rheumatoid group was 58.18% and in control group was 27.27%. IgM antibody was not positive in none of the patients and control group.
In Arthritis Rheumatoid patients using immunosuppressive drugs, especially steroids, can provide a context for opportunistic infections. Therefore, it is recommended timely screening more accurate and management of decrease prevalence of toxoplasmosis among Arthritis Rheumatoid patients.

SY05.P.02

POSITIVE ASSOCIATION OF TOXOPLASMA GONDII INFECTION AND ANISAKIS SIMPLEX PARASITISM IN CHRONIC URTICARIA

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In the last decades a rise has been observed in the prevalence of allergic disease, most noteworthy in westernized populations. Within the important role of environmental factors suggested as an explanation, the hygiene hypothesis has received an outstanding attention. *Toxoplasma gondii* is a food-borne and orofecal microorganism which produces chronic infection and its negative association with atopy has been tried to prove previously in the context of the hygiene hypothesis. *Anisakis simplex* is a fish-parasite associated with chronic urticaria (CU) in endemic regions. As in our region A. simplex sensitization associated CU accounts for a high proportion of CU, we hypothesized that Th2-associated A. simplex sensitization and Th1-associated *T. gondii* infection could be negatively associated. Therefore we studied patients with CU with respect to these immunologically antagonistic infectious agents. We included 42 patients with chronic urticaria (18 patients with *A*. *simplex* sensitization associated CU and 24 not sensitized CU patients). Nineteen native subjects without history of urticaria served as controls. Patients were assessed for atopy by Skin Prick Test (SPT) against common aeroallergens [animal dander (cat, dog), house dust mites (*Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*), pollen of *Cupressus arizonica*, *Olea europea*, *Lolium perenne*, weed mix and mould *Alternaria alternata*] and for respiratory symptoms. CU patients were analysed with respect to *T. gondii* seropositivity, *A. simplex* sensitization and atopy. *A. simplex* sensitization was assessed by SPT and specific IgE by CAP-FEIA. If patients displayed positive SPT and specific IgE against A. simplex, they were included in the CU+ group. CU- patients showed neither a positive SPT nor detectable serum specific antibodies against *A. simplex*. Anti-*T. gondii* IgG levels were measured by ELISA (NovaLisaTM Toxoplasma gondii IgG-ELISA system). The seroprevalence of T. gondii was 40.5% in CU patients and 42.1% in the control group. Anti-*T. gondii* IgG antibodies were associated with past A. simplex parasitism (Odds ratio 6.73; p=0.03) and independently with atopic sensitization (Odds ratio 5.85; p=0.04). In CU patients, *T. gondii* has no protective effect on atopic sensitization or *A. simplex* sensitization. In conclusion, our data show in CU a positive and independent association of *T. gondii* infection with past *A. simplex* parasitism as well as with an atopic status.

Mutua-Madrileña, SEAIC and Ramón-Areces Foundations.

SY05.P.03

ELISA AND IFA IN TESTING THE SEROLOGIC PROFILE OF TOXOPLASMOSIS AMONG A SAMPLE OF MUNICIPALITY WORKERS IN DUBAI – UAE

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Toxoplasma gondii infection is a growing worldwide health concern, with estimates of up to one third of the world's population infected with the parasite. The paucity of data on the prevalence of *T. gondii* and associated risk factors in UAE has prompted us to undertake this study to evaluate the seo-prevalence and risk factors of *T. gondii* in 200 healthy workers seen at Dubai municipality in Dubai- UAE in an attempt to define the epidemiology of this infection in a sample of this population comparing two serological assays. Workers were interviewed about socio-demographic

characteristics and risk factors for *T. gondii* infection such as area of residency, agricultural activity, professional contact with animals and history of blood transfusion or consumption of raw meat. Blood samples were collected to document their *T. gondii* antibody status using *Toxoplasma* IgG ELISA and IgG IFA test system. Sensitivity and specificity of the two tests were calculated using western blot as a gold standard. Overall, 42/ 200 workers (21%) were positive using IgG ELISA, 36 (18%) and 40 (20 %) were positive using *Toxoplasma* IgG IFA and western blot analysis respectively. There was a high significant association between the prevalence of toxoplasmosis and the history of contact with animals, history of consumption of raw meat and agricultural activity with p <0.0001. However the association between the prevalence of infection and history of blood transfusion or areas of residency were found insignificant with p = 0.863 and 0.948 respectively. The sensitivity of *Toxoplasma* IgG ELISA and *Toxoplasma* IgG IFA test was found to be 95% and 85% respectively, while the specificity of the both tests was found to be 97.5 and 98.8% respectively.

SY05.P.04

EFFECT OF BEE VENOM ON *TOXOPLASMA GONDII* TACHYZOITES IN VITRO

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Toxoplasma gondii an intracellular parasite infection cause acute or chronic toxoplasmosis among mammalian. Widespread transmission routes of *Toxoplasma gondii* have led to global prevalence of this disease. The pathogenesis of *Toxoplasma* is now double that in immunocompromised patients with acute, diffuse and systemic disease. Annually many studies on anti-parasitic plant and synthetic drugs on the *Toxoplasma gondii*. Studies show that beekeeper in compare of other people significantly and are much less likely to suffer from infectious diseases. The aim of the study is measuring anti-parasitic properties of bee venom in the culture medium on the *Toxoplasma* tachyzoite.

In order to isolated RH strain, *Toxoplasma* tachyzoite was injected in a peritoneal cavity of six laboratory mice. After one week of peritoneal fluid was removed and transferred to specific culture of *Toxoplasma* parasit. The affect of bee venom on *Toxoplasma* tachyzoite in a different doses (0.01 mg, 0.1 mg and 1.0 mg) at different times (30 min,2 hrs and 3 hrs) was done in case & control group All phases of laboratory research was done in sterile conditions. At last all obtained data were analyzed using the statistical package for social sciences (SPSS, version 18.0) statistical software.

Bee venom has notable anti-parasitic properties of on the *Toxoplasma* tachyzoite. Results indicated that there was a significant difference between case &control group (p<0.05) at 1 mg cons after 2 hours of exposure.

Bee venom is very potent in destruction *Toxoplasma* tachyzoite *in vitro*. The study recommended a wider phase is performed on animal models *Toxoplasma gondii*.

SY05.P.05

TOXOPLASMOSIS AND IMMUNOSUPRESSION: REACTIVATION IN A CASE OF DUAL (GENETIC AND TRANSPLANTATION-INDUCED) IMMUNODEFICIENCY

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Toxoplasmosis is a well-known opportunistic infection, and bone marrow transplant (BMT) recipients are particularly prone to reactivation of a latent infection. We present a case of severe disseminated toxoplasmosis early after allogenic hematopoetic stem cell transplantation. The patient was a 16-year-old boy treated for acute T-cell lymphoblastic leukemia, with an underlying Nijmegen breakage syndrome (NBS). NBS is a rare genetic disorder at the level of DNA repair, resulting in chromosomal instability, combined T and B-cell immunodeficiency and predisposition to lymphoma development at childhood age. After a reduced-intensity conditioning regimen, which included prophylaxis with cotrimoxazole, BMT was carried out from an HLA-identical sibling, his brother. Pre-transplantation serology for toxoplasmosis showed that the donor was seronegative, while the recipient was previously infected (IgG 578 IU/ml, IgM negative). Signs of infection including fever, pleuropneumonia and acute respiratory distress syndrome appeared as of post-transplantation day (ptd) 12, and cotrimoxazole was initiated on ptd 14. Initial work-up ruled out other infections, but suggested toxoplasmosis (tachyzoite-like structures on Giemsa-stained bronchoalveolar lavage fluid (BAL) smears), and on ptd 16 the patient's blood and BAL samples were sent to the NRLToxo for confirmation. Serological testing showed high-avidity specific IgG antibodies without specific IgM. Real-time (RT) PCR targeted at the *Toxoplasma* AF146527 gene revealed parasite DNA, and the parasite burden was quantified, according to a standard curve, at 15.000 parasites/ml in the blood, and at a staggering 100.000 parasites/ml in the BAL. This triggered immediate (ptd 16) introduction of intravenous cotrimoxazole and clindamycin, followed with pyrimethamine and sulfadiazine at ptd 23. Control serology (ptd 24) showed a two-fold rise in specific IgG antibody without the appearance of specific IgM, while RT-PCR revealed a dramatically reduced parasite load of 200 parasites/ml (BAL was not further sampled due to the patient's poor condition). Despite a transient improvement, the patient deteriorated and died of multiple organ failure at ptd 37. Direct genotyping of both the blood and BAL sample with PCR-RFLP using SAG1, SAG2, GRA6 and GRA7 as markers revealed the infecting *Toxoplasma* strain to be of type II. The presented case shows the detrimental consequences of a donor/recipient mismatch in the serological status to *Toxoplasma* infection in a dually immunosuppressed patient. On a good note, the presented results show the benefit of using highly sensitive and quantitative molecular methods for the diagnosis and monitoring of toxoplasmosis.

SY05.P.06

INFECTION OF HUMAN NERVOUS CELLS BY DIFFERENT STRAINS OF *TOXOPLASMA GONDII* IN VITRO: ANALYSIS OF NEURONAL CYTOKINE AND CHEMOKINE EXPRESSION PROFILES

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Toxoplasmosis is caused by an intracellular protozoan parasite, *Toxoplasma gondii* which causes either congenital or acquired toxoplasmosis. Two types of acquired toxoplasmosis are generally described. The first is characterized by an asymptomatic benign infection in immunocompetent subjects. At this stage of infection, the host's immune response induces the conversion of the

parasite from the virulent form into tissue cysts which persist in the center nervous system throughout the host life. The second form results from reactivation of latent toxoplasmosis in immunocompromised subjects. In AIDS patients, encephalitis is the most frequent clinical manifestation that results from progressive impairment of immune cell function. The severity of toxoplasmosis also depends on the type of *T. gondii* strains. Several haplogroups of *T. gondii* are described: types I, II, III were the first recognized, but other haplogroups are now described, named atypical. These types differ by their virulence profile. In this context, the relationship between human host and parasite has not yet been elucidated because few studies have been conducted on the nature of human immune response in brain, and the genetic diversity of the parasite which could explain the different clinical evolutions. Objective: the principal aim of this study was to determine the immune mechanisms of human nervous cells (HNC) in vitro which were infected with different strains of *Toxoplasma gondii* that caused neurological diseases. Materials and methods: HNC (microglia CMH5, neuroendothelial cells HBMEC and neurons SH-SY5Y) were infected by two strains of *Toxoplasma*: RH (type I) and PRU (type II) for 14h and 24h (ratio 1:2). The immune response was analyzed by determination of the cytokine and chemokine expression profile produced during infection. Thirty six cytokines and chemokines were tested by Proteome Profiler array and RT-PCR techniques. Statistical analysis was performed using the ANOVA test. Values of $p<0.05$ were considered significant. Results and discussion: Data has showed principally expression of the inflammatory mediators including: IL6, IL8, MIF, MCP1, Serpin E1 and growth factors: G-CSF and GM-CSF. The comparison of these protein expression profiles was significantly different between the strains (PRU and RH) and among different HNC. These results suggest that the different protein expression profiles depend on the parasite strain that could be at the origin of diverse brain lesions caused by *T. gondii*. Nevertheless, HNC seem able to inhibit parasite replication, so the role of these cells can explain the immune response activated during toxoplasmosis encephalitis.

SY05.P.07

SEROPREVALENCE OF TOXOPLASMA GONDII ANTIBODIES AND PROFILE OF CD4+ COUNTS IN HIV/AIDS PAITENTS IN NORTH OF IRAN 2009-2010

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Introduction: *Toxoplasma gondii* is as an important opportunist pathogenic agent, among the Human immunodeficiency virus (HIV) and acquired immunodeficiency Syndrome (AIDS) patients.
Abjectives: This study has been designed to determine the prevalence of anti-*Toxoplasma* antibodies and profile of CD_4^+ counts among these individuals.
Method: A total of 142 serum samples (64 HIV^+/AIDS patients and 78 non-HIV infected individuals) collected form Mazandaran province were screened for anti-*Toxoplasma* IgG and IgM by Enzyme-linked Immunoassay (ELISA). Each person in this study was examined for CD_4^+ counts.
Result: The seropositivity of IgG anti-*Toxoplasma* antibodies in HIV^+/AIDS was 48/64 (75%) and there was 4 cases (6.25%) with IgM antibodies. In the sera of control group we found 59/78 (76.92%) with IgG and 7 (12.82%) cases with IgM anti-*Toxoplasma* antibodies. The mean of CD_4^+ counts in HIV^+/AIDS group (430 cells/microliter) was remarkedly less than controls (871 cells/microliter) ($p= 0.001$).
Conclusion: Although there was not any different in seroprevalence of anti-Toxoplasma antibodies between HIV-positive patients and control group, but a high seroprevalence of anti-*Toxoplasma* IgG has found in both group the risk of damage to the CNS by reactivation of previously latent *T.gondii* infection type of patients,it is most important to find these patients and treated them to reduce morbidity in these patients.

SY05.P.08

TOXOPLASMA GONDII EVOLVES A NEW ARCHITECTURE FOR THE MULTI-AMINOACYL-TRNA SYNTHETASE COMPLEX

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Mechanisms of protein translation are poorly understood in Apicomplexa, a phylum that encompasses deadly human pathogens like *Plasmodium* spp. and *Toxoplasma gondii*. Toxoplasmosis treatment needs urgent improvement as only rare new treatments have been described in the last decade and most of them display strong side effects. Similarly, rapid development of drug resistance in malaria parasites needs a continuous stream of new generation of molecules which may lead to new antimalarial drugs. Detailed structure-function studies of crucial parasite proteins may provide new targets for drug discovery against malaria and toxoplasmosis. Aminoacyl-tRNA synthetases (aaRSs) are key players in protein translation as pivotal in determining how genetic code is interpreted. Bioinformatics revealed that apicomplexans possess high fraction of aaRSs relative to their proteome size when compared with bacteria and human counterparts. This fact provides an opportunity to target this vital function in the parasite as a route to killing the protozoan. AaRS can be found in high-molecular weight complexes – the multi-aminoacyl-tRNA synthetases complexe (MSC) – where aaRS are embedded with auxiliary proteins like p43. Here we provide the proof of the existence of a *T. gondii* MSC complex whose composition was determined by mass spectrometry. Besides the p43 subunit used as a bait for its purification, the complex harbors three aaRSs. The subcellular localization of p43 suggests that the complex is highly expressed and localized in the parasite cytosol. Genetic analysis in virulent, non-cystogenic type 1 (RH strain) background reveals that the above cited complex is not essential to the survival of *T. gondii*. The virulence of the Tgp43 mutant was monitored by intraperitoneal injections in Swiss mice. Mice inoculated with parasite mutants died between D9 and D11 while those inoculated with the wild-type died on D8 and D10. No differences in invasion or proliferation between the two strains were noticed. A key result is the determination of the molecular composition of MSC of *T. gondii* while its *modus operandi* is still under study. Anyhow, our results pave the way to a better understanding of this complex in *Apicomplexa* and beyond in eukaryotic cells. Our main focus on the protein translation machinery in these parasites was initially justified by novelty of the proposed targets due to their established ability to become potential antiparasitic drugs. Thus, we recently identified a new compound targeting a specific aaRS that reduces *T. gondii* proliferation in human fibroblasts without affecting the growth or viability of the host cells.

SY05.P.09

VALIDATION OF THE ELECSYS® TOXO IGG AVIDITY ASSAY FOR TOXOPLASMOSIS: NEW INSIGHTS IN EVALUATION OF THE TIME OF INFECTION?

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Detection and treatment of acute toxoplasmosis during pregnancy can avoid severe disease of the foetus. In this situation, assessment of anti-*Toxoplasma* IgG avidity plays a critical role in the

interpretation of serological results. A high avidity value excludes a recent infection and thus allows treatment to be avoided or stopped; it also removes the need for follow-up for *Toxoplasma* infection during the pregnancy and to consider termination. The Roche Elecsys® Toxo IgG and IgM assays have been validated for screening and monitoring of immune status in pregnant women and a new additional test, the Elecsys® Toxo IgG Avidity assay has now been developed. The aims of our study were to assess the performance characteristics of this new avidity assay and to explore whether additional helpful information could be provided by avidity testing.
The Elecsys® Toxo IgG Avidity assay was compared with the bioMérieux VIDAS® and Abbott ARCHITECT® avidity assays using two sets of serum samples taken from pregnant women (n=291 and n=255, respectively). For all samples the time of infection onset had been determined previously by analysing sequential sera using commercially available routine diagnostic techniques.
None of the assays detected high avidity antibodies in serum taken <4 months after infection onset. Hence all three assays were able to exclude a recent primary infection (infection within the last 4 months) in samples with high avidity antibodies. Avidity values >90% were reported by Elecsys® and Architect® assays only in sera taken >9 months after infection. Similarly, avidity values >0.6 were reported by the VIDAS® assay only in sera taken >9 months after infection. Almost all avidity values <19% using the Elecsys® assay and <17% using the Architect® assay corresponded to sera taken <3 months or <2 months after infection, respectively.
We conclude that the Elecsys® Toxo IgG Avidity assay can be used to exclude recent infection. In addition, new ways of interpreting the avidity results are suggested: using avidity assays very high or very low avidity values could support the exclusion of infections within the last 9 months or assist in the confirmation of a recent infection, respectively. However, these potential interpretations require further investigation in a larger number of sera.

SY05.P.10
ROLE OF *TOXOPLASMA GONDII* INFECTION IN SERUM LEVEL OF TESTOSTERONE

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Toxoplasmosis is the disease caused by an intercellular protozoan called *Toxoplasma gondii*. *Toxoplasma* has global expansion and infects a wide range of warm blood animals. Presence of parasites inside cells affects cellular various activities and causes changes in cellular mechanisms.This study was carried out in order to evaluate the changes in testosterone level due to *Toxoplasma gondii*.
This cohort study was performed on individuals who referred to one of Tehran military hospitals in year 2009. 180 people were selected by simple random sampling. 5 ml of blood was taken from individuals and 1 ml serum was separated and used in order to determine toxoplasmosis infection and testosterone concentration by ELISA method. Hirsutism, hair loss, weight and height were also evaluated and documented. The results were analyzed by SPSS 16 software using independent t-test and Pearson correlation.
Of 180 serum samples (73 females and 107 males), 24femals (13/33%) and 39 males (21/66%) had anti *Toxoplasma* IgG antibody. The results showed significant correlation between *Toxoplasma* and testosterone increase in women (p=0.002), testosterone increase in men (p<0.0001), hair loss in women (p=0.002), hirsutism in women (p=0.001) and height increase in women and men, but there was no significant correlation between weight and *Toxoplasma* infection in women and men.
There is a significant correlation between *Toxoplasma* infection and serum increase of testosterone, hirsutism, hair loss and height in patients. Results of this research can be beneficial in evaluation of disease symptoms and treatment protocol of toxoplasmosis patients for research centers and physicians.
Keywords: *Toxoplasma gondii*, Serum Level, Testosterone.

SY05.P.11

RESISTANCE INDUCTION OF *TOXOPLASMA GONDII* STRAIN BY SULFADIAZINE PRESSURE

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Toxoplasma gondii, the causative agent of toxoplasmosis, is an obligate intracellular protozoan parasite that infects more than one-third of the world's human population. Toxoplasmosis is an infection generally benign but which can cause severe damage when the pathogen is contracted congenitally or in immunocompromised individuals, particularly transplants and AIDS patients. Treatment of *T. gondii* generally uses a combination of a sulfonamide with pyrimethamine; however several failures have been reported. In previously work, we have evaluated the sulfadiazine susceptibilities of 17 *T. gondii* strains on MRC5 cells *in vitro* by calculating the 50% inhibitory concentration (IC_{50}) according to a method developed by Derouin (1988), and we have shown the existence of three resistant strains on sulfadiazine, a drug currently prescribed in treatment of toxoplasmosis: TgH 32006 (Type II), TgH 32045 (Type II variant) and TgA 103001 (Type I) (Meneceur, 2008). However, none link could be established with resistance mechanism shown in other parasites such as *Plasmodium falciparum*. The aim of this study is to induce *in vitro* sulfadiazine resistance on the sensitive strain RH (Type I) to better understand resistance mechanisms.
Tachyzoïtes of RH strain were maintained on Vero cells (ATCC, CCL-81). To induce sulfadiazine resistance, RH strain was cultivated with drug pressure: sulfadiazine concentration was doubled every three passages from 50 µg/mL to 800 µg/mL. Sulfadiazine susceptibilities of the sensitive RH strain, "RH resistant to sulfadiazine" strain called RH-R^{SDZ} and the naturally resistant type I strain TgA10301 were evaluated according to the method developed by Derouin (Derouin *et al*., 1988). Briefly, tachyzoïtes were maintained for 72h on Vero cells in 96-well culture plates, and then sulfadiazine at various concentrations was added. *Toxoplasma* growth was quantitated by an enzyme-linked immunosorbent assay (ELISA) performed directly on the fixed cultures. The sensitive RH strain has an IC_{50}=75µg/mL of sulfadiazine, the RH-R^{SDZ} has an IC_{50}>1000µg/mL of sulfadiazine like the naturally resistant strain TgA10301. Our results show that we developed a RH-R^{SDZ} which was resistant to the maximum dose of the drug the host could tolerate and equivalent to the TgA10301's drug susceptibility. Moreover, this resistance was stable after several passages and decongelation without drug pressure. Further analysis of RH, RH-R^{SDZ} and TgA 103001 strains could allow us to understand resistance phenomena.

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SY07.P.01

FASCIOLOSIS IN MEXICAN PEDIATRIC PATIENTS

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Fasciolosis is an infection caused by *Fasciola hepatica*, geographical distribution of human fasciolosis is found in all continents which has made this parasitosis in a major health problem, and is presented importantly in bovines in México; human fasciolosis is present for a long time and has been the cause of disease and damage to the Mexicans, who in the past has been a diagnostic problem disease however there are few cases in humans, and many of them in pediatric patients.

In Mexico the first human case was reported in 1936 in an 11 year-old boy, since then many cases have been reported: two in 1942, ten in 1992, one in 1999, four in 2000, one complicated case in 2002 and 5 in 2006.
The diagnostic of these 24 cases were made by isolation of the parasite in 14 and by immunologic test in 10.
The parasitologic isolation were made by coproparasitologic test in 13 patients, in the 2002 case, the isolation was made by identification of the parasite directly from the gallbladder tissue, obtained by quirurgic procedure.
Last 3 years we diagnosed 3 pediatric patients by coproparasitologic studies where we observed *Fasciola hepatica* eggs.
In clinical evaluation two of these patients presented unspecific symptoms, only one presented symptoms as weight loss, hepatomegaly, hepatic pain, intermittent jaundice in last 10 months.
We consider the human fasciolosis more frequent than reported in literature, by the etiologic diagnosis is not made, is important that physicians suspect this parasite; it is advisable to include a concentration coproparasitoscopic as routine test in geographic zones where the fasciolosis is an endemic disease in bovines and ovine.

SY07.P.02

CHARACTERIZATION OF *FASCIOLA HEPATICA* STRAINS FROM SHEEP SUSCEPTIBLE AND RESISTANT TO ANTHELMINTICS USING MITOCHONDRIAL DNA MARKERS

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In the present study we have characterized three different sheep *Fasciola hepatica* strains by sequencing the cytochrome C oxidase subunit 1 (Cox1) and the NADH dehydrogenase subunit 1 (Nad1). The strains were isolated from sheep flocks with different levels of resistance after carrying out the Faecal Egg Count Reduction Test (FECRT): LS, susceptible to albendazole, triclabendazole and clorsulon; CS, resistant to the three drugs; SV resistant to albendazole and clorsulon. The characterization was done in 9-12 individual eggs collected from faeces before the treatment of sheep in the FECRT, in the case of LS and CS, and before (SV0) and after treatment with albendazole (SVA) and clorsulon (SVC) in the case of SV. A nested-PCR was carried out in each egg to amplify a 798 bp fragment of Cox1 and an 870 bp fragment of Nad1. After the analysis of the sequences we found different Single Nucleotide Polymorphisms (SNPs) in all strains, although, when the SNP was only described in one egg, this was not considered significant. Regarding Cox1, the following SNPs and frequencies were described for each strain: LS, D258A (2/12); CS, G379E (2/12) and A429S (4/11); SV0, A457V (2/12); SVA, A457V (4/12); SVC, A457V (2/9) and Y264N (2/9). In relation to Nad1, we found the following SNPs: CS, P45L (2/12); SVA, T271N (2/12) and R274 (2/12); SVC, F27L (2/12). We can conclude that the genetic variability in Cox1 is higher than in Nad1, where no SNP was described in LS or SV0. It is worth noting that in Cox1 the SNPs with the highest frequencies were shown in the resistant isolates, CS and SVA. Moreover, the SNP A457V was described before and after treatment in SV, although its frequency was increased after the administration of albendazole. Therefore, a higher genetic variability and a higher susceptibility of the parasite to adapt to the selection pressure, exerted by the anthelmintic drug, result in greater likelihood of the anthelmintic resistance phenomenon taking place.

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SY07.P.03

MOLECULAR CLONING AND CHARACTERIZATION OF CATHEPSIN L3 FROM *FASCIOLA HEPATICA*

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Fasciola hepatica infections cause significant global problems in veterinary and human medicine. The fluke causes huge losses in cattle and sheep production. During host infections flukes express cysteine proteases (cathepsins) which play pivotal roles in parasite feeding, migration through host tissues and immune evasion. Expression of cathepsins L is developmentally- regulated. Excystment of the infective larvae is dependent on FhCB and FhCL3 and together these enzymes account for over 80% of total protease activity in *F. hepatica* newly excysted juvenile (NEJ).
We focus on members of the cathepsin L gene family, belonging to the CL3 clade. The cDNA of two novel CL3 proteases – Fh-CL3-1 and Fh-CL3-2 were cloned. We identified differential level of mRNA transcript expression for these enzymes in various life stages obtained *in vitro*, ie. metacercariae at 0 time, metacercariae 1h post induction, NEJ briefly after excystment and NEJ 24 h after excystment. Expression of Fh-CL3-2 was at higher level in all stages compared to Fh-CL3-1, indicating that there was also regulation within the clade. The ability of antibody responses from rats and sheep challenged with *F. hepatica* to recognize recombinant Fh-CL3-1 and Fh-CL3-2 was shown to differ. Differences were also shown by the use of anti-FhCL3-1 and anti-FhCL3-2 sera in Western blot analysis of juvenile excretory/secretory (ES) material separated by 2D electrophoresis. Computational analysis was performed and used to examine immunogenicity of CL3 cathepsins. Immunohistochemical analysis showed the secretory nature of the proteases.

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SY07.P.04

LIVER FLUKE PHENOTYPIC CHARACTERIZATION IN ANDEAN HUMAN ENDEMIC AREAS: ALTIPLANIC VERSUS VALLEY PATTERNS

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Fascioliasis is a parasitic disease of humans and livestock caused by fluke species of the genus *Fasciola*. In South America, this disease is caused by *Fasciola hepatica* and gives rise to a serious public health problem. Given the necessity to characterize *F. hepatica* populations involved, the phenotypic features of fasciolid adults infecting sheep present in human fascioliasis endemic areas were analysed in the Cajamarca Valley and Mantaro Valley (valley transmission patterns) and the northern Bolivian Altiplano (altiplanic transmission pattern). A computer image analysis system (CIAS) was applied on the basis of standardized measurements. To complete the characterization, all these South American highland populations were compared to standard lowland populations of (i) *F. hepatica* natural infection from Valencia, Spain, and (ii) *F. hepatica* experimental adults from Bialowieza NP, Poland. *F. hepatica* size was studied by multivariate analyses. Two phenotypic patterns could be distinguished in *F. hepatica* adult size: the valley pattern (Cajamarca and Mantaro, Peru) and the altiplanic pattern (northern Altiplano, Bolivia). Within-comparison of the values of the *F. hepatica* populations shows a general overlap between them, regardless of the geographical area of origin. The first common principal component of the five populations can be interpreted as a measure of overall size. The results show that *F. hepatica* populations of the Cajamarca and Mantaro valleys (Peru), from Spain as well as the experimental *F. hepatica*

standard population have both a similar maximum and minimum size. On the contrary, the northern Bolivian Altiplano population shares its maximum size with the aforementioned *F. hepatica* populations but presents a lower minimum size. Our results indicate that liver flukes from Peru and Europe (natural and experimental) have a common minimum size from which the parasites begin to be gravid. However, this minimum size is smaller in Bolivian liver flukes. There was no consistent relationship between the size-free pattern of variation and altitudinal differences. No significant correlation between Mahalanobis distances and geographic distances was detected. The results of this study demonstrate that there is no apparent relationship between the shape of fasciolid adults with regard to altitudinal difference or geographical origin and that allometry-free shape appears as a more stable trait than size in fasciolid species. Results are analysed in terms of intensity/crowding effect aspects and permanent/seasonal transmission characteristics.

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SY07.P.05

UTERUS DEVELOPMENT CORRELATED WITH EGG-SHEDDING IN *FASCIOLA HEPATICA*

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Fascioliasis is an important human and animal disease caused by two trematode species, *Fasciola hepatica* and *F. gigantica*. At present, fascioliasis is emerging or re-emerging in numerous regions. The highest human fascioliasis prevalence and intensities are encountered in the Northern Bolivian Altiplano, where *F. hepatica* is the only fasciolid species present, and *Galba truncatula* the only intermediate snail host species. In this endemic region, sheep and cattle may be considered the main reservoir host species, with pigs and donkeys playing a secondary role. The emission of *F. hepatica* eggs in faeces is usually subject to oscillations along time in animals as well as humans. Thus, looking for alternative biological markers reflecting eggs shed per gram of faeces (epg) with lower oscillations may be useful. This study analyzes the possible relationship between liver fluke uterus area (UA) and epg. Uterus area development of adult *F. hepatica* obtained at different days post infection (dpi) in a Wistar rat model with isolates obtained from cattle, sheep, pigs and humans from the endemic human fascioliasis area of the northern Bolivian Altiplano was analyzed and compared with the epg as obtained through the Kato–Katz technique. The morphometric study of the UA of liver flukes was carried out using image analysis software. The multiple regression model shows that UA is dependent on dpi and isolate. The evolution of UA vs dpi followed a damped model. This work shows a positive relationship between liver fluke UA and egg production. The complete absence of eggs in the uteri of some parasite individuals at 300 dpi was observed, which corresponds to the cessation of egg shedding in the advanced chronic stage. This study shows a relationship between liver fluke UA and egg production, which is consistent with similar findings in other helminths. In this experimental study it was demonstrated that *F. hepatica* UA development along time fitted a saturated model, while body growth follows a logistic model characterized by two phases: the 'exponential' part of logistic growth corresponds to body development during migration through the abdominal cavity and liver parenchyma as well as to development and sexual maturation in the biliary duct system up to the onset of egg production. From this moment onwards, development follows the 'saturated' part of logistic growth with a considerable persistence of body growth after sexual maturity. Oviposition is the inflection point of the logistic growth marking the end of the 'exponential' period and the beginning of the 'saturated'

period, i.e. the beginning of egg shedding to the external environment constitutes the biological factor that marks the inflection point. The results obtained suggest the necessity to characterize the isolates employed with regard to geographical as well as host origin in fascioliasis studies in which egg production is used as a biological tag.

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SY07.P.06

EFFICACY OF TRICLABENDAZOLE POUR-ON AGAINST *FASCIOLA HEPATICA* IN COWS IN TROPICAL MEXICAN REGION

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Spanish Society of Parasitology.

Fasciolasis is the main hepatic disease of cattle worldwide. Its control is carried out by chemical, physical and biological methods. Currently, chemical control is still the best option. The objective of this study was to evaluate the effect of triclabendazole (TRZ) epicutaneous application in bovine natural fasciolasis. The experiment was performed at a cattle production ranch, in the municipality of San Rafael, state of Veracruz, Mexico. The evaluation criterion was the percentage of trematode egg-reduction after treatment with TBZ. The sedimentation technique was used in each feaces sample individually obtained, in order to determine the quantity of eggs in 5 g of feaces. A herd of 30 heads was used, all positive to F. hepatica eggs; two groups of 15 animals each were formed, balanced with regard to egg quantity. Group 1 was the control and Group 2 was the treated. TBZ was applied along the mid line of the back from the withers to the base of the tail, at a dose of 12 mg/kg equivalent to 1 ml per 10 kg. The feaces samples were collected and individually examined at days 14, 21, 35 and 56. On day 1, TBZ was applied after weighing the animals in order to use the exact quantity. The effect of the treatment on the percentage of egg-reduction was statistically analyzed using the U Mann Whitney test. The percentage of egg-reduction after treatment was 100%. It is concluded that under the conditions in which the study was carried out, the application of triclabendazole by epicutaneous route was highly efficacious.

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SY07.P.07

AMPLIFICATION AND EVALUATION OF A PHAGE DISPLAY CLONE AS IMMUNOGEN AGAINST *FASCIOLA HEPATICA* IN SHEEP

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With the objective to obtain an immunogen against *Fasciola hepatica*, three clones selected with antibodies obtained against cathepsin L, from a phage-display combinatorial library (clones 1, 5 and 20), were characterized. For this, clones were propagated and DNA nucleotide sequence was obtained, their encoding was analyzed and compared with cathepsin L sequences published in the

GenBank, using the Clone Manager Professional Suit program. Clone 5 was selected and amplified, growing it in 300 ml of *E. coli* culture in LB medium, obtaining a titer of 8.3 × 1014 pfu/ml, enough material for 2500 doses. This clone was evaluated in sheep using a dose of 1 × 1014 phages in 3 ml of PBS. For the immunogen evaluation, a lot of 20 sheep, parasite-free, were used; four groups of 5 sheep each were formed. Group (G) 1 was the control without immunization. G2 was individually immunized with a dose of 0.6 mcl; G3 with 1.2 mcl; and G4 with 1.8 mcl in 1 ml at days 1, 43 and 90. On days 115 and 171, sheep from the four groups were inoculated with metacercarie of F. hepatica. The percentage of F. hepatica egg-reduction in biliary conducts was 4.9% in G2, 0% in G3 and 26.1% in G4. The percentage of reduction in miracide formation was 79.8% in G2, 38.7% in G3 and 76.8% in G4. It was observed that clone 5, which represents a mimotope of cathepsin L, showed an effect on the reduction of the parasite charge in biliary conducts and in miracide formation. Fasciola reduction seems to be dependent on the administered dose. There was no dose-response relation on miracide formation. However, there was no egg-reduction in Group 3. This may be due to immunogen management inconsistencies. In conclusion, although it is necessary to further evaluate this immunogen, it is possible to notice that the use of cathepsin L mimotopes, obtained from phage display libraries, are promising immunogens in the control of *Fasciola hepatica*.

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SY07.P.08

FREQUENCY AND INTENSITY OF ADULT AND IMMATURE *FASCIOLA HEPATICA* IN VERACRUZ, MÉXICO

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Spanish Society of Parasitology.

Fasciolosis or liver fluke disease is caused by *Fasciola hepatica* in a wide range of mammals, also causes important economic losses in the livestock industry. In Mexico is the most important cause of decomise of livers in the tropical regions. The fasciolosis epidemiology knowledge in warm climate regions is scarce; however, it is necessary to know transmission periods, which could be interpreted by the frequency of immature and mature stages. The immature flukes are found in the liver parenchyma and the adults in the bile ducts. The young fluke is 1-2 mm length and lancet-like, when it has become mature in the bile ducts it is leaf-shaped, grey-brown in colour and is around 3.5 cm in length and 1.0 cm in width. The aim of this study was to determine frequency and intensity of adult and immature stages of *Fasciola hepatica* in livers of slaughtered bovines in an abattoir located on federal road Tlapacoyan-Martinez de la Torre, Veracruz, Mexico. During a period of 6 weeks (January to February) 492 bovine livers were examined, from which 58 were seized with macroscopic lesions of F. hepatica. The origins of bovines with positive livers were from the municipalities of Nautla, San Rafael, Martinez de la Torre, Atzalan and Misantla. The frequency in bovines from the municipalities of Nautla was 40%, San Rafael 25% and Martinez de la Torre 13%; while the average intensity was 94.57 ± 136.04 in Nautla; 58.3 ± 47 in San Rafael and 176.71 ± 130.05 in Martinez de la Torre; however, in regard to the intensity of immature forms this was 20.06 ± 28.35 in the ones coming from Nautla, 22.58 ± 49.76 from San Rafael and 19.83 ± 13.03 from Martinez de la Torre. Considering the 58 animals from the 10 municipalities, the frequency was 10.6% and the adult to immature forms ratio was 6:1.

SY07.P.09

FASCIOLIASIS IN CATTLE FROM WESTERN ARGENTINA: STUDY OF HEMATOLOGICAL AND BIOCHEMICAL PARAMETERS

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Fascioliasis is currently expanding in Argentina. In the Midwestern province of Mendoza, fascioliasis is endemic with high prevalences in livestock, human cases reported and three species of snail vectors: *Lymnaea viator*, *Galba truncatula* and *Lymnaea neotropica*. Due to the lack of publications of cattle fascioliasis in the region, our objective was to detect the presence of *Fasciola hepatica* in cattle and describe the haematological and biochemical parameters in infected animals. Faecal samples were collected from the rectum and blood samples were obtained from thirty-three bovines from two regions: 1) an endemic mountainous region with animal and human cases reported and the presence of vectors; 2) a non-endemic plains region with no human or animal reports or snail vectors. Faecal samples were analyzed by a rapid sedimentation technique to determinate the presence and number of eggs per gram (EPG) of *F. hepatica*. Haematological studies where performed in an automated blood cell counter (Abacus Junior Vet®) and blood smears stained with Giemsa for the differential leukocyte count. For biochemical parameters an automatic analyzer (INCCA®) was used according to standard procedures. F. hepatica eggs were found in nineteen (60%) of the animals from the endemic region (15 ± 9.49 EPG) and no positive animals were detected from the non endemic region. In haematological studies infected animals had significantly lower (p<0.05): hemoglobin, total leukocytes, monocytes and neutrophil counts. Infected animals had significantly higher values of urea, creatinine, GT, albumin, A/G ratio, bilirubin and lower values of AST, ALT and globulins. Both anaemia and hypoalbuminaemia are common in heavy infections; the low EPG counts might explain why these changes were not present. In the infected animals, the absence of elevation in AST and ALT activity, and the presence of significant high levels of GT, can indicate a biliary stage, since ALT and AST increase during the parenchymal stage of the disease. The most outstanding result was the low white blood cell count and low globulins in parasitized animals, contrary to the leucocytosis and hyperglobulinaemia described by other authors. A possible explanation would be an immune suppression caused by *F. hepatica*.

SY10.P.01

SPECIFIC ANTI-HYDATID IGG SUBCLASSES FOR THE DIAGNOSIS OF PRIMARY INFECTION AND RELAPSES OF CYSTIC ECHINOCOCCOSIS

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Background: Hydatidosis is a public health problem in Tunisia responsible of a high annual surgery incidence, which is estimated at 15 cases per 100000 inhabitants. Serological diagnosis of hydatid disease faces problems of sensitivity. Moreover, the long time persistence of IgG after surgical excision of cystic echinococcosis complicates also the postoperative monitoring making difficult the early detection of recurrences. The use of IgG subclasses seems to overcome these difficulties. The aim of the current study was to evaluate the contribution of IgG subclasses in the diagnosis of both primary infested (PI) and relapsed hydatid cysts (RC) patients.
Methods: Sixty eight patients operated for liver hydatid cyst were recruited from surgery units. Thirty four had no previous history of surgery for hydatid disease (PI) and thirty four presented a recurrence within the 5 years after an anterior resection of liver hydatid cysts (RC). Twenty healthy volunteers were enrolled as healthy controls (HC). They had no past history of hydatidosis and had a normal abdominal ultra sound image. Enzyme-linked immunosorbent assay based on fluid anti-hydatid antigens (HA) was performed to detect specific IgG1, 2, 3, 4 subclasses. Sensitivities and specificities of IgG subclasses were estimated using ROC curves.

Results: Anti-HA IgG1, anti-HA IgG2 and anti-HA IgG4 antibodies were able to distinguish between PI patients from HC whereas only anti-HA IgG2 and anti-HA IgG4 antibodies discriminate RC patients from HC. ROC curves analysis demonstrated that IgG4 antibodies are also statistically efficient to discriminate between RC (sensitivity of 97.1%) and PI (sensitivity of 70.6%) patients (*p=0.008*).
Conclusions: Anti-HA specific IgG2 antibodies revealed suggestive of primary hydatid infestations while IgG4 were significantly associated to relapses. The characterization of the antigens involved should be useful for early detection of post-surgical hydatid recurrence.

SY10.P.02

MITOCHONDRIAL SEQUENCE DIVERSITY OF *ECHINOCOCCUS MULTILOCULARIS* ISOLATES FROM HUMANS IN SOUTHERN GERMANY

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Echinococcus multilocularis exhibits rather low genetic diversity on a worldwide scale. Based on mitochondrial sequences and microsatellite profiles, three main clusters have previously been identified, which can approximately be associated with a geographical occurrence in Europe, North America and eastern Asia, respectively. However, minor variants (mt haplotypes and ms profiles) can be distinguished even within these clusters, and this has already provided valuable information and hypotheses on the geographical history of this parasite in Europe. Most of the isolates characterized so far were of animal origin. Samples from human patients are difficult to obtain and process, because most specimens had been fixed in formalin and stored as paraffin-embedded tissue blocks which often prevents extraction of high quality DNA. Here, we present mitochondrial sequence data from a series of 32 histological specimens, kept in paraffin, from *E. multilocularis* metacestode tissue of human origin from the University Clinic of Ulm. In all of these samples, a 373 bp fragment of the 12S-rRNA gene could be amplified, while longer fragments (530 bp of nd1, 806 bp of atp6 and 875 bp of co1) could be amplified in 26, 18 and 16 of these samples. Based on the co1 fragment, on which the largest body of comparative data is available, most of our isolates (n=12) were identified as a haplotype which was previously shown to be the most frequent variant in animal hosts in Europe (Šnábel et al.; Schroer et al., unpublished), and which, within our sequence, is identical to the haplotype E5 of Nakao et al., 2009. Three of our isolates showed a variant co1 sequence (1 fixed base exchange) that was found before only once by Šnábel et al. (unpublished) in a fox from Hungary, the sequence of one isolate was not reported before. Some variability was also found within the atp6 fragment. To be able to draw conclusions on possible correlations between haplotypes and host preference, or geographical distribution, studies are in progress to characterize a larger panel of isolates from selected regions of Europe.

SY10.P.03

CROSS REACTION BETWEEN THE CRUDE HYDATID CYST FLUIDE (HCF) ANTIGENS OF HUMAN AND ANIMALS ORIGIN (MICE, SHEEP, CATTLE) IN RESPONSE TO HUMAN IGG CLASS, IGG SUBCLASSES AND IGM ANTIBODIES

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The use of crude hydatid cyst fluid (HCF) antigen for the diagnosis of hydatid cystic is one method which along with immediate serological investigation can be helpful and effective in rapid treatment of the disease. However, there is still no standard, highly sensitive, and specific serological test for antibody detection in cases of human cystic echinococcosis (CE). The current study aimed to evaluate the cross-reactivity of human sera against crude hydatid fluid antigens of animals in order to find the

target antigens with the highest IgG class, IgG subclasses and IgE response from the human immune sera.
Human and animal crude HCF used as the source of antigen for performing ELISA, Western blotting and immunization the mice. Sample sera were collected from patients who recently had hydatid surgery in hospitals as human cases group together with some human or animal sera with no history of hydatidosis with negative HC using ELISA and IFAT as control groups. Totally 30 positive samples sera from each animal and human sources were used as the case together with 30 healthy sera from each as control group. SDS PAGE gel electrophoresis was carried out under reducing conditions. ELISA was carried out as described by Verastegui.
The highest mead OD value of the human IgG class antibody was against antigen B (0.93) and the lowest against cattle HCF antigen (0.32). The difference between responses to these antigens were statistically significant (p<0.001). ELISA revealing that the highest mean OD value in response to human , sheep and mice HCF antigen was related to IgG4 while the lowest to IgG3. The sensitivity and specificity of ELISA test that was used for evaluating the responses of human total IgG to different HCF antigen was 100% and 95.8% respectively. Cross –reaction of human IgG class and subclasses and IgE response was found almost for all antigens with the best reaction against human HCF antigen and antigen B.
Human sera showed a considerable cross-reactivity against human, sheep, cattle and mice HCF antigens by ELISA test. The human HCF and antigen B were superior over other antigens in that their mean OD values and their OD ratio significantly higher than that for the others.
Keywords: hydatid cyst fluid, antigen, human, animals.

SY10.P.04
USE OF THE RECOMBINANT ANTIGEN 2B2T IN A COMMERCIAL IMMUNOCHROMATOGRAPHIC TEST FOR THE DIAGNOSIS OF CYSTIC ECHINOCOCCOSIS

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The lateral flow immunochromatographic assay is a simple diagnostic technique with low production costs, easy to transport and handle, therefore with potential applications in the diagnosis of parasitic diseases.
An immunochromatographic test based on the detection of specific serum/plasma antibodies against native echinococcal antigens has been developed for the diagnosis of cystic echinococcosis (CE) by Vircell SL (VIRapid® HYDATIDOSIS; Santa Fe, Spain). However, diagnostic tests based on native antigens have low specificity due to frequent cross-reactions, and native antigens themselves may be difficult to obtain. Replacement of native antigens by recombinant polypeptides could overcome these drawbacks and increase the specificity and sensitivity of the test.
A new recombinant antigen called 2B2t, constructed by cloning tandems of two units of the antigen B2t (Hernández-González et al, 2008) was applied in the test line to the above-mentioned immunochromatographic strips. These were compared with the VIRapid® HYDATIDOSIS original test containing the native antigen in the test line using a panel of 385 sera from patients with CE of the liver in different stages (active –CE1, CE2-, transitional –CE3- and inactive –CE4 and CE5-), 36 sera from healthy donors, 50 sera from patients with alveolar echinococcosis and 4 pools of sera from neurocysticercosis patients.
Test performances were compared using ROC curves (sensitivity *vs.* 1-specificity) and the corresponding area under the curve (AUC). The sensitivity of the strips was evaluated in relation to the clinical characteristics of patients by multivariate logistic regression. Results were also compared with those obtained with the 2B2t antigen in ELISA.
The ROC curve analysis showed that the results from the strips containing the 2B2t antigen produce the largest AUC, compared with those from the strips containing the native antigen and the 2B2t-ELISA. The sensitivity of the three tests was strongly influenced by the cyst stage according to the

WHO IWGE ultrasound classification, with higher number of positives for active and transitional cysts than for inactive cysts. The use of recombinant antigen 2B2t in the immunochromatographic assay developed in collaboration with Vircell improves the overall diagnostic usefulness over that based on native antigens, showing similar sensitivity for active and transitional cysts (~95%), and higher specificity (81.1% vs. 57.8%) than strips containing native antigens.

SY10.P.05

GENOTYPES OF ECHINOCOCCUS GRANULOSUS VOMPLEX IN CENTRAL-EASTERN EUROPE

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Cystic echinococcosis (CE) is regarded as re-emerging zoonosis in several countries of Eastern Europe and Eastern Asia. To obtain a more complex view about genotype spectrum circulating of the causative agent *Echinococcus granulosus* complex, three mitochondrial genes were used to identify in four countries of Central-Eastern Europe. In Northeastern Ukraine (Sumy region), in three of 5 examined pig isolates the G7 microvariant was detected, manifesting two nucleotide differences in mitochondrial *cox1* gene compared with characteristic G7 structure (currently attributed to *E. canadensis*, lower infectivity to humans). The identical non-synonymous substitutions were also recorded in one G7 isolate from Southeast Slovakia (Trebišov district). Remaining 20 Slovak isolates under study (18 pig and 2 human isolates) bear sequence profiles with a typical G7 pattern. Among isolates from two provinces (Caras-Severin, Timis) in Western Romania, in human isolates 2 displayed G7 genotypes and 2 *E. granulosus* sensu stricto (G1, G3 genotypes, attributed to *E. granulosus* sensu stricto with high infectivity to humans). In addition, cattle isolate had G1 microvariant and pig isolate exhibited G7 genotype. In Eastern part of the country (Vaslui and Iasi provinces), both human isolates exhibited G1 genotype (sample from the former province had microvariant G1E). G1 genotype (G1A microvariant) was also found in Hungary in Sheghalom (Békes County) in a Central part of the country with the extensive sheep breeding.
In Central-Eastern Europe, transmission of G7 genotype in sylvatic conditions is provided also by wild boars as was documented by findings of Kedra et al. (2000) who detected this intermediate host harbouring this variant in Sumy region in Ukraine, and Dinkel et al. (2006) who confirmed parasite in isolated focus of North-Eastern Germany (Brandenburg county). A relatively high proportion of G7 in humans in Romania pointed out for its establishment in this host in areas highly contaminated with G7 by farm animal hosts. The study indicated the trend of spread of the infective *E. granulosus* sensu stricto from endemic Mediterranean area Northern into the Eastern-Central European territory as was corroborated by several records in Hungary and Romania in areas previously free of this species.

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SY10.P.06

INTER- AND INTRASPECIFIC DIVERSITY OF *ECHINOCOCCUS* SPP. IN SOUTHERN KENYA

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Considerable genetic polymorphism of *Echinococcus granulosus* sensu stricto (cluster G1-G3) was recently demonstrated in parts of Europe, Asia and South America. A rather large number of mitochondrial haplotypes based on the cytochrome c oxidase subunit 1 (cox1) gene was found in western Asia and Eastern Europe, while this diversity was decreased in Southern Europe, Eastern Asia and Peru. This has lead to tentative hypotheses concerning the origin and zoogeography of this taxon. Here, we contribute preliminary data on the haplotype diversity of *E. granulosus* s.s. from sub-Saharan Africa, using a panel of livestock isolates from Southern Kenya (Maasailand). The genetic polymorphisms were analysed by partial sequencing of an 1123 bp long segment of the mitochondrial cytochrome c oxidase subunit 1 (cox1) gene. A total of 251 hydatid cysts had been obtained in two abattoirs (Suswa and Kitengela) from different organs (liver, lung, heart, spleen, kidney) of 141 goats (n=4), sheep (n=21) and cattle (n=68). As a first step, the *Echinococcus* species were determined by RFLP-PCR of the NADH dehydrogenase subunit 1 (nad1) gene. The dominating species was *E. granulosus* s.s., (245 samples), followed by *E. canadensis* (5) and *E. ortleppi* (1). For the analysis of the genetic polymorphisms within *E. granulosus* s.s., partial cox1 gene sequences were obtained from 141 of these isolates. In total, 42 haplotypes could be found in this very restricted part of Kenya alone. These preliminary results are compared with data from other regions, and implications concerning the role of sub-Saharan Africa in the distribution history of this parasite are discussed.

SY10.P.07

CYSTIC ECHINOCOCCOSIS IN SLAUGHTERED CATTLE IN SARDINIA: A RETROSPECTIVE EPIDEMIOLOGICAL STUDY AND SPATIAL ANALYSIS

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Cystic echinococcosis (CE), caused by *Echinococcus granulosus*, is an important zoonotic, parasitic infection causing morbidity and mortality in humans apart from significant economic losses in livestock. The parasite lifecycle includes dogs and other canids as definitive hosts, whilst sheep and numerous ungulates (cattle, goats, pigs, etc.) are intermediate hosts harbouring the hydatid cysts . Dogs usually acquire the infection from hydatid-carrying livestock (especially sheep) as a result of being fed with infested offal (liver and lungs) by owners who practise homeslaughter .Cystic echinococcosis (CE) in cattle was found in 246 out of all 377 municipalities in Sardinia, Italy. Out of 32,685 bovines slaughtered in Sardinia in 2009, 1,360 were found to be positive for CE with a registered average prevalence of 4.2%. Of these animals, 896 (66%) had lived on the same farm from birth to slaughter, thus linking the infection to the farm with certainty, while 413 (30%) had lived on two different farms (one transfer) and 51 (4%) on three (two transfers). As it was not possible to assess in which farm the animals acquired the infection, all farms having kept infected cattle were considered as suspected sources of CE infection. Based on this classification, 534 farms were listed as definitely infected with a further 495 suspected to also be infected. Scan statistics was used with the Bernoulli model to detect and evaluate clusters of infected farms and also clusters of "non-cases". For the spatial analysis, 1,029 farms (534 + 495) were considered as positive with the number of non-infected farms from which negative results were available (8,457) as controls. A most likely cluster was detected at latitude 39.47861 N and longitude 8.58216 E in a centroid of 97.92 km radius and a secondary cluster was detected at latitude 40.58890 N and

longitude 8.98400 E in a centroid of 15.44 km radius. To address the issue of sensitivity and consistency of the results, we ran multiple scans with various max-sizes as this allowed us to achieve more valid, consistent results and to highlight the core clusters.

SY10.P.08

A RETROSPECTIVE STUDY ON BURDEN OF HUMAN ECHINOCOCCOSIS BASED ON HOSPITAL DISCHARGE RECORDS FROM 2001 TO 2009 IN SARDINIA, ITALY

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The purpose of this study was to estimate the burden of human CE in Sardinia. Such an estimate is imperative since it can be used as a tool to prioritize control measures for this essentially preventable neglected disease. Recent studies suggest that this disease has a large social impact on endemic areas, and estimates of burden in terms of monetary and no-monetary impact on human health are essential to allocate financial and technical resources. In this work, a retrospective study was carried out using public Hospital Discharge Records drawn from the regional database between 2001 and 2009. During these years, a total of 1409 discharges were recorded, 1196 (84.88%) records corresponding to primary diagnosis, that is patients hospitalized for symptoms directly correlated to CE, and 213 (15.11%), records corresponding to secondary diagnosis, that is patients hospitalized for symptoms not directly correlated to CE and with an afterwards or concurrent diagnosis of echinococcosis made during the hospitalization, with an annual regional average record of 9.5 per 100,000 inhabitants. Direct cost associated with diagnosis, surgery or chemotherapy, medical care, and hospitalization in humans were evaluated in this work. Furthermore, indirect costs were also evaluated by using the disability-adjusted life years (DALYs), the preferred disease-burden measure of the World Health Organization. During the reporting period, the direct cost for 1266 OH was € 6,625,453.40 distributed as € 4,561,244.00 (range € 381,555.77 in 2005 and € 783,628.43 in 2001) for 515 OH with surgical procedures and € 2,064,209.40 (range € 87,660.83 in 2001 and € 320,444.86 in 2009) for 751 OH with medical care. The mean cost of a single OH was € 5316.85 (range € 4171,43 in 2006 and € 7161.37 in 2002) considering an average length of hospital stay of 15.1 days. The direct cost for 143 DH was € 91,396.53 (range € 5175.35 in 2003 and € 15,996.88 in 2006) with a mean cost for each DH of € 653.77 (range € 345.02 in 2003 and € 1143.00 in 2009). From 2001 to 2009 the total direct cost (OH plus DH) for Echinococcosis in Sardinia was € 6,716,849.93 corresponding to a mean cost of € 746,316.65 per year. These data confirm the high prevalence of human echinococcosis in Sardinia and highlight the importance of implementing a continuous and more effective control programme. More accurate data on CE prevalence in humans (particularly undiagnosed or asymptomatic cases) are needed, and the activation of correct reporting measures for this infectious disease, together with the implementation of the Community Network under Decision n° 1219/98/EC of the European Parliament and Council, is of considerable importance.

SY14.P.01

DISCRIMINATION OF *CULICOIDES* OF *AVARITIA* SUBGENUS BY MULTIPLEX PCR

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Culicoides species of the *Obsoletus* group are important vectors of viral diseases as *Bluetongue*, *Schmallenberg virus*, *African horse sickness,* etc. Considering the rapid spread of these vector-borne pathogens in recent years we examined the *Culicoides* population in eastern Slovakia with

the aim to confirm the potential presence of biting midges that serve as vectors of important pathogens.
Whereas the diagnostic characters of two undistinguishable species *C. obsoletus* and *C. scoticus* are morphologically overlapping, we performed the multiplex PCR based on analysis of ITS-2 sequences of 6 *Culicoides* populations sampled in 3 localities across eastern Slovakia. The molecular analysis of 5 samples from 2 localities showed explicit results for *C. obsoletus* and *C. scoticus*. In the third trapping site, 5 individuals showed band characteristic for both, *C. obsoletus* and *C. scoticus* species. For genotyping we performed the multiplex PCR based on ITS-1 sequences. Three investigated individuals belonged to *C. scoticus* and two to *C. obsoletus*. We analysed ITS-2 sequences in 4 individuals assumed to be *C. chiopterus*, 2 of them were identified as *C. scoticus*, whereas the ITS-1 analysis diagnosed them as *C. chiopterus*. One individual showed ITS-2 pattern typical for *C. obsoletus*, while ITS-1 analysis revealed pattern typical for *C. scoticus*. The ITS-2 analysis of fourth individual did not identify any species and ITS-1 sequences were characteristic for *C. scoticus*.
Due to ambiguous genotyping results, further sequencing analyses are needed to confirm the species of investigated *Culicoides* individuals.

The work was supported by the Slovak Grant Agency VEGA No. 1/0236/12, under the basic research project NRL UVLF for pesticides and Italian Ministry of Health.

SY14.P.02

FIRST DETECTION OF TICK-BORNE ENCEPHALITIS VIRUS IN *IXODES RICINUS* TICKS COLLECTED IN FRANCE SINCE THE 70S

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Tick-Borne Encephalitis (TBE) is caused by a virus, belonging to the *Flavivirus* genus. In the natural environment, TBE virus (TBEV) is maintained in a cycle involving *Ixodes ricinus* for Western European subtype and small wild rodent hosts. Moreover, ticks remained infected throughout their life cycle and transmit virus to uninfected ticks when co-feeding on small wild rodents. Human are accidental hosts and the maximum incidence of human infections coincides with seasonal peaks of feeding activity of the ticks. Usually, TBE endemic areas are limited to strict regions (foci) where TBEV circulates through the tick and the vertebrate populations. Unlike Germany, Switzerland and Italy, the number of human cases in France has not increased last decade. Although *I. ricinus* is present over a large area of France, the majority of French cases of TBEV infection have been described in Alsace (East Department of France). Unfortunately last data about TBEV detection in French ticks had occurred in the 70s.
To better evaluate TBE situation in France, an epidemiological study has been performed in human foci in Alsace. Prevalence of TBEV in ticks has been estimated in three collecting sites (Wasselonne, Hohbuhl and Murbach woods) defined from three recent French human cases infected by tick bite.
I. ricinus (adults and nymphs) were collected by dragging low vegetation three times per year in 2010 and 2011. TBEV has been detected by Realtime RT-PCR amplifying a fragment of the TBEV 3'-UTR. Among the 776 nymphs, 20 females and 27 males collected in 2010, three batches of five nymphs from Murbach wood were positive for TBEV, with a minimal infection rate (MIR) (if we considered only one positive tick per batch) of 0.54% (3/558 nymphs). In 2011, 5467 nymphs, 129 females and 159 males were collected. Again, TBEV was only detected in ticks from Murbach wood, with a MIR of 0.48% for nymphs (19 batches of 15 nymphs among 3980 nymphs), 2.25% for females (two batches of three females among 89 females) and 2.75% for males (three batches of three males among 109 males).
Results are under confirmation by nested PCR targeted the envelop gene of the virus and sequencing. Moreover, TBEV isolations are attempt by passages on mammalian cells and

embryonic eggs. To conclude, this work will be the first detection of TBEV in *I. ricinus* ticks in France since the 70s.
Keywords: TBEV, *Ixodes ricinus*, Alsace, France.

SY14.P.03
SOME EPIDEMIOLOGICAL AND CLINICO- BIOLOGICAL ASPECTS IN HUMAN NEUROBORELIOSIS

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Lyme disease is a multisystem inflammatory disease, with uneven geographical distribution and seasonal occurrence of cases, influenced by environmental conditions, the climatic changes, the presence of vegetation, the people in outdoor activities and uneven due to the presence of different *Borrelia* genospecies, the most common vector-borne disease, that tick. Romania belongs to countries with favorable climate for infection. Like other infections transmitted by spirochetes, Lyme disease evolves in stages with a wide range of signs and symptoms of each stage: localized infection with erythema migrans accompanied by flu-like symptoms, fever, headache, myalgia, fatigue, with the emergence of disseminated infection in weeks/months from the onset of the disease neurological and cardiac manifestations, about 15% of patients and persistence infection, when patients develop chronic arthritis, chronic cardiac or neurological manifestations. The study was conducted on a group of 32 patients hospitalized with Lyme disease in 2007-2011, at the Hospital of Infectious Diseases and Neurology Hospital of Braşov, monitoring only those patients with neurological manifestations that have been pursued clinical features, biological, imaging found in various stages of the disease evolution. Neurologic examination have revealed a variety of clinical signs, most people cast more than one neurological manifestation, with a varied clinical picture and age distribution. Age group most affected was over 40 years, migratory erythema, being present at only 50% of patients at onset, type IgG ELISA serology, being present in 62% of patients with long neuroboreliozis which confirms the evolution of the disease.

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SY14.P.04
CURRENT DISTRIBUTION AND PREDICTED RANGE EXPANSION OF THE SAND FLY VECTOR *PHLEBOTOMUS NEGLECTUS* (DIPTERA, PSYCHODIDAE) IN THE EASTERN MEDITERRANEAN

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Phlebotomine sandflies are exclusive vectors of *Leishmania* parasites, the causative agents of leishmaniasis, a worldwide vector borne zoonotic disease. Visceral and canine leishmaniasis are particularly important, which have an increasing incidence in the last decades, with more than 700 autochthonous human cases reported in the Mediterranean region each year. The research conducted within the scope of this study focused on defining the distribution of *Phlebotomus neglectus* the most important vector of *Leishmania* parasites in the Mediterranean region. Previous research has suggested that this species is synanthropic and peridomestic, which may have particular epidemiological significance after successful isolation of *Lesihmania infantum* from females of *P. neglectus* from endemic foci of visceral leishmaniasis in Greece and Montenegro.

This finding provided the impetus to conduct a detailed study of the biology of this sand fly species. Data on *P. neglectus* published previously were combined with our latest findings to generate a detailed distribution map, setting the western most point of its distribution range in northern Italy, the eastern most point in southeast Turkey, spreading northwards up to Budapest, Hungary, while the area of West Bank, Palestine, delineates its south most border. Comparison with previous data, there is a noticeable expansion of the distribution range towards the western Mediterranean and Central Europe. Maps that have been generated using the ArcView software, based on Geographical Information Systems (GIS), were founded on faunistic studies of sand flies collected in 78 localities in the Central and Eastern Mediterranean, as well as on epidemiological data for 29 main epidemiological foci of visceral leishmaniasis from the same region. These maps strongly suggest the connection between the distribution of *P. neglectus* and endemic foci of both visceral and canine leishmaniasis in the Central and Eastern Mediterranean. They also show an apparent correlation of the distribution pattern of this species and the geographic characteristics of the area, such as the temperature and the presence of larger water bodies. The generated GIS maps also allow for the prediction of the range expansion of this species further inland in Europe and the Middle East along the basins of the main rivers, if the global climate warming trend continues, and consequently, the expansion of the pathogen range and emerging of autochthonous leishmaniasis in areas where it was not recorded previously.

SY14.P.05

SANDFLIES OF THE SUBGENUS *LARROUSSIUS* IN THE REGION OF EL HAOUZ, MOROCCO

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In Morocco, leishmaniasis is a parasitic disease, known since the years 1900s (Foley *et al.,* 1914; Colonieu, 1931). Transmitted by phlebotomine sand flies (Diptera: Psychodidae), it still presents a real public health problem (Moroccan Ministry of Health, 2011).
The aim of the current study is to evaluate the risk for *Leishmania infantum* in the region of El Haouz, declared as a focus of cutaneous leishmaniasis caused by *Leishmania tropica* (Ramaoui *et al*., 2008; Boussaa *et al*., 2009). For this, an entomological survey was carried out between 2006 and 2008, by using sticky traps, at three localities (Akhlij, Amkhlij and Khmiss).
Three species of the subgenus *Larroussius* are incriminated for *L. infantum* transmitting. There are*Phlebotomus perniciosus* and *P. ariasi* proven vectors of *L. infantum* and *P. longicuspis* suspected one (Rioux *et al*., 1984, Killick-Kendrich,1990).
Our investigations showed the presence, in El Haouz region, of *Phlebotomus (Larroussius) perniciosus* (59.5 %), the most prevalent species, followed in decreasing order of prevalence by *P*. (*L*.) *longicuspis* (36.5%) and *P*. (*L*.) *ariasi* (4%). The sex ratio was in favor of males for all species.
All *Larroussius* collected on the three stations and for 3 years is 689 sand flies (20.66%), so the percentage of each species relative to the total is 12.32% (*P. (L.) pernicious*), 8% *P. (L.) longicuspis*, 0.32% *P.(L.) ariasi*.
No obvious morphological anomalies were observed in the sandflies collected but all specimens of *P. perniciosus* examined showed single-pointed aedeagi curved at their apices, indistinguishable from the atypical morph of *P. perniciosus* (Pesson *et al*., 2004). The count of the number of coxite hairs for *P. perniciosus* (mean = 13) and *P. longicuspis* (mean = 25) confirms our identification (Boussaa *et al*.,2008).
The statistical analysis, using ANOVA test, showed significant differences (p<0.05) in species distribution according to localities, months and study years.

SY14.P.06

PREVALENCE OF IXODID TICKS ON CATTLE AND SHEEP IN SISTAN AND BALUCHESTAN PROVINCES, IRAN

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Ticks are hematophagous arthropods belonging to the Class Arachnids. Once they attach to a host for a blood meal, they can cause skin irritation and anemia. Ticks are also one of the major vectors of pathogens, such as *Babesia*, *Theileria*, and *Anaplasma* spp., to animals in the world. It is important to know the prevalence of the tick species involved on the transmission as well as their geographical distribution for the control of tick and tickborne diseases. and tick-borne diseases affect animal and human health worldwide and are the cause of significant economic losses. A survey was carried out to investigate the prevalence of hard tick species (Acari: Ixodidae) on cattle and sheep in southeast of Iran. A total of 1403 ticks were collected from 332 infested cattle and 1480 ticks were collected from 602 infested sheep during activating seasons of ticks in 2010–2011. The species collected from cattle were *Hyalomma marginatum marginatum* (46.04%), *Hyalomma anatolicum excavatum* (25.51%), *Hyalomma anatolicum anatolicum* (10.33%), *Hyalomma asiaticum asiaticum*(6.34%), and *Rhipicephalus sanguineus* (11.76%) while the species collected from sheep were *R. sanguineus* (34.66%), *H. marginatum marginatum* (25.60%), *H. anatolicum excavatum* (27.97%), *H. asiaticum asiaticum* (9.45%), *Hyalomma detritum* (2.29%), The results show that, *H. marginatum marginatum*, *H. anatolicum excavatum*, and *R. sanguineus* are dominant tick species in the surveyed area.
Keywords: Ixodidae, Ticks, Sistan and Baluchestan, Iran.

SY14.P.07

THE PRESENCE AND GENETIC VARIABILITY OF *ANAPLASMA PHAGOCYTOPHILUM* AND CANDIDATUS *NEOEHRLICHIA MIKURENSIS* IN RODENTS AS RESERVOIR HOSTS IN SLOVAKIA

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Rodents are important reservoir hosts in the circulation of many tick-borne pathogens including *Anaplasma phagocytophilum*. Granulocytic anaplasmoses represent a group of emerging tick-borne zoonotic diseases caused by the intracellular bacterium transmitted mostly by *Ixodes ricinus* ticks. In US, rodents are reservoir hosts of *A. phagocytophilum* genotypes that are pathogenic for humans. Recently described *Candidatus neoehrlichia mikurensis* is another tick-borne pathogen of public health importance from this family. The aim was to characterize the presence and genetic variability of bacteria from the *Anaplasmatacae* family circulating in natural foci between the rodents and two tick species *I. ricinus*, *I. trianguliceps* and to study their ecologic associations. *Candidatus* N. mikurensis was detected in questing *I. ricinus* and spleens of rodents. *A. phagocytophilum* was detected in questing *I. ricinus* ticks from all studied sites, rodent feeding *I. trianguliceps*, ear and spleen biopsies of rodents (*Myodes glareolus, Apodemus flavicollis*). In areas where *I. trianguliceps* were absent we did not detect *A. phagocytophilum* in rodents. None of the feeding *I. ricinus* ticks from rodents were infected with *A. phagocytophilum* even though it was feeding on the infected rodent. Phylogenetic analysis of four genetic loci in positive samples has shown that genotypes in questing *I. ricinus* were distinct from genotypes found in rodents and feeding *I. trianguliceps*. Msp4 and DOV sequence of *A. phagocytophilum* genotypes showed considerable heterogeneity but none of the positive questing *I. ricinus* tick was infected with the

rodent genotype that was identical to the genotype found in *I. trianguliceps*. The 16S rRNA gene in rodents was represented by two variants. Our study from Central Europe confirms the previous findings from UK that in Europe *A.phagocytophilum* variants associated with rodents are transmitted by *I. trianguliceps* and not *I. ricinus* ticks.

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SY14.P.08

BREED CHARACTERISTICS OF HOST INFLUENCE THE INTENSITY OF TICK INFESTATION IN RUMINANT LIVESTOCK

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Tick (Acari: Ixodidae) infestation is a serious menace to the livestock industry of temperate countries. We investigated the influence of breed of host on the quantitative tick burden in ruminant livestock population of lower Punjab, Pakistan and found a statistical association with the intensity of hard tick infestation. In the target sites of lower Punjab province, Pakistan, available breeds of cattle (*Bos indicus* and *Bos taurus*) include: Jersey, Friesian, Sahiwal and Cross-bred; buffaloes (*Bos bubalus bubalis*) include: Nili-Ravi and Kundi; Goats (*Capra hircus*) include: Beetal, Nachi, Dera Din Pannah (DDP) and Teddy. The peculiar breed characteristics observed in relation to tick intensity were skin thickness, hair length and hair density. In cattle, the prevalence was found to be highest in Friesian (*B. taurus*) (86.16%; 172.3/200), followed in order by cross-bred (*B. indicus* x *B. taurus*) (84.16%; 168.3/200), Jersey (*B. taurus*) (71.83%; 143.60/200) and Sahiwal (*B. indicus*) (23.16%; 46.33/200). In buffaloes, there was an insignificant difference (p = 0.513) in prevalence percentage of Nili-ravi (36.55%; 71.33/200) and Kundi (44.5%; 89/200) breeds. In goats, the difference in prevalence percentage was found to be significant (p = 0.00) being highest in Beetal (71.3%) followed in order by cross-bred (70.3%), Nachi (62.6%), DDP (31%) and Teddy (23%). Determination of logarithmic relationship of hair length, hair density and skin thickness of animals with the intensity of tick infestation revealed a negative statistical association (RR=5.69). The study provided a baseline data for planning of prophylactic measures for specific herds.
Keywords: Breed; Ticks; Goats; Buffalo; Cattle; Pakistan.

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SY14.P.09

QUESTING TICKS (IXODIDA) COLLECTED DURING EXPEDITION IN CROATIA 2011

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Ticks collected at 18 locations of Croatia by flagging method during expedition in May 2011 (5.-11 May 2011) are presented. The aim of the expedition was the mapping of tick communities and their relative density in Croatia on the locations with important recreational exploitation. More intensive research has been concentrated in two recreational areas - around the Plitvice lakes and the Adriatic coast (Dalmacia) in the vicinity of Promajna and Makarska Riviera.

Together were registered 1702 ticks belonging to five species: *Ixodes ricinus*, *Dermacentor reticulatus*, *D. marginatus*, *Haemaphysalis punctata*, *Rhipicephalus sanguineus*. The predominant species was *Ixodes ricinus* (376 males, 357 females, 630 nymphs), tick species was recorded at 10 sites and occurs mainly in the northern part of Croatia as well as in surroundings of Plitvice Lakes area. Next two ticks, *D. reticulatus* (69 males, 100 females) were registered at 5 locations and *D. marginatus* (18 males, 30 females) at 6 locations. Interestingly is the co-occurrence of both species at 4 sites (Draganić, Bunic, Grabovac, Stara Krslja), habitats were represented by grassland and meadows with different level of humidity. At three xerothermic grassland sites (Mašvina, Bunic, Gospic) we recorded together 25 individuals of *H. punctata* and occurred with other 3 tick species - *D. marginatus*, *I. ricinus* and partially with *D. reticulatus*. For offshore, coastal regions was a characteristic only one species *R. sanguineus*, we recorded 97 specimen at 7 localities studied.
Tick fauna of Croatia in the spring reflected the predominant habitat type, i.e. in mountain type with dominance of beech-forests dominated *I. ricinus*, in the central part of country with a predominace of plains and pastures with the co-occurrence of *D. marginatus*, *D. reticulatus* and *H. punctata*. The seaside area with a predominance of dry xerothermophilous formations is typical of the occurrence of *R. sanguineus*.

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SY14.P.10

DETECTION AND PREVALENCE OF SIBLING SPECIES OF THE *ANOPHELES MACULIPENNIS* COMPLEX (DIPTERA: CULICIDAE) IN THE REPUBLIC OF MOLDOVA

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Anopheles maculipennis complex comprises of reproductively isolated but morphologically similar species in the northern hemisphere with different medical importance as vectors for malaria parasites. It is known that *An. sacharovi* is one of the most important malaria vectors which prefer feeding on humans, followed by *An. atroparvus* which prefers domestic animals but readily feeds on humans. *An. messae* and *An. maculipennis* s.s. are less important malaria vectors feeding mainly on cattle and domestic animals. *An. melanoon* is not considered as malaria vector. In the past *An. messae*, *An. maculipennis* s.s. and *An. atroparvus* were supposed as principal vectors of *Plasmodium vivax*, *P. falciparum* and *P. malariae* in the Republic of Moldova.
The aim of the study was to establish the presence of sibling species of the *Anopheles maculipennis* complex by PCR assay and reveal their prevalence in different locations of the country.
Anopheles mosquitoes were collected from April to November in 2009, 2010 and 2011 from 10 locations from the northern, central and southern parts of the republic, including three natural reserves: “Codrii”, “Plaiul Fagului” and “Prutul de Jos” and seven localities: Chisinau, Chirileni (Ungheni), Iablona, Cotovsc, Cotul Morii, Ceadir-Lunga and Cantemir. Larval sampling was conducted using standard dipping techniques. Adult mosquito collections were conducted using aspirators and entomological nets for mosquito catches from humans, vegetation, manmade and livestock shelters.
Species genetic differentiation was done using six species-specific primers for the second internal transcribed spacer (ITS2) of the ribosomal DNA and one universal primer for conserved region 5.8S rDNA (Proft et. al., 1999). A total of 438 specimens of adults of both sexes and 4th instars larvae were examined by PCR assay, preliminary stored in 70% ethanol until DNA extraction by Fermentas DNA purification kit.
Our data showed the next prevalence and abundance of identified sibling species depending on collecting sites: “Prutul de Jos” Reserve - *An. messae* (64.7%), *An. sacharovi* (23.5%), *An. melanoon* (6.0%) and *An. maculipennis* s.s. (5.8%); “Codrii” Reserve - *An. melanoon* (78.9%), *An. maculipennis* s.s. (21.1%); “Plaiul Fagului” Reserve - *An. melanoon* (77.7%), *An. messae* (12.0%), *An. maculipennis* s.s. (10.3%); Chisinau - *An. melanoon* (64.2%), *An. maculipennis* s.s. (21.4%), *An. messae* (14.4%); Chirileni: *An. atroparvus* (58,4%), *An. melanoon* (33,3%), *An. maculipennis* s.s. (8,3%); Ceadir-Lunga:

An. melanoon (33.2%), *An. atroparvus*: (54.5%), *An. maculipennis* s.s. (12.3%); in Cotovsc and Cantemir only *An. messae* has been identified from our collections; in Iablona – only *An. melanoon*. Preliminary results revealed the presence of five sibling species: *An. messae*, *An. maculipennis s.s*, *An. atroparvus*, *An. sacharovi* and the first records of *An. melanoon* for Moldova. *An. labranhiae* was not identified. Large population of *An. sacharovi* was detected in the south of the country.

SY14.P.11

ENZYMATIC AND FUNCTIONAL CHARACTERIZATION OF THE *LEISHMANIA MAJOR* PROTEIN DISULFIDE ISOMERASE (LMPDI) AS A DRUG TARGET

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Leishmaniasis is a heterogeneous group of diseases that affect millions of people in tropical and subtropical areas of the world. Tools available for leishmaniasis control are limited: effective vaccines are still lacking, drugs are toxic and expensive, and parasites develop resistance to chemotherapy. Previous studies strongly suggest that molecules involved in assisting folding of newly synthesized proteins could constitute suitable candidates for drug development against protozoan infections. Protein Disulfide Isomerase (PDI), a member of the thioredoxin superfamily, is a central player of this process. The *Leishmania major* Protein Disulfide Isomerase (LmPDI) could constitute an interesting drug target for leishmaniasis; in fact, gene deletion causes loss of infectivity of parasite in experimental model.
LmPDI reductase activity was tested using a turbidometric assay based on insulin interchain disulfide bond reduction. LmPDI chaperone activity was assayed using rhodanese renaturation assay. Known inhibitors of PDI isomerase, reductase, and chaperone activities were tested in LmPDI enzymatic assays versus promastigotes in in vitro growth and versus amastigote multiplication inside infected macrophages. High-throughput turbidometric assay was set up for the identification of novel LmPDI inhibitors. A pilot screen was performed on 1920 compounds. Toxicity of compounds was tested and their anti-proliferative effect on intracellular amastigotes was determined.
LmPDI displays similar functional properties to other PDI family members: (i) the same domain structure organization; (ii) the three functional activities, isomerase, reductase and chaperone; (iii) and it form homo-oligomers. Bacitracin inhibited both isomerase and reductase activities and blocked in vitro promastigote growth. At 5µM, Bacitracin partially inhibited amastigote multiplication inside macrophages by 30%.
The screen of a small library of 1920 compounds was performed in 384-well format and led to the identification of 27 compounds with inhibitory activity against LmPDI. We further tested the cytotoxicity of these compounds using Jurkat cells as well as their effect on Leishmania donovani amastigotes using high content analysis. Hexachlorophene and a mixture of Theaflavin monogallates inhibit Leishmania multiplication in infected macrophages derived from THP-1 cells.
In conclusion, the assay performed well in this pilot screen, allowing for expansion, and screening of larger libraries in a similar manner covering broad chemical space will help identify other inhibitors and help in evaluating the catalytic mechanisms of target enzymes such as protein disulfide isomerase.

SY14.P.12

LEISHMANIA MAJOR LARGE RAB GTPASE (LMRAB) PROTECTS BALB/C MICE AGAINST A *L. MAJOR* CHALLENGE AND IS HIGHLY IMMUNOGENIC IN IMMUNE LEISHMANIASIS INDIVIDUALS

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Human leishmaniasis is a disease caused by *Leishmania*, an intracellular protozoan parasite associated with considerable morbidity and mortality throughout the world. Depending on the parasite species and on the host immunological response, leishmaniasis ranges from an asymptomatic infection to a self-limiting cutaneous lesion(s) or a fatal visceral form. In Human and experimental leishmaniasis , acquired resistance to *Leishmania* is mediated by T cells, Healing is generally associated with the development of a life-long immunity to reinfection, and correlated with the development of antigen-specific Th1 cell responses and IFNg production, Based on these data , vaccine development for *Leishmania* infection has been focusing on generating protective T-cell responses. To date, there is no vaccine against leishmaniasis.
In the present study, we evaluated the vaccine efficacy of a novel LmRAB protein in BALB/c mice, an intracellular antigen which plays a crucial role in exocytosis/endocytosis process and highly conserved protein among *Leishmania* species. Furthermore, we evaluated the immunogenicity of both proteins in cured cutaneous leishmaniasis and asymptomatic visceral leishmaniasis individuals.
We analyzed the protective effect of LmRAB (610aa) in the BALB/c mice model after a virulent challenge; the progression of infection was followed by measuring the thickness of footpad swelling.
We analyzed also the cellular immune response to LmRAB and its divergent carboxy-terminal part (LmRABC) in 9 cured CL, 15 Asymptomatic VL subjects compared to 11 healthy donors, cytokines levels were measured in culture supernatants using ELISA.
Interestingly, recombinant LmRAB protein with CpG is able to confer a strong protection in vaccinated mice against a virulent L. major challenge (2.106 metacyclics). This protection is observed up to 20 weeks post-challenge.
LmRAB and LmRABC induce higher IFN-γ levels in both groups when compared to control group (p<0.05). In the other hand, significant amounts of IL-10 are observed in response to both proteins in all groups with no significant difference (p>0.05).
In conclusion, our results show that LmRAB and LmRABC induce a dominant Th1 profile in cured CL subjects and suggest that this protein may constitute a potential vaccine against leishmaniasis.

SY14.P.13

SEROLOGIC SCREENING OF VECTOR-BORNE INFECTIONS IN DOGS FROM WESTERN ROMANIA: CURRENT STATUS

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Information regarding the spreading of canine vector borne diseases in dogs from Romania is limited. The wide spectrum of disease and the emergence and re-emergence of ''new'' pathogens constitute a diagnostic challenge for human and veterinary doctors.

The aim of this study was to provide data on the seroprevalence of *Anaplasma, Babesia, Borrelia, Ehrlichia*, and *Dirofilaria* infection in a dog population from western Romania.
Blood samples from 321 randomly selected dogs, living in five counties (Arad, Bihor, Timiş, Caraş-Severin and Hunedoara) from western Romania, were collected and serologic screened for the presence of five vector borne infections. The presence of antibodies to *Borrelia burgdorferi* sl., *Ehrlichia canis*, *Anaplasma phagocytophilum*, *Babesia canis* and *Babesia gibsoni* was detected with indirect fluorescent antibody test (IFAT), using a commercially available antigen kit (Mega Cor Diagnostic GmbH, Hörbranz, Austria). Additionally, the screening of specific antigens for *Dirofilaria immitis* was performed using enzyme-linked immunosorbent assay (ELISA, DiroCHEK® Canine Hearthworm Antigen Test Kit) method.
The seroprevalence of screened pathogens through IFAT was 11.8% for *B. canis*, 2.8% for *B. gibsoni*, 12.1% for *E. canis*, 27.7% for *Borrelia burgdorferi* sl. and 4% for *A. phagocytophilum*, respectively. 4% of the sampled dogs showed specific antigens to *Dirofilaria immitis*.
The results of this study highlighted the importance and widespread occurrence of these pathogens in western Romania. However, for a more comprehensive etiological picture further studies, supported by molecular tools, are still required.

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SY14.P.14

OLFACTOMETRY PHYTO PREFERENDUM FOR IXODID TICKS IN THE STEPPE LANDSCAPE OF ARMENIA

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In the farms of Vayots Dzor Region (Armenia) research has been done the main point of which was to develop and test biological methods to control ixodid ticks. During the study of the ecology of Ixodoidea ticks in different biotopes, we discovered that they ignore certain types of herbs while others serve as means for cattle invasion. The absence of different species of ticks on certain plants helped us to discover plants with negative phyto preferendum (in the sense of tick-plant).
The collection of such plants and plant associations and preparation of various infusions from them aimed to exert influence on Ixodoidea ticks in laboratory conditions really led to the state that the infusions received made a repellent influence on ticks. Ticks were running away from such infusions, which contained phytoncides of ticks under test. The following plants collected directly from pastures were chosen for the purpose of getting repellent influence. Common wormwood (*Artemisia absinthium* L.), Wild chamomile (*Matricaria chamomilla* L.), Milfoil (*Achillea millefolium* L.) were selected for experiment. The following species*, Ixodes ricinus* (L.)*, Dermacentor marginatus* (Sulzer), *Hyalomma* sp., were subjected to experiment. The experiment was followed by studyimg effect extracts of Wild chamomile, Common wormwood and Milfoil on behaviour of ticks. Statistic data include laboratory and field investigations. The data of laboratory experiments are worked out statistic method Analysis of Varians “ANOVA”.
The table make the average data on behaviour of ticks the essence of which is that ticks run away from the source of smell, which has repellent influence on them. Only 160 ticks among the offered examples took part in the experiment called “circus-polygon”. We received the following results using the method of “ANOVA”. Extracts of all tested plants have repellent influence on ixodid ticks but they differ by the degree of effect. Thus among the list presented the extract of Milfoil is the most effective one. There is much more difference between the efficiency of influence made by Wild chamomile and Common wormwood on ticks. Thus Milfoil is the dominant plant in the list of those which have repellent influence on ticks. The extract of Milfoil has repellent influence on *D. marginatus* and *Hyalomma* sp. and it exerts the least influence on *I. ricinus.*

We can see that summer means are significantly smaller, than autumn means and that there is no significant difference between autumn and summer means. In the case of use of Wild chamomile extract the percentage of decrease will be 25 to 55%, the use of Wormwood extract - 33– 63%, and Milfoil - 40 – 70%. On the whole when calculating the effect using all plants the decrease of the average percentage will 32.4 – 62.7%. As it was mentioned earlier the method of trustworthy neighbourhoods was used. Another purpose of the research was to work out practical anti-tick measures directly in field conditions that differ by a number of biotic and abiotic factors.

SY14.P.15

DETECTION OF NATURALLY INFECTED VECTOR TICKS BY DIFFERENT SPECIES OF *BABESIA* AND *THEILERIA* AGENTS IN THREE DIFFERENT ENZOOTIC PARTS OF IRAN

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Diagnostic study of vector ticks for different pathogens which are transmitted specifically have been done by Iranian old scientists working on the basis of biological transmission of pathogens. Some of ixodid vectors have been confirmed when their biological cycle has been studied. Natural infection of ixodid ticks was not clear before. Then it was decided to confirm natural infection of different collected ticks from three different provinces of Iran. Ticks have been collected from livestock (sheep, goats and cattle) during suitable seasons. They have been diagnosed according to specific characteristic keys. Salivary glands of ticks were pulled out and wet mount slides were prepared. Some preparations were stained by Giemsa and Feulgen. Slide preparations were studied searching for any trace of infection. DNA was extracted from different selected tick samples. Extracted DNA from ticks was confirmed by a specific primer (ITS2). Positive DNA from infected blood or tissue samples was provided and have been used as positive control. PCR optimization for positive DNA was done. Eleven pairs of primers were designed for detection of *Theileria*, *Babesia* and *Anaplasma* spp. Totally 21 tick samples were detected to be infected with protozoa. *Hyalomma anatolicum ana*. and *Rhipicephalus turanicus* from Fars province were infected with *T. lestoquardi* at two different places. *Hyalomma detritum* was infected with *T. lestoquardi* in Lorestan province and *Rhipicephalus turanicus* was infected to *Babesia ovis* from Fars province. Since there are complex relations of vectors and their relevant protozoa, different procedures are presented for future studies.
Keywords: Tick; Natural Infection; Vector; Ixodidae; Classical method; Molecular method.

SY14.P.16

FIRST CASES OF *BARTONELLA BOVIS* INFECTION IN CATTLE FROM POLAND

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Bacteria of genus *Bartonella* are increasingly being recognized as important human and animals pathogens and are responsible for a wide range of clinical manifestations including cat scratch diseases or endocarditis. *Bartonella* are intracellular parasites of erythrocytes and endothelial cells. The blood-sucking arthropods, mainly lice and flies, are thought to be the main vectors of these bacteria. Cattle constitutes the reservoir for *B. chomelii,* isolated for the first time in France, and for *B. bovis*. Although *Bartonella* infections in natural host are usually asymptomatic, *B. bovis* was identified as a cause of bovine endocarditis and long lasting bacteremia. The *Bartonella* infections are often misdiagnosed and only discovered during the slaughtering process or at necroscopy. In Europe, *B. bovis* infections in cattle were reported only in France and Italy. Up to date little is known about dispersion of *B. bovis* infection in cattle from the northern and eastern parts of

Europe. Therefore, the aim of our study was to investigate for the first time the distribution the *Bartonella* infection in cattle in Central Europe (Poland).
Blood samples were collected aseptically from cattle herds pastured on the meadows that were situated near the woodlands in Eastern Poland (n=177). Detection and genotyping of *Bartonella* isolates were performed by amplification and sequencing of fragments of three genes, *gltA*, *rpoB*, *groESl* and 16S-23S intergenic spacer (ITS) region. From PCR positive samples, 100 µl of previously frozen blood were inoculated on Columbia agar containing 5% sheep blood. DNAs of *Bartonella* were detected in 12 blood samples (6.8%), but bacterial growth on plates was not observed. The phylogenetic analysis has shown that our isolates are closely related to *B. bovis* strain originally isolated from cattle in France. According to our the best knowledge, this is the first report on *B. bovis* infections in cattle in Poland and in Central Europe.

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SY14.P.17
NEW DATA ON *DERMACENTOR RETICULATUS* EXPANSION IN CENTRAL EUROPE

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Dermacentor reticulatus (ornate cow tick) is a vector of many pathogenic microorganisms like *Borrelia burgdorferi s. l.,* TBE virus, *Anaplasma phagocytophilum*, rickettsieae, *Listeria monocytogenes* or *Salmonella* sp. It's the main vector of piroplasm *Babesia canis* in Central Europe - an etiological agent of canine babesiosis causing multisystemic clinical manifestation. In Europe there are two populations of ornate cow tick: the Western and the Eastern. The 'gap' separating those populations is localized in Poland and due to the field studies conducted 20 years ago has covered the area between Vistula (Central Poland) and Oder river (Western border) valleys. However, during last 10 years veterinarians have been reporting new cases of canine babesiosis among dogs inhabiting the regions in the 'gap'. These reports suggested *D. reticulatus* expansion to the new regions of the country.
The aim of our study was to verify the actual range of *D. reticulatus* distribution, especially in the area of the 'gap'. The field work conducted in autumn 2011 and spring 2012 provided new piece of data on new localizations of ornate cow ticks. First, the border of eastern population moved to west direction, about 60-80 km behind the Vistula river. Second, ticks originating probably from the western population were found in several localizations near the western border of Poland: by the Kwisa river - a tributary of Bóbr river, and by the Oder river, near tributary of Kaczawa. Thus, the 'gap' between two tick populations seems to be now much more narrow and the risk of tick-borne diseases increases in the area of Central Europe. The role of the new populations of ticks as the vectors of *B. canis* and other tick-borne pathogens needs further investigations.

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SY14.P.18

LOA LOA AND MANSONELLA PERSTANS INFECTION ASSOCIATED WITH FATAL PROGRESSIVE MALIGNANCY

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Loa loa and Mansonella perstans infection often co-exist in endemic regions. Versus L. loa infection most patients infected with M. perstans are asymptomatic. Response to L. loa infection appears to differ between residents and nonresidents in endemic areas. Most infected travelers are amicrofilaraemic or with low microfilaraemias. We report the case a 52-year-old man, a forest worker from Serbia, who had worked in Western Africa, with bifilarial infection associated with fatal malignancy. This is, to our knowledge, the first case of a patient with refractory filarial infection and concurrent disseminated renal cell carcinoma. A feeling of weakness started five months before admission during his holiday in Serbia. He returned to Equatorial Guinea when his condition was deteriorated. Malaria and filariasis were assumed and specific therapy was introduced. Because therapeutic effects was delayed, he returned to Serbia fifteen days later. On admission at the our Clinic on Ferbruary 2008, the patient was afebrile, adynamic, moderately dehydrated, walked painfully and had mild edema of the lower part of the left leg. He reported general malaise, headache, arthralgia, especially in left shoulder, increasing sweating and widespread abdominal pain. Giemsa-stained blood smear and Knott's concentration technique showed the presence of L. loa microfilariae (90 mf/ml). On the fifth day, very rare microfilariae of M. perstans were also found. Repeated smears for malaria were negative. On the first day of hospitalization, the patient received 3×50 mg/day DEC, and therapy was continued by increasing gradually to 8 mg/kg bw. Despite of three weeks of therapy by DEC (sixth day of hospitalization at our Clinic), level of L. loa microfilaraemia had maximum (1650 mf/ml). Microfilaraemia was resolved successfully after introduction of albendazole in therapy schema. But, the patient developed progressive arthralgia and myalgia. Abdominal CT showed primary tumor located in the left kidney. Bone metastases in spine and pelvis were discovered by CT and confirmed by MRI. Twenty days after admission, the patient developed encephalopathy associated with respiratory failure and transferred to the ICU. Despite of rehydration therapy and respiratory support by noninvasive ventilation, a month after hospitalization, rapidly progressive deterioration was resulted in cardiorespiratory insufficiency and death.

SY14.P.19

A NEW REAL TIME PCR BASED ON OMPA GENE FOR SPECIFIC RICKETTSIA CONORII DETECTION AND QUANTIZATION

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The genus *Rickettsia* belongs to the family of Rickettsiaceae in the order of Rickettsiales and constitutes a group of obligate intracellular endosymbionts of eukaryotic cells. *Rickettsia* is an important cause of emerging infectious disease in people and animals and rickettsiosis is one of the oldest known vector-borne diseases. Currently used diagnostic tests have limitations. Serological tests are the easiest methods for the diagnosis of rickettsioses but the interpretation of serological data can be complicated by the cross-reactivity among the rickettsiae. Molecular methods based on PCR utilize different primer sets targeting various rickettsial genes and constitute sensitive and rapid tools for rickettsiae detection. Recently quantitative methods based on Real Time PCR were developed for diagnosis of Spotted Fever Group Rickettsia. This study was aimed to develop a quantitative PCR assay targeting the OmpA gene and involving the use of SYBR Green method for the diagnosis of *Rickettsia conorii* infection in order to simultaneously detect and quantify the parasite from blood samples. Analyses were conducted to test sensitivity and specificity of the assay. OmpA gene sequences from many different Rickettsia species

(*R.conorii, R.israelensis, R.slovaca, R.monacensis, R.massiliae, R.aeschlimannii, R.raoultii, R.felis*) were selected from GenBank and aligned using Clustal W to identify the appropriate region for primer design using the Primer Express 3.0 software. The assay was utilized to test the presence of pathogen DNA belonging to different species of *Rickettsia* (*R.conorii, R.massiliae, R.aeschlimannii, R.raoultii, R.monacensis R.slovaca and R.felis*) and also in presence of DNA from pathogens others then *Rickettsia* such as *Anaplasma*, *Ehrlichia*, *Babesia* and *Theileria*. The customary values obtained for the standard curve (r =0.995; slope = -3.292) indicate that the reaction was well optimised. The reaction resulted positive only for R.conorii with the unique exception of R.aeschlimannii, while there wasn't amplification for all the other analyzed Rickettsia species. Furthermore, all the samples positive for the other related pathogens resulted negative. The sensitivity of the Real Time PCR was also calculated and the limit of detection was of 0.01 pg of DNA per reaction. This study allowed developing a new and powerful diagnostic method able to detect and quantify pathogen DNA of R. conorii (and R.aeschlimannii) species species . The assay is rapid, easy to perform and also sensitive and specific.

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SY14.P.20

THE PROMISING USE OF BABESIA BIGEMINA APICAL MEMBRANE ANTIGEN-1 IN A NEW ELISA DIAGNOSTIC TEST

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The babesiosis due to *Babesia bigemina* is a relevant constraint in tropical and subtropical regions worldwide. The tick-borne pathogen infects bovine and causes a severe disease, producing significant economic losses. For vaccine and diagnostic purposes, many surface antigens of the pathogen have been analyzed, among these, the recently identified Apical Membrane Antigen 1 (AMA-1). This study is focused on the *B. bigemina* AMA-1 and its use for a diagnostic test assessment . AMA-1 sequence was amplified from a *B. bigemina* Italian strain, cloned into an expression vector and the plasmid was used to transform *E. coli* competent cells. Protein expression was induced by IPTG 0.75 mM and bacterial cells were collected after 2 hours of incubation at 37°C. After cells sonication, the recombinant protein was purified by chromatography thanks to a histidine-tag in its N-terminus. The foreign tag of the protein was enzymatically cleaved, the protein was quantified by spectrophotometer and used to assess an ELISA test. The adsorption of the antigen to the plate well was performed diluting the protein in a carbonate buffer, over night at 4°C. Twenty Italian field sera, collected from naturally infected and non infected bovine, were selected using a commercial *B. bigemina* ELISA kit and their positivity and negativity were confirmed by PCR from the correspondent DNA sample. Horseradish peroxidase conjugated anti-bovine IgG monoclonal antibodies were used to detect the presence of antibodies anti-*B. bigemina*. The optical density (OD) was measured at 405 nm. The *B. bigemina* antigen was obtained in a good amount (400 µg/ml). The best conditions for the IgG detection resulted to be 0.1µg/ml of AMA-1 antigen adsorbed to the well and field sera samples diluted 1:100. The overall average OD405 of positive sera samples diluted 1:100 was of 1.202, while for the negative sera the overall average OD405 was of 0.499. The background signal, derived from the empty well, was negligible. Hereby is described the successful method of the in *E. coli* synthesis of *B. bigemina* AMA-1 protein and are showed the data of the purified antigen application in a new diagnostic test. The results showed that the OD405 values of positive sera significantly differed from negative ones, suggesting the presence of antibodies against AMA-1 in naturally infected bovine, and that AMA-1 is suitable as antigen in a diagnostic test.

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SY14.P.21

PREVALENCE AND ABUNDANCE OF CULICOIDES IMICOLA, C.OBSOLETUS AND C.PULICARIS IN PALERMO PROVINCE, ITALY

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The genus *Culicoides* (Diptera: Ceratopogonidae) contains important vectors of pathogens responsible for several diseases with veterinary and public health significance, including bluetongue (BT) in ruminants, African horse sickness (AHS) in equids, epizootic hemorrhagic disease (EHD) in deer, filarial diseases in various species including humans and the recently isolated Schmallenberg virus in ruminants. In this study, seasonal abundance and prevalence of *C. imicola, C.pulicaris* and *C.obsoletus* are evaluated. The monitoring was performed at a sheep and goat mix breeding in Sicily, Italy, from 2005 to 2011, 491 light-trap collections were made, using Onderstepoort-type blacklight trap. For all the catches, *C. imicola, C. obsoletus* and *C. pulicaris* were counted, the abundance and the prevalence were calculated. The results showed that *C. obsoletus* had a very high prevalence of positive catches throughout the year with a peak in May, while *C. pulicaris* showed lower prevalence values, but was present during the whole year. *C. imicola* was present with poor prevalence values during the year with an increase from August to November. As regarding the abundance, *C. obsoletus* showed high values all over the year with a peak in May, *C. pulicaris* had poor abundance values with the highest presence from March to June. *C. imicola* showed very low values with a peak in September - October. Abundance and prevalence of these species were also estimated for each Sicilian province. Concerning the abundance, the highest values for *C. obsoletus* and *C. pulicaris* were reported in Messina province, while *C. imicola* had the major value in Palermo. The highest values of prevalence were reported in Messina and Caltanissetta for *C. obsoletus*, in Messina and Palermo for *C. pulicaris*, in Palermo and Siracusa for *C .imicola*. These data suggest a different temporal and spatial distribution of *C imicola*, *C .pulicaris* and *C. obsoletus* species since different values of abundance and prevalence can be seen during the year in the Sicilian provinces. To facilitate the formulation of predictive risk maps for a disease transmitted by *Culicoides*, it is essential to identify whether novel vector species such as are sufficiently widespread and abundant to act as vectors and to assess the relative role of these novel vectors in disease transmission. This paper reports the seasonal abundance and prevalence values for *C. imicola, C. pulicaris* and *C. obsoletus/scoticus* obtained in a seven-years monitoring in Sicily.

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SY17.P.01

TEM STUDY OF THE TEGUMENT OF ADULT *MARITREMA FELIUI* (DIGENEA: MICROPHALLIDAE)

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The tegument of the adult digeneans is of particular importance in any consideration of the host-parasite relationship. The tegument is specialized mainly for absorption and secretion, and surface features are consistent with these functions. Its other functions in excretion, osmoregulation and lipid release have not been studied extensively. Regional specialization of the tegument is common in most trematodes and is often correlated with particular function.

Live adult specimens of this species were collected from the intestine of the shrew *Crocidura russula* (Insectivora: Soricidae) captured around La Ricarda, estuary of the River Llobregat (Barcelona, Spain). Live digeneans, fixed in cold (4°C) 2% paraformaldehyde and 2.5% glutaraldehyde in a 0.1 M sodium cacodylate buffer at pH 7.4, and post-fixed in cold (4°C) 1% osmium tetroxide with K_4FeCn_6, were prepared for TEM examination using standard techniques.
The syncytial tegument of *Maritrema feliui* Gracenea et al., 1993 is of the neodermatan type, where junctions between cells are broken down and a single continuous cytoplasm surrounds the entire body and the tegumental nuclei are located not within the surface syncytium but within subtegumental perikarya situated beneath the muscle layers (Fig. 1). The much infolded tegumental surface, with numerous surface pits and two types of spines, inevitably increases the total surface area, which is probably important for absorption. The two types of deeply embedded tegumental spines differ in the ultrastructure of their extremities projecting through the tegument; in the type-1 spines the sharp ends are conical, whereas in type-2 spines they are comb-shaped with 3–5 teeth. The tegumental cytoplasm consists of an electron-dense granular, but ribosome-free, matrix with three main types of inclusion: small mitochondria, discoid densely granulated bodies, and large, irregular, moderately electron-dense bodies. The tegumental perikarya are irregular in shape and sometimes multinucleate. Their large nuclei contain numerous heterochromatin islands in moderately electron-dense karyoplasms, and their cytoplasm contains Golgi complexes, GER, free ribosomes and a few mitochondria. In addition to these cell organelles, they also contain two characteristic types of tegumental inclusions, i.e. the same discoid and irregularly-shaped bodies as occur in the tegument, which are secreted by these cells. The tegumental perikarya are linked to the tegument by one or more long, tortuous cytoplasmic connections, which are reinforced by a single row of numerous longitudinally arranged microtubules. A direct continuity between these perikarya and the outer cytoplasmic layer of the tegument is seen only rarely in ultra-sectioned material.

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SY17.P.02

ULTRASTRUCTURE OF THE EARLY EMBRYONIC STAGES OF *MARITREMA FELIUI* (DIGENEA: MICROPHALLIDAE)

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The origin, differentiation and fine structure of the early embryonic stages of the digenetic digenean trematode *Maritrema feliui* Gracenea, Montoliu & Deblock, 1993 have been studied by means of transmission electron microscopy (TEM) and ultracytochemistry.
Live adult specimens of this species were collected from the intestine of the shrew *Crocidura russula* (Insectivora: Soricidae) captured around La Ricarda, estuary of the River Llobregat (Barcelona, Spain). Live digeneans, fixed in cold (4°C) 2% paraformaldehyde and 2.5% glutaraldehyde in a 0.1 M sodium cacodylate buffer at pH 7.4, and post-fixed in cold (4°C) 1% osmium tetroxide with K_4FeCn_6, were prepared for TEM examination using standard techniques. The Thiéry cytochemical technique with PA-TCH-SP was used to detect glycogen at the ultrastructural level.
The eggshell is formed within the ootype from the shell globule material of the vitelline cells, probably with the aid of Mehlis' gland secretions. During consecutive cleavage divisions of the zygote, three types of blastomeres are formed: macromeres, mesomeres and micromeres. As the cleavage divisions frequently take place simultaneously, it was impossible to follow their detailed sequence. The outer envelope is of cellular origin and is formed by a cytoplasmic fusion of two

macromeres situated in the peripheral layer of the early embryos, just beneath the eggshell. During embryonic development, the other blastomeres multiply and differentiate, while some micromeres undergo a simultaneous degeneration or apoptosis. In some respects, ultrastructural features of early *M. feliui* embryos resemble to some extend those of previously studied *Mediogonimus jourdanei,* but differ somewhat from those of some other digeneans studied.

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SY17.P.03

PRO-INFLAMMATORY CHANGES IN THE INTESTINE DETERMINE NUMBER AND PROTEIN COMPOSITION OF *HELIGMOSOMOIDES POLYGYRUS* L4

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The *H. polygyrus*- mouse system is widely used for studies of parasite immunomodulation. Primary exposure of mice to *H. polygyrus* infection significantly reduced the inflammation in experimental model of *colitis* and interestingly the inflammation promotes the development of the nematodes. Parasite excretory/secretory and somatic extracts contain immunomodulatory molecules that help parasites survive in the host. In addition, different host intestinal environments can induce protective or pathogenic immune responses. In this study, we examined if the higher level of *H. polygyrus* L4 infection in BALB/c mice with DSS-induced *colitis* is a consequence of different nematode protein production and various nematode proteins recognized by immune sera. *H. polygyrus* L4 at 6 days post infection from DSS-treated and untreated mice intestines were separated by 2D gel electrophoresis. Selected spots were identified by automatically using a MassOrbitrap and MASCOT program. The production of pro-inflammatory, anti-inflammatory and regulatory cytokines (IL-2, IFN-γ, TNF-α; IL-4, IL-6, IL-17 and IL-10) differed in the small intestine of mice with *colitis*. *H. polygyrus* larvae in the pro-inflammatory *milieu* provoked by DSS-treatment changed somatic proteins and had a profound effect on immune response. Western-blotting with *H. polygyrus* immune serum showed reduced numbers of immunogenic proteins in L4 antigens from DSS treated mice; only 6 proteins were recognized by IgG1. Although the pro-inflammatory cytokines increased the number of immunogenic proteins recognized by IgG1 what could affect in the weak recognition and better adaptation of the nematode larvae in the intestine.

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SY17.P.04

THE INFECTION BIOLOGY OF A DIPLOZOID FOUND ON *LABEO UMBRATUS* SMITH, 1841 IN THE VAAL DAM, SOUTH AFRICA

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Diplozoidae are oviparous macroscopic ectoparasites which occur primarily on the gill lamellae of cyprinid fish. These diplozoids are unique among the *Platyhelminthes* as they display precocious sexual behaviour with two hermaphrodite individuals forming a permanent cross-copula in the adult stage. Little information is available on the diplozoid parasites of the indigenous cyprinids of South Africa in terms of infection biology. As these parasites show great potential as sentinels as well as a tool for identifying hosts, their study is essential. *Labeo umbratus* Smith, 1841 were thus collected from the Vaal Dam, around the UJ Island, and the Vaal River, in the Visgat area, using gillnets. The fish were examined for diplozoids while noting the position of the parasites on the gill

as well as the gill arch on which each parasite is found. The data was then be analysed statistically using SPSS 2.0 to determine whether these parasites show any specificity when selecting an attachment site. The parasites showed a prevalence of 28.71%, a mean intensity of 2.31 and an abundance of 0.66. The results showed that the parasites did not prefer one side of the gill chamber over the other. In terms of the specific sites on the gills, the parasites showed a 100% preference of the central area of the gills while the dorsal and ventral areas where almost equally selected for attachment with 50.7% and 47.7% preference respectively. The first and fourth gill arches were the most highly parasitized with 41.8% and 46.2% preference respectively. It was hypothesised that this selection pattern could be as a result of feeding, better opportunities for egg distribution as well as some sites providing more protection than other and as such a closer look needs to be taken at the internal construction of the gill chambers. This data is substantially different from other species studied and thus supports the hypothesis that these diplozoids may be a new species. Thus further taxonomic, morphological and genetic studies will be performed to describe these parasites.

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SY17.P.05

A COMPARATIVE STUDY OF THE SECOND INTERNAL TRANSCRIBED SPICER (ITS2) OF RIBOSOMAL DNA OF SPECIES *HAEMONCHUS CONTORTUS* AND *H. PLACEI* (NEMATODA: TRICHOSTRONGYLIDAE)

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Nematodes of the genus *Haemonchus* (Cobb, 1898) parasitize the abomasums of animals and are common in terrestrial ecosystems. Currently, according to literature, thirteen Haemonchus species have been recorded in the world fauna. The ruminants from the families Cervidae, Antilocapridae, Giraffidae, Bovidae, as well as Camelidae have been recorded as definitive hosts. These nematodes are widespread and cause serious diseases in animals. Losses caused by these diseases to cattle husbandry are significant. Our objective was to a morphological study and molecular identification based on ITS-2 spacer of the rDNA of *H. contortus* from sheep and H. placei from cattle, obtained from different regions of Uzbekistan, with the purpose of identifying differences between and obtaining additional data on the rDNA. We determined of nucleotide sequences from the second internal transcribed spacer region (ITS-2) of nuclear ribosomal DNA in adults;. contortus and *H. placei* revealed six nucleotide differences. The differences between the studied parts of ITS-2 region of these nematodes constituted 2.6%. The level of the intraspecific difference in ITS-2 in *Haemonchus* is not high. *Haemonchus* of sheep morphologically differ from those of cattle. A number of morphological criteria and clear distinction in PCR pattern indicates the independence of the species *H. placei* in the genus *Haemonchus*. The recorded level of polymorphism variation in adults;. *contortus* and *H. placei* is probably the result of the effect of different evolutionary factors affecting the structure of this parasite population at different stages of its ontogeny and host population. Of these, the most important factors should be the selection of respective definitive hosts.

SY17.P.06

DARKLING BEETLES (COLEOPTERA) AS INTERMEDIATE HOSTS OF SPIRUROIDEA (NEMATODA) PARASITES OF MURINAE (RODENTIA) IN EL HIERRO (CANARY ISLANDS, SPAIN)

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The Canary Archipelago forms a volcanic island chain of seven main islands 110 km offshore from Cape Juby in Northwest Africa. El Hierro is the youngest, smallest, most occidental and meridian island, with an area of 268 km^2 and is located 17º53'-18º09'W and 27º38'-27º50'N. Prospection area was located in the surroundings of the ancestral village of Guinea, in the northeast of the island near the coast, and is characterized as a stony area with semi desert vegetation were murine rodents (*Rattus rattus*, *Mus musculus domesticus*) were previously caught, showing a high infection prevalence by stomach nematodes of Spiruroidea. In order to look for the intermediate hosts of these nematodes, endemic darkling beetles of *Pimelia laevigata costipennis* Solier, 1835 (97 specimens) and *Hegeter amaroides* Wollaston, 1864 (150 specimens) (Tenebrionidae), were collected in February and May of 2010. Detected nematodes were studied morphometrically under microscope, essentially *in vivo* but also fixed in 70º ethanol and cleared in lactophenol. Identification was based on morphology and biology of Spiruroidea nematodes: eggs hatch in the gut of insects; larvae develop in the haemocel or in other tissues and undergo two moults; second and third larval stages eventually become encapsulated; third-stage larvae are generally large and possess some of the cephalic characteristics of adults. In this study, *P. l. costipennis* resulted infected in 17.5% of prevalence with Spiruroidea larvae: *Streptopharagus* larvae (16.5%), mostly free, but also encapsulated; *Gongylonema* encapsulated larvae (4.1%); and *Mastophorus* encapsulated larvae (2.0%). The smaller darkling beetle, *H. amaroides*, showed a lower prevalence of parasitation (11.3%), and was mostly infected with *Streptopharagus* free larvae (7.3%), followed by *Gongylonema* (2.6%) encapsulated larvae. Experimental infection of laboratory rats with *Streptopharagus* larvae allowed us to obtain adults in the stomach that were identified as *S. greenbergi*, one of the predominant nematode detected in *R. rattus* naturally infected. The high intensity rate of this nematode in *Pimelia* (1-50 larvae) would explain the also high intensity detected in the stomach of its natural host. Further studies are needed to confirm the identification of *Mastophorus* larvae as *M. muris,* also very prevalent in *R. rattus* and *M. m. domesticus,* and *Gongylonema* larvae as *G. neoplasticum,* another Spiruroidea infecting the rat, in order to confirm the role of species of *Pimelia* and *Hegeter* as naturally intermediate hosts of Spiruroidea nematodes. Other Spiruroidea larvae were detected with low prevalence in the tenebrionids studied, which possibly are parasites of birds.

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SY17.P.07

MOLECULAR AND MORPHOMETRIC ANALYSIS OF HETEROPHYID TREMATODES; COLLECTED FROM FRESHWATER FISHES IN NAN PROVINCE, THAILAND

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Haplorchis sp., heterophyid trematodes, was one of the food-borne zoonotic trematodes. This is a common infection disease, which is human public health in Southeast Asia. Human and animals were infected by eating raw or unproperly cooked freshwater fish. Infected fish were found metacercariae, larval stage of trematodes. The metacercaria of trematode was infective stage,

which cause parasitic diseases. In this study, metacercariae were investigated from collected freshwater fishes in April 2010 and January 2011 in Nan Province, the North Thailand. The collected fishes were categorized by their morphology. The metacercariae were examined by compression method. A total of 102 fishes were identified into 5 species, *Garra cambodgiensis, Porpuntius normai, Opsarius pulchellus, Systomus orphoides* and *Cyclocheilichthys lagleri*. The infection rates of digenean trematode were 41.67% (10/24), 100% (3/3), 91.43% (32/35), 50% (16/32) and 50% (4/8) respectively. First, metacercariae were identified based morphology. Three species of trematodes were found*, Haplorchis taichui, Procerovum cheni* and *Centrocestus caninus.* Only metacercariae of *H. taichui* were analyzed using Internal Transcribe Spacer subunit I and II. The molecular results revealed that all of them were *H. taichui*.
Keywords: Heterophyid, *Haplorchis*, metacercariae.

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SY17.P.08

SYNANTHROPIZATION OF SAND FLIES AS A FACTOR IN INCREASING THEIR EPIDEMIOLOGICAL SIGNIFICANCE IN THE FORMER USSR

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In the former USSR sand flies recorded in Central Asia and Caucasus Turkmenistan, Uzbekistan, Tajikistan, Armenia, Azerbaijan, Georgia. There are more than 40 species. Sand flies are vectors of leishmaniasis, as well as pappatachi fever.
Some species of sand flies are showing varying degrees of synanthropization. In the former Soviet Union reported 28 such species, of which *Phlebotomus papatasi*, *Ph*. *sergenti*, *Ph*. *cancasicus*, *Ph*. *alexandri*, *Ph*. *mongolensis* abound in the towns. Dwelling sand flies rural areas and cities increases their ability to transmit pathogens. In the villages inhabited sand flies in the mud fences, livestock buildings, fly into houses. In urban areas they are hiding in a residential area on the first floor. Sand flies, which are found in cities, have the greatest potential for development of new settlements.
Control measures of sand flies in populated areas are divided into public and conducted by a professional. An example might be the work carried out by us in the south of Tajikistan, in rural areas on irrigated lands. In the residential population of fumigant applied the device - elektrofumigators with plates, spirals and rods based on pyrethroids.
Places hatching of larvae and adult sand flies hiding from the treated groups of drugs sprayers organophosphorus compounds and pyrethroids. The residual effect of insecticides was short (from 5-7 to 12-15 days), due to high air temperatures (40 -45ºC). Of great importance were the sanitary and technical measures to eliminate open waste household water as well as in the soil moistened by these flows, a massive hatching of immature stages of sand flies.

SY17.P.09

SPERMATOLOGICAL CHARACTERS OF THE TRYPANORHYNCHA, WITH NEW DATA ON THE LITTLE-STUDIED SUPERFAMILY TENTACULARIOIDEA

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Trypanorhynchs are polyzoic cestodes, readily recognised by their rhyncheal apparatus, which are among the most common metazoan parasites of elasmobranchs. They have been one of the most chaotic and confusing tapeworm groups, but recent work has shed light on their systematics. Both morphological and molecular data indicate that this order is polyphyletic and consists of two well-supported clades.

Ultrastructural studies on cestode spermatozoa have proved useful for interpreting their phylogenetic relationships within the Platyhelminthes and could, therefore, offer useful morphological indicators of the phylogeny and/or classification of *Trypanorhynchs*. However, studies of spermiogenesis and/or spermatozoa are limited to *Grillotia erinaceus* and *Lacistorhynchus tenuis* (Lacistorhynchoidea), *Aporhynchus menezesi* (Gymnorhynchoidea), and *Dollfusiella spinulifera* and *Parachristianella trygonis* (Eutetrarhynchoidea). The aim of this study is to analyse, for the first time, the spermatological patterns of two species of the superfamily Tentacularioidea, *Nybelinia queenslandensis* and *Kotorella pronosoma*.

Live adult specimens of *N. queenslandensis* and *K. pronosoma* were collected by Dr Ian Beveridge, University of Melbourne, from *Carcharhinus melanopterus* and *Himantura granulata,* respectively, caught off Lizard Island (Queensland, Australia). Mature proglottids were routinely processed for TEM examination.

Spermiogenesis in trypanorhynchs is type 1 (with both rotation and proximodistal fusion of flagella). The intercentriolar body consists of a variable number of electron-dense plates (three, five or seven) according to the species. The spermatozoon is a long filiform cell, tapered at both ends, which lacks mitochondria. Its cytoplasm contains: (1) two axonemes of different lengths with the 9+'1' trepaxonematan pattern, (2) an arched row of thick, parallel cortical microtubules near the anterior extremity, (3) two rows of thin, parallel cortical microtubules, (4) a parallel nucleus, and (5) glycogen in the form of α-glycogen rosettes and/or β-glycogen particles. Anterior and posterior spermatozoon extremities exhibit cortical microtubules and a single axoneme, respectively. Unlike the majority of cestodes, trypanorhynch spematozoa lack crested bodies, and, consequently, the postulated synapomorphy of crested bodies for the Eucestoda is questionable. The results presented both here and in previous studies indicate only insignificant ultrastructural differences between the spermatological characters of the seven species studied and offer no additional support for the hypothesis, suggested by some authors, that the trypanorhynchs are polyphyletic.

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SY17.P.10

ULTRASTRUCTURE OF VITELLOGENESIS AND VITELLOCYTES IN THE TRYPANORHYNCH CESTODE *APORHYNCHUS MENEZESI*, A PARASITE OF THE VELVET BELLY LANTERNSHARK, *ETMOPTERUS SPINAX*

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The synthetic activity of vitellocytes plays two important functions in the developmental biology of cestodes: (1) their shell globules serve in eggshell formation; and (2) their accumulated reserves of glycogen and lipids represent a food source for the developing embryo.
Live adult specimens of *Aporhynchus menezesi* were collected from the spiral valve of *Etmopterus spinax* (L.) caught off Faial Island (38º31'N, 28º37'W) (Azores, Portugal) and processed for TEM examination.
In *A. menezesi,* vitelline follicles consist of cells at various stages of development, from peripheral, immature cells of the gonial type to mature cells toward the centre. These stages are (I) immature, (II) early differentiation, (III) advanced maturation and (IV) mature. Gradual changes involved in this process occur within each stage. Vitellogenesis involves: (1) an increase in cell volume; (2) the development of a smooth endoplasmic reticulum and an accelerated formation and accumulation of both unsaturated and saturated lipid droplets, along with their continuous enlargement and fusion; (3) the formation of individual β-glycogen particles and their accumulation in the form of glycogen islands scattered among lipid droplets in the cytoplasm of maturing and mature vitellocytes; (4) the rapid accumulation of large, moderately saturated lipid droplets accompanied by dense accumulations of β-glycogen along with proteinaceous shell-globules or shell-globule clusters in the peripheral layer during the advanced stage of maturation; (5) the development of cisternae of granular endoplasmic reticulum that produce dense, proteinaceous shell-gobules; (6) the development of Golgi complexes engaged in the packaging of this material; and (7) the progressive, continuous enlargement of shell-globules into very large clusters in the peripheral layer during the advanced stage of maturation.
Vitellogenesis in *A. menezesi*, only to some extent, resembles that previously described for four other trypanorhynchs. It differs in: (i) the reversed order of secretory activities of the differentiating vitellocytes, namely the accumulation of large lipid droplets accompanied by glycogenesis or β-glycogen formation during early differentiation (stage II), i.e. before the secretory activity, predominantly protein synthesis for shell-globule formation (stage III); (ii) the very heavy accumulation of large lipid droplets during the final stage of cytodifferentiation (stage IV); and (iii) the small number of β-glycogen particles present in mature vitellocytes. Ultracytochemical staining with PA-TSH-SP for glycogen proved positive for a small number of β-glycogen particles in differentiating and mature vitellocytes. Hypotheses, concerning the interrelationships of patterns of vitellogenesis, possible modes of egg formation, embryonic development, life-cycles and their phylogenetic implications, are commented upon.

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SY17.P.11

IDENTIFICATION AND CLASSIFICATION OF PROTEINS EXPRESSED IN THE PROTOZOAN PARASITES *PENTATRICHOMONAS HOMINIS* USING TWO-DIMENSIONAL GEL ELECTROPHORESIS

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Pentatrichomonas hominis is a commensal anaerobic flagellate inhabiting the large intestine of humans, dogs and cats. Infection in human is usually asymptomatic, but under certain unbalanced conditions in the digestive tract, the parasite can cause abdominal discomfort, persistent diarrhea, colitis. Recent advances in proteomic approaches have created great opportunities for mapping and characterization of protein populations. A *P. hominis* (ATCC30000) expressed sequence tag (PhEST) project was conducted to generate 5000 randomly selected EST clones from a trophozoite cDNA library and served as a database for proteomic analysis. In the present study, we use two-dimensional electrophoresis combined with MALDI-TOF-MS to profile, identify and characterize proteins expressed in the trophozoite stage of *P. hominis*. We established the proteome reference maps (p*I* 4-7, p*I* 6.2-7.5) and analyzed highly expressed proteins of *P. hominis.* A total of the 210 protein spots were processed to MALDI-TOF-MS analysis and 201 protein spots were successfully identified, representing 108 unique proteins. Identified proteins were further classified into 19 groups according to their biological process. This study provided the most comprehensive and extensive proteomic analysis for *P. hominis* .The establishment of the proteome reference maps for *P. hominis* will serve as a basis for future comparative proteomic and functional genomics studies between different developing stages of *P. hominis.*

SY17.P.12

CERCARIAL INFECTIONS OF FRESHWATER MOLLUSKS AT PASAK CHOLASID RESERVOIR, THAILAND

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Cercarial infections of freshwater mollusks at Pasak Cholasid Reservoir were studies between April 2011 and July 2011. Pasak Cholasid Reservoir is one of the important public reservoirs for local residents in the central part of Thailand. The aim of this study was investigated mollusks intermediate host of trematodes. Freshwater mollusks were collected by using counts per unit of time sampling method. The samples of mollusks were collected every 20 minutes per sampling by five collectors, collecting samples of freshwater mollusks by hand picking. The samples were brought back to the laboratory for species identification and investigating trematode infections. A total of 2,465 mullusk samples were collected from 21 sampling stations, they were identified into 15 species. They were 90 *Pomacea* sp.*,* 36 *Pila ampullacea*, 190 *Filopaludina sumatrensis polygramma*, 47 *Filopaludina javanica*, 564 *Clea (Anentome) helena*, 971 *Bithynia siamensis siamensis*, 230 *Melanoides tuberculata*, 3 *Tarebia granifera*, 71 *Corbicula arata,* 5 *Corbicula gustaviana,* 3 *Corbicula blandiana ,* 48 *Indonaia substriata*, 86 *Limnoperna supoti*, 76 *Scabies crispata* and 45 *Scabies phaselus*. Cercarial infections were investigated using shedding and crushing methods. Two species of snails were found trematode infections. They were *M. tuberculata* and *B. s. siamensis*. The infection rate were 26.96 % (62/230) and 1.75 % (17/971), respectively. The cercariae were categorized into four types and four species. The first type, Echinostome cercariae, consisted of *Echinochasmus pelecani.* The second type,

Xiphidiocercariae, consisted of *Loxogenoides bicolor.* The third type*,* Paraplerophocercous cercariae, consisted of *Haplorchis taichui* and the forth type*,* Allocreadiidae, consisted of *Allocreadium isoporum*. One snail of *M. tuberculata* was found double infection with *H. taichui* and *L. bicolor*.

Keywords: Trematode, Cercariae, Freshwater molluaks, Pasak Cholasid Reservoir.

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SY17.P.13

THE NEURO-MUSCULAR SYSTEM IN FRESH-WATER FURCOCERCARIA. COMPARATIVE STUDY

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The neuromuscular system (NMS) in cercariae of *Diplostomum pseudospathaceum* (Diplostomidae), *Cotylurus szidati*, *Australapatemon burti* (Strigeidae), *Holostephanus volgensis*, *Paracoenogonimus ovatus* (Cyathocotylidae) and *Bilharziella polonica*, *Trichobilharzia szidati*, and *Trichobilharzia franki* (Schistosomatidae) was studied with immunocytochemical methods and confocal scanning laser microscopy. The patterns of F-actin in the musculature, 5-HT immunoreactive (−IR), FMRF-amide-IR neuronal elements, and α-tubulin-IR in sensory receptors were investigated. No major structural differences in the musculature, the 5-HT-IR or FMRF-IR neuronal elements were noticed between the cercariae. The minor variations observed in the musculature were related to the size and organization of the muscle fibers. A trend in the differentiation of the longitudinal muscle fibers in the furca from evenly distributed fibers in *H. volgensis* and *P. ovatus* to many bundles in D. pseudospathaceum and two well-organized lateral bundles in *C. szidati*, *A. burti*, and *Trichobilharzia* spp*.* was observed. The transverse muscle fibers in the furca follow the same trend. The number of 5-HT-IR neurons in the cercarial bodies varied between 10 and 16. In cercariae of *H. volgensis* and *P. ovatus*, the central nervous system (CNS) was less centralized compared to the CNS in the other species studied, with only two 5-HTIR marker neurons in each brain ganglion and the other neurons distributed evenly along the main cords. In the tails of *H. volgensis* and *P. ovatus*, many transverse 5-HTIR comissures were found. In the tails of higher strigeidid cercariae, only a few crosslinks were observed. The number and distribution of sensory receptors on the bodies and tails of the cercarial species differed from each other. A trend in the differentiation of the sensory receptors in the tails was discerned. A process of grouping and decrease in number of ciliated receptors in the stem and in the furca from *H. volgensis* and *P. ovatus* to Schistosomatid cercariae took place.

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SY19.P.01

SEQUENCE DIVERSITY IN THE GALECTIN LOCI FROM *TELADORSAGIA CIRCUMCINCTA*

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The abomasal nematode *Teladorsagia circumcincta* is the most common nematode in sheep from cool temperate areas of the world. Sheep antibodies of the IgA and IgE classes have been associated with reduced worm fecundity and establishment. Galectins are beta-galactoside-binding proteins produced by ruminant gastrointestinal nematodes that can potentially bind galactose residues on IgA and IgE. Therefore galectins may play a role in immune evasion. The aim of our research was to investigate the galectin genes of *T. circumcincta*. One (ID) and two dimensional (2D) western blotting has shown that galectin binds to or is bound by IgA and IgE from infected sheep. Galectin is the only parasite molecule recognised by all sheep. In 2D gels galectin appears as multiple spots of similar molecular weight suggesting the existence of multiple genes with distinct sequences. Sequencing of multiple clones from single nematodes revealed multiple single nucleotide polymorphisms (SNP). Non synonymous substitutions were identified in 69 positions: 9 in exon 2, 9 in exon 3, 10 in exon 4, 10 in exon 5, 9 in exon 6, 12 in exon 7 and 10 in exon 8. SNP and AA changes were confirmed by sequencing of exon 1 and exon 2 genomic DNA of single adult nematodes. These polymorphisms suggest the existence of multiple polymorphic loci that code for galectin. Duplication of galectin loci may allow the rapid production of galectin to neutralise host antibodies.

SY19.P.02

REPORT OF A NEW GENOTYPE OF *EHRLICHIA* SPECIES FROM CATTLE AND CERVIDS

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Tick transmitted *Ehrlichia* and *Anaplasma* are closely related blood-borne rickettsia. Several species are important pathogens of domestic and wild ruminants, and some infect monogastric hosts, including humans. Although *Anaplasma* spp are common in livestock in many parts of the world, *Ehrlichia* infection in cattle occurs only in a few regions, including sub-Saharan Africa and a few Caribbean islands. In a recent investigation of unexpected reactors from a serological survey for bovine anaplasmosis, we detected the first natural ehrlichial infection in cattle that was not due to *Ehrlichia ruminantium*.

In a series of two bioassay studies, splenectomised and intact cattle were inoculated with fresh blood collected from naturally or experimentally infected cattle. Serially collected blood samples were analyzed by blood smear examination, PCR, IFA, C-ELISA, and cell culture. Molecular and serological data showed the presence of a novel genotype of rickettsial species that was distinct from *Anaplasma* spp and *E. ruminantium*. A few of the experimentally infected animals manifested mild clinical disease and histological evidence of mild encephalitis. Bacterial morulae morphologically consistent with *Ehrlichia* were detected in cultured leucocytes.

Phylogenetic analysis of gene sequences of 16S ribosomal RNA (16S rRNA), citrate synthase (*gltA*) and heat shock protein (*GroEL*) showed that the *Ehrlichia* found in cattle is a distinct genotype, with *Ehrlichia canis* as the closest clade and *E. ruminantium* as the furthest removed within the genus. The novel *Ehrlichia* genotype was also detected in blood collected from mule deer in the region where infected cattle were found. A molecular and serological study of blood

collected from the experimentally infected cattle and naturally infected cattle and cervids showed cross reactivity on IFA and a commercial C-ELISA for bovine anaplasmosis.
Although the clinical significance for animals infected with the novel *Ehrlichia* genotype appears to be minimal, the zoonotic potential is unknown, and the implications of the serological cross reactivity are significant for the diagnosis and control of bovine anaplasmosis. Further research is needed to elucidate the biology and transmission of this novel *Ehrlichia* genotype, and to develop reliable and specific diagnostic tools for rickettsial pathogens.

SY19.P.03
DETECTION OF THE MOST IMPORTANT SPECIES OF *CRYPTOSPORIDIUM* FOR HUMAN HEALTH IN RIVER WATER OF IRAN BY GP60 PRIMER

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Human cryptosporidiosis is mainly caused by *Cryptosporidium parvum* and *Cryptosporidium hominis*. *C. hominis* is found almost exclusively in humans, whereas *C. parvum* is found in domestic livestock, wild animals, and humans. These important species have been identified in cases of cryptosporidiosis outbreaks and these species are represents as a potential risk of cryptosporidiosis from water for humans and livestock. Totally 25 water samples collected, 20 samples from river in north of Iran and 5 samples from 2 water treatment plant in Tehran. 5 liter of each River water sample filtrated by membrane filter then purified by sucrose flotation method and 50 liter of each water treatment plant sample filtrated by Filta-Max xpress filters and purified by IMS method. Genomic DNA was isolated from concentrated oocysts by a QIAamp DNA mini kit protocol (Qiagen GmbH, Hilden, Germany) as recommended Jiang et al. (2005). As previously described *C.homonis* and *C. parvum* were determined by nested PCR of the GP60 gene as described by Abe et al 2006 (Abe et al., 2006). PCR products were visualized by electrophoresis in 1.5% agarose gels stained with ethidium bromide. 15 out of 20 river water samples and 2 out of 5 water treatment plant were positive by Gp60 primer. As this primer only detected *C. parvum* and *C.homonis*, so a positive PCR result indicated to presence of important species of *Cryptosporidium* for human health. These results, although limited by the small number of isolates studied, but suggest that the occurrence of the *C. parvum* or/and *C.homonis* are frequently in water samples study area, so humans could potentially infected by using this water during entertainments activity or drinking unfiltrated water.

SY19.P.04
RANDOM PRELIMINARY SCREENING OF AN EXPRESSED SEQUENCE TAG LIBRARY OF *DICROCOELIUM DENDRITICUM*

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Dicrocoeliosis, caused by *Dicrocoelium dendriticum*, is an important hepatic trematodosis which affects a wide range of mammals, mainly ruminants, and occasionally infects humans. However, in spite of the financial and health significant of Dicrocoeliosis its immunological diagnosis is still highly unsatisfactory, as well as its control. A search on NCBInr databases for the family Dicrocoelidae only retrieves 3 proteins and no expressed sequence tag (EST). These findings indicate the need for further research on both, new diagnostic methods and parasite molecular

biology. Our main goal was to clone diagnosis *D. dendriticum* genes to be used as recombinant antigens in the specific immunological diagnosis of the disease. A cDNA library was constructed, with mRNA extracted from *D. dendriticum* adults, using the cDNA synthesis system ZAP-cDNA® (Stratagene). A random screening was performed to identify characteristic ESTs of the trematode by PCR (T3 and T7 primers). The generated amplicons were sequenced and compared with those in GenBank. The selected cDNAs were subcloned into the pGEMT vector (Promega), and competent XL1-Blue cells were transformed with the ligation mixture. Plasmid DNA was extracted with the Qiaprep (QIAGEN) kit, and sequenced using D and SP6 universal primers.
The recombinant percentage of the amplified library was 90% and the size of the inserts ranged from 496 bp to 2000 pb, with most over 700 bp. A preliminary screening of 250 phage plaques from the library resulted in the identification of 113 different cDNAs. According to the literature some of these proteins have been described as possible vaccine targets in other trematodes, and/or as relevant diagnosis antigens: (a) *D. dendriticum* myoglobin (GI: 122064715), proven to be reactive against sera from infected sheep with high specificity against other trematodes; (b) *Clonorchis sinensis* 7KD protein (GI: 21489590) which a potential diagnostic role in this parasitosis, and (c) *Fasciola hepatica* homologous cystatin (GI: 55978577). These cDNAs were subcloned in expression vectors using specific primers with the restriction enzyme sites. PCR assay was carried out and the purified products were cloned into both the Glutation-S-Transferase (GST) pGEX6P vector (Health Care), and the His6-tag pRSET vector (Life Technologies), with the recombinant proteins as diagnosis antigens.
This is the first study conducted for identification and characterization of *D. dendriticum* ESTs. A total of 103 different proteins were identified, and three of them subcloned in expression vectors to be tested as potential diagnostic targets.

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SY19.P.05
STAGES OF INTERSPECIFIC AND INTRASPECIFIC INTERACTIONS BETWEEN HELMINTHES

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The using of measurement analysis is correct and universal method for valuation of interspecific and intraspecific interactions between helminthes. The objects of our exploration were nematodes *Syphacia obvelata*, *Aspiculuris tetraptera* from wood and house mice, *Ivaschkinonema alticola*, *Citellina alatau* from silver vole (*Alticola argentatus*), *Heterakis galinarum*, *Ascaridia galli* from home hens, *Ganguleterakis dispar* from home aquatic birds, nematodes *Oswaldocruzia filiformis*, *Rhabdias bufonis* and trematodes *Opisthioglyphe ranae*, *Hahlometra cylindracea*, *Pleurogenes intermedius* from moor frog (*Rana arvalis*). Measured exemplars of every worm species we united dependently on the number of parasites specimens in the host’s organism (for the studying of intraspecific relationships) and on the presence of other worm species (for the research of interspecific interactions). The results of investigations showed that helminthes of own and other species may be both synergists and antagonists proceeding from the possibilities of host and parasites. Incidentally we may classify the next stages of interactions. Neutralism, when individual specimens of small and low-pathogenic helminthes are not detected by the host’s organism, don’t cause the damage by their feeding and living activity, don’t compete with each other when the sources of organism is sufficient; they usually have the large sizes. Opposition to the host’s organism with the mutual synergism between parasites of one or different species – when the sources of the host’s organism are sufficient, but difficulty accessible, and the parasites’ synergism is directed to the overcoming the immune barriers and making the accessible to the source. Parasites usually are not numerous, and their sizes are small. Stage of optimal balance – when

parasites in the certain degree overcome the resistance of the host's organism and make the accessible to the source. Quantity of parasites is usually middle, their sizes are maximal. Stage of interspecific and intraspecific competition – when in the host's organism take place the immune weariness, sources of the host's organism decrease, feed and space competition between parasites and their co-inhibition by the products of metabolism begin. The result of this stage is the decreasing of absolute sizes of parasites, which dependents not only from the size, and also on the taxonomic status of helminthes. Described stages of parasites' interaction take place on the individual level. And precisely on the level of host's specimen the regulating mechanisms, emerging then in the total interactions to the level of populations and species, begin.

SY19.P.06

DETECTION AND MOLECULAR CHARACTERIZATION OF *CRYPTOSPORIDIUM* SPECIES IN RECREATIONAL WATERS OF SHAHR-E-KORD DISTRICT OF IRAN USING NESTED-PCR-RFLP METHOD

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Cryptosporidiosis is a diarrheal disease caused by microscopic parasites of the genus *Cryptosporidium*. Over the two last decades, a large number of outbreaks of waterborne human cryptosporidiosis have been occurred in different parts of the world.
The parasite may be found in drinking water and recreational water in every region of the world. Large-scale surveys reveal prevalence rates that range from 0.6 to 20% in developed countries and 4 to 32% in underdeveloped countries. Fecal-oral transmission of *Cryptosporidium* oocysts occurs through ingestion of contaminated drinking or recreational water, consumption of contaminated food, and contact with infected persons or animals. People who are most likely to become infected with *Cryptosporidium* include children who attend daycare centres, including diaper-aged children; childcare workers; parents of infected children; international travellers; backpackers, hikers, and campers who drink unfiltered, untreated water; swimmers who swallow water while swimming in swimming pools, lakes, rivers, ponds, and streams to detect and characterize *Cryptosporidium* spp. in water samples collected from recreational waters of the area, we used the SSU rRNA-based PCR-RFLP technique. Out of thirty water samples examined, 6 (20%) were positive for different *Cryptosporidium* spp. Restriction pattern analysis showed that *C. parvum* bovine genotype had been the most prevalent genotype, followed by *C.parvum* human genotype and *C. canis*, respectively. This region is not only one of the main poles of animal husbandry in the country, but also one of the most beautiful regions for tourists .Therefore, according to the findings of the study the risk of cryptosporidial infections may be noticeable for people or tourists who live or travel to this region. On the other hand, the study suggests that farm animals, particularly cattle are the main source of cryptosporidial contamination for the recreational waters.

SY20.P.01

PREVALENCE OF ANTIBODIES TO *NEOSPORA CANINUM* AND *TOXOPLASMA GONDII* IN RED FOXES (VULPES VULPES) FROM SLOVAKIA

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Neospora caninum and *Toxoplasma gondii* are two closely related protozoan parasites with cosmopolitan occurrence. They have indirect life cycle with carnivores as definitive hosts, dogs and canine carnivores for *N. caninum* and cats and feline carnivores for *T. gondii*. Oocysts are excreted in the feces of the definitive host to the environment where under appropriate conditions mature and can cause infection in several host species, including wild animals. Neosporosis has been ranked a leading reason of abortions in cows in some countries. Its zoonotic potential has not been resolved yet. Toxoplasmosis is a zoonotic disease and can cause serious illness in humans. People become infected due to consumption of insufficiently cooked meat and milk, especially from goats. Both parasitoses circulate in sylvatic and domestic cycle. The infections can be spread to stables due to poor zoohygiene by rodents or by contact with free living animals on unfenced pastures.
Within the serological survey in Slovakia, a total of 94 blood serum samples of foxes and 3 sera of wolves were examined by competitive ELISA and indirect ELISA for detection of antibodies to *N. caninum* and *T. gondii*. The results revealed 45.7% seropositivity to neosporosis and 93.6% seropositivity to toxoplasmosis in foxes. Lower seropositivity was noticed in wolves, 33.3% and 66.7%, respectively. The presence of *N. caninum* was confirmed in 3 isolates out of 45 tested fox muscle samples, indicating a low incidence of acute stage of the disease. Presence of *T. gondii* has been detected in 30 tissue samples of foxes out of 108 tested. The alarmingly high seroprevalence highlights the constant occurrence of these serious protozooses in Slovakia. The results suggest the circulation of both protozooses in sylvatic cycle with the risk of their spread to domestic animals.

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SY20.P.02

POSITION OF *PHOLETER GASTROPHILUS*, *BRAUNINA CORDIFORMIS* AND *OGMOGASTER ANTARCTICUS*, PARASITES FROM CETACEANS, IN THE MOLECULAR PHYLOGENY OF THE DIGENEA

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Cetaceans harbor a specific fauna of digeneans that include *Braunina cordiformis* (Brauninidae), *Pholeter gastrophilus* (Heterophyidae), species of *Ogmogaster* (Notocotylidae) and species of the Brachycladiidae. *Braunina cordiformis* and *P. gastrophilus* are gastric parasites of odontocetes, species of *Ogmogaster* inhabit the intestine of baleen whales and species of the Brachycladiidae occur in the digestive tract, bile ducts or air sinuses of odontocetes or baleen whales. Data about the phylogenetic position and evolutionary history of digeneans from cetaceans is currently available only for the Brachycladiidae. In this study we determine the position of *B. cordiformis*, *P. gastrophilus* and *O. antarcticus* in a previous molecular phylogeny of the Digenea. The analysis was based on the 18S and 28S genes of 164 digenean taxa sequences comprising 78 families. Species of Aspidogastrea were used as the outgroup. A Bayesian inference was made under a model of nucleotide substitution (general time reversible GTR with among-site rate heterogeneity)

that was considered the best estimate of phylogeny. Results showed two major clades with good nodal support based on posterior probabilities: Diplostomida and Plagiorchiida. *Braunina cordiformis* was placed within the Diplostomata, together with Strigeidae and Diplostomidae, whose members parasitize birds and mammals, whereas *Pholeter gastrophilus* and *O. antarcticus* were included within the Plagiorchiida. *Pholeter gastrophilus* shared its phylogenetic position with members of the families Opisthorchiidae, Cryptogonimidae and Heterophyidae, whose members are parasites of birds, mammals and marine and freshwater fishes. In a second phylogenetic analysis based on both 18S and ITS2 regions that included nine taxa of the Heterophyidae and Opisthorchiidae, *P. gastrophilus* was placed as the sister taxa of *Pygidiopsis* and *Phagicola*, which are common parasites of aquatic birds and carnivores. Interestingly, *Ogmogaster antarcticus* was included within a clade along with the Rhabdiopoeidae, the Labicolidae and the Opisthotrematidae, which are all parasites of sirenians. Overall, these results would suggest that the associations between cetaceans and their digeneans would have originated via host switching events, lending support to the hypothesis that the ancestors of cetaceans probably lost parasites of terrestrial origin during the hosts' transition from the land to the sea.

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SY20.P.03

GASTROINTESTINAL PARASITES IN NORTHERN FUR SEALS (*CALLORHINUS URSINUS* L.) ON ST. PAUL ISLAND, ALASKA

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The population of northern fur seals (*Callorhinus ursinus* L.) (NFS) inhabiting the Pribilof Islands Archipelago (St. Paul and St. George islands) constitutes about 50% of the world's current population with about 900,000 NFSs in 2000. The bulk of parasitological studies of NFSs in the Pribilof Islands were performed in the 1950–1960s and 1988–1992; but few parasitological examinations of NFSs older than pups have been made since. We examined of 105 subadult (3-4 year old) NFS males (SAMs) that were humanely harvested during the annual Aleut subsistence collection period (July and August, 2011) at four haul-out areas on St. Paul Island: Lukanin (n=26), Polovina (n=28), Gorbatch (n=30) and Morzhovyj (n=21). Gastrointestinal tracts were examined visually; all helminths were collected manually, fixed in 70% ethanol, and identified by morphological criteria. We collected and identified 675 specimens of nematodes, 72 of acanthocephalas and 1373 of cestodes.
All SAMs examined were infected with gastrointestinal helminths. Prevalence with anisakid nematodes was 89.2% (range 86.9% – 100%). Four species of anisakids from three genera *Anisakis* (*A. simplex*), *Contracaecum* (*C. osculatum*) and *Pseudoterranova* (*P. decipiens* and *P. azarazi*) were found. Three specimens of *Uncinaria lucasi* (1 male and 2 females) were found in one SAM (prevalence 0.95%). It is the first finding of U. lucasi in a NFS older than a pup.
Prevalence of SAM infection with acanthocephalas was 29.5% (from 7.7% to 47.6% at different haul-out areas). Seven acanthocephalan species in two genera *Corynosoma* (*C.strumosum*, *C. alaskensis*, *C. semerme*, *C. similis*, *C. validum*, *C. villosum*), and *Bolbosoma* (*B. nipponicum*) were found. Prevalence of cestodes was 99.1%. *Diphyllobothrium pacificum* was the most common species (prevalence 93.3%). Three more species of *Diphyllobothrium* (*D. lanceolatum*, *D. hians* and *D. romeri*) were also documented in SAMs; however, identification of all cestodes has not been completed.

Comparison of our results with data from previous reports revealed changes in prevalence and biodiversity of gastrointestinal parasites in the NFS population on St. Paul Island. Further studies are necessary to determine the reasons for current differences in prevalence and biodiversity of NFS parasites.

SY20.P.04

ANTIMALARIAL THERAPY AND CLINICAL MANIFESTATION OF *PLASMODIUM RELICTUM* INFECTION IN GYR FALCONS (*FALCO RUSTICOLUS*)

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Captive - bred Gyr falcons are the most valuable species of falcons used for a traditional Arab falconry. Falcons were shipped from North America to Middle East for traditional falconry purposes. Of all *Plasmodium* sp. infecting raptors only *P. relictum* is considered to be a virulent and highly pathogenic. Clinical history of four malaria cases caused by *Plasmodium relictum* in captive - bred Gyr falcons (*Falco rusticolus*) treated in Abu Dhabi Falcon Hospital is reviewed. Neat stain of blood smears of these falcons showed high parasitaemia in two cases (44% and 36%), one moderate (18%) and one case of low parasitaemia (9%). Packed Cell Volume 55-58% was confirmed. Clinical examination revealed severe dehydration, reduced performance, decreased appetite and thickened, grey discoloration of urates. Radiological examination showed splenomegaly, nephromegaly and hepatomegaly. Endoscopic examinations showed small nodules in lungs parenchyma, later identified as exoerythrocytic schizonts. Histopathology of liver biopsy revealed numerous intraendothelial schizonts. Treatment consisted of primaquine (Primaquine phosphate, 0.75 mg/kg) and chloroquine (Malarex, 25 mg/kg initial loading dose continued with 15 mg/kg). In two cases relapses occurred. Repeated treatment with increased dose of primaquine (1.9 mg/kg) and chloroquine (37.5 mg/kg) resulted in another relapse of parasitaemia two weeks after the last treatment. Treatment with pyrimethamine (12 mg/kg) and sulphadiazine (25 mg/kg) for four days cleared the parasitaemia. Repeated sampling three months after treatment did not showed *Plasmodium* parasites in a peripheral blood. No falcon died due to *Plasmodium relictum* infection. Because of a persistent decrease of performance, birds were not suitable for falconry use.
The presence of the vector species in Middle East and large number of imported captive - bred falcons are questioning the importance of malaria screening in birds traded from North America.

SY20.P.05

METASTRONGILIDS OF WILD BOARS IN UZBEKISTAN

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The analysis of data obtained enabled us to record three Metastrongylidae species in wild boars inhabiting Uzbekistan, namely *Metastrongylus elongatus* (Dujardin, 1846), *M. pudentotectus* (Wostokov, 1905); *M. salmi* (Gedoelst, 1923), are found in the form of association. The infection of wild boars occurs mainly in lowland areas. The extent of infection by *M. pudendotectus* reached 92.2%, *M. salmi* and *M. elongatus*, 84.6%. The intensity of infection by these species in the mountainous area on average was 192, 101.6 and 69.5 individuals, respectively. In this case, the maximum extent of infection and intensity of infection was recorded in the species *M. pudendotectus*. It should be noted that the highest degree of infection by metastrongylids of the

animals was as the follows: in the autumn, 92.3 and 88.8% in winter; the intensity of infection, in autumn (506.5 individuals) and in the spring, 358.8 individuals, in the summer, it was minimal and was 50% (75 individuals). The degree of infection of animals by season ranges from 50 to 92.3%; the intensity of infection on average ranged from 75 to 506.5 individuals. The age dynamics of extensiveness of metastrongilids ranged from 50.0 to 87.5%; the intensity, ranged from 93 to 338.2 individuals. The highest infection was recorded in the animals from one to 4 years of age. This is likely due to the physiological activities of animals (the way of life, migration, seasonality, nature of soil cover, etc.) and their low immune-morphological reactions. A high infection of wild boars by Metastrongylus depends on their lifestyle, environment and migration. The intensity of infection in the wild boars is the result of the autumn seasonal infection of earthworms in summer.

SY20.P.06

MOLECULAR EVIDENCE FOR THE EXISTENCE OF TWO FURTHER SIBLING SPECIES OF THE *CONTRACAECUM RUDOLPHII* COMPLEX (NEMATODA: ANISAKIDAE) FROM THE SPOTTED SHAG, *PHALACROCORAX PUNCTATUS*, A SPECIES OF CORMORANT ENDEMIC TO NEW ZEALAND

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Two sibling species of *Contracaecum* have been detected in sympatry in the Spotted Shag *Phalacrocorax punctatus*, a species of cormorant endemic to New Zealand. This is based on sequences analysis of multiple loci, i.e. mitochondrial cytochrome oxidase 2 (mtDNA *cox-2*), the small subunit of the mitochondrial ribosomal RNA gene (*rrnS*), the ITS-1 and ITS-2 of the nuclear ribosomal DNA. The worms morphologically resemble the nominal species *C. rudolphii s.l.* The genetic relationships between the two sibling species and the related congeners from fish-eating birds, previously characterized genetically by the same genetic markers, i.e. *C. rudolphii* A, B, C, D, E, *C. septentrionale*, *C. microcephalum, C. bioccai*, *C. pelagicum, C. micropapillatum*, *C. gibsoni* , *C. overstreeti, C. chubutensis, C. australe* and *C. fagerholmi* have been carried out based on those multiple loci. Concatenated phylogenetic analysis inferred from mitochondrial genes (*cox-2*, *rrnS)* were congruent in depicting the two sibling species as forming two distinct clades, highly supported at the bootstrap analysis, from the remainder of the *Contracaecum* taxa considered; thus, it validates their specific status. Further, analyses of the ITS-1 and ITS-2 sequence data from the two taxa supported their distinction from all the *Contracaecum* previously sequenced at that loci and deposited in GenBank.
Phylogenetic trees support the two species as sister taxa, and included in the same clade with the previously detected species of *Contracaecum* from cormorants (i.e. *C. rudolphii* A, *C. rudolphii* B, *C. rudolphii* C and *C. septentrionale, C. rudolphii* C, *C. chubutensis, C. australe*). That these two new sister taxa parasites of *Ph. punctatus* form a supported clade with *Contracaecum* spp. from other *Phalacrocorax* spp. is in agreement with the results of seabird phylogenetic analysis in which closely related Seabird taxa appear to have closely related *Contracaecum* spp.

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SY20.P.07

PREVALENCE OF ENDOPARASITES IN BROWN BEARS (*URSUS ARCTOS*) FROM NATURAL HABITATS IN ROMANIA

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Half of the brown bear population from Europe (6500-7000) live in Romanian Carpathian Mountains. The aim of this study was to establish the structure of the endoparasitic population of the brown bear (*Ursus arctos*) in Romania. A number of 48 faecal samples have been collected from brown bears that were caught for microchip implantation, in a program that monitors the wildlife population in Romania. All the samples were examined using the classical techniques (flotation and sedimentation). In order to identify *Cryptosporidium* spp. oocysts, we used modified Ziehl-Neelsen stain technique. The overall prevalence was 45.8 % (22/48). 8 parasites species were identified, with different level of prevalence, as follows: *Isospora fonsecai* 10.4 %; *Dicrocoelium* spp. 10.4 %; *Bayliascaris* spp. 29.2 %; *Toxocara mystax* 2.1%, *Toxocara canis* 2.1%, *Spiroptera ursi* 4.2 %, and *Crenosoma* spp. 6.3%.
This is the first study the evaluates the structure of the endoparasitic population of the brown bear (*Ursus arctos*) in Romania.
Keywords: brown bear, endoparasites, Romania.

SY20.P.08

THE HELMINTH FAUNA OF WILD UNGULATES ANIMALS IN NATURAL ECOSYSTEMS OF THE INNER TIEN-SHAN

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It is known that helminthes are wide spread and affect all the species of wild hoofed animals. WHO data show that helminthiasis occupies the third position among the most significant infectious and parasitic diseases, the number of cases reaches 1.4 bn. Therefore, the specification of animal helminthofauna within constantly changing ecological conditions has a significant medical and veterinary importance for population health protection, efficient nature management, animal resources protection and development of antiparasitic action system. The helminthofauna of wild cloven-hoofed animals under the conditions of highland ecological systems of Inner Tien-Shan in terms of parasitology is insufficiently explored.
The focused helminthological examinations were launched in 2001 in the natural conditions of Naryn State Reserve. The objects of helminthological examination were red deers *(Cervus elaphus sibiricus S.)* and roe deers *(Capreolus capreolus L.).* Such examination method was applied to the liver and digestive system of 87 red deer and 102 roe deer total.
The helminthological examinations held have shown that within the red deers population the parasitizing helminths are of the families of Dicrocoeliidae *(Dicrocoelium lanceatum)* - 64%, Trichostrongyloidea (*Trichostrongylus capricola, Haemonchus contortus, Dictyocaulus filaria),* - 58.7%, Anoplocephalidae *(Moniezia expansa)* -23.1%, Ascaridoidea *(Neoascaris vutulorum)* – 17.8%, Trichocephalidea *(Trichocephalus ovis)* -16.5%, Paramfhistomatidea *(Paramfhistomum cervi)* – 13.6%, Fasciolidae *(Fasciola hepatica, Parafasciolopsis fasciolaemorpha)* – 8.7% and parasitic protozoa – Eimeriidae *(Eimeria crandallis, Eimeria ovinoidalis*)- 38.9%. Nematodirus invasion prevails. Nematodiruses were found most frequently in young animals. The basis of parasite complex is made of trichostrongylidae and eimeria oocysts that are dominant in terms of frequency of occurrence and that have epizootic importance.
The intensity of helminth invasions among roe deer *(Capreolus capreolus L.)* constituted 74.1%. Helminthofauna included: Fasciolidae (*Parafasciolopsis fasciolaemorpha)* – 3.7%, Dicrocoeliidae

(Dicrocoelium lanceatum) – 48%, Paramphistomatidae (*Paramphistomum cervi)* – 15.4%, Taeniidae (*Echinococcus granulosus*, *larvae)* – 7.6%, Anoplocephalidae *(Moniezia expansa)* – 19.6%, Strongyloidea *(Chabertia ovina, Oesophagostomum venulosum)* – 16.7%, Trichostrongyloidea (*Trichostrongylus capricola, Haemonchus contortus, Dictyocaulus eckerti, Ostertagia ostertagi)* – 13.5%, Ancylostomidea (*Bunostomum trigonocephalum)* – 5.4 %, Filaroidea (*Setaria labiato-papillosa)* – 7.4%, Trichocephalidea (*Trichocephalus ovis)* – 4.1%.

The received research results show that the wild cloven hoofed animals in the reserve are mainly affected by the helminthiasis, the causative agents of which are developed with the help of intermediary host. The spread of such helminthiasis is connected to the special features of ecology of terricole molluscum of *Bradybaena* genus and ants of *Formica* and *Proformica* genera. During our research *Bradybaena* species were encountered in various biotypes (the sections of mixed forest; the shores of basins covered with bushes; water meadows). They dwelled in leaf litter¸ in hollows¸ under stones¸ in grass¸ in floodplain bushes and trees. The population density per 1 m² reached 1.3 to 17.5 species.

A specific variety helminthofauna wild cloven-hoofed animals in territory of Naryn State Nature Reserve includes 20 species parasitizing helminths, 12 families. In structure helminthofauna the share nematode trematode fauna makes wild cloven-hoofed animals 50 %, trematode fauna – 25%, cestode fauna – 16.7%. The faunistic structure of communities parasitic helminth *Dicrocoelium lanceatum* is defined by ecological features of a habitat of intermediate hosts – land overland molluscs *Bradybaena* genus and ants *Formica* and *Proformica* genera.

Thus, the analysis of information on helminthofauna of wild cloven hoofed animals signifies the importance of performing ecological and parasitological research, aimed at comprehensive examination of up-to-date composition and development cycles of parasites of wild animals and the role thereof in interspecies interactions.

SY20.P.09

STUDY ON THE ECTOPARASITES OF CHUKAR PARTRIDGE, *ALECTORIS CHUKAR* FROM SHAQLAWA DISTRICT, KURDISTAN REGION, IRAQ

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A total of 96 specimens of chukar partridge *Alectoris chukar* were collected from Shaqlawa district, northeast of Erbil city, Kurdistan region, north of Iraq, during the period from May 2009 to the end of April 2010 and inspected for ectoparasites. The study revealed the existence of six species of lice (arthropodans) namely: *Amyrsidea perdicis*, *Cuclotogaster heterographus*, *Goniocotes gallinae*, *Goniocotes chrysocephalus*, *Goniodes colchici* and *Lipeurus maculosus*. Four species of these parasites (*A. perdicis*, *G. Chrysocephalus, G. colchici* and *L. maculosus*) are considered as first records in Iraq. Also, *A. chukar* was regarded as a new host for *G. gallinae*.

SY20.P.10

THE HELMINTHES FAUNA OF ANURAL AMPHIBIANS IN KAZAKHSTAN

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In the work the original authors' materials on the helminthes fauna of anural amphibians in the different point of Kazakhstan per 1986-2011 years were representative. In the moor frog (Rana arvalis) from Pavlodar region 5 mature helminthes species: *Opisthioglyphe ranae*, Ha*plom*etra *cylindracea*, *Pleurogenes intermedius*, *Rhabdias bufonis* and *Oswaldocruzia filiformis* – were registered, which earlier were recorded by V.G.Vakker and N.E.Tarassovskaya Among the larval forms mesocercaria *Alaria alata*, metacercaria *Strigea* s*trigi*s and *S.falconis*, larvae of acanthocephalan *Sphaerirostris teres* were discovered. In the neighbourhood of Leninogorsk, in

flood-land of Ulba River in this host three helminthes species - trematode *Haplometra cylindracea*, nematodes *Rhabdias bufonis* and *Oswaldocruzia filiformis*, and in the city agglomeration of Ust-Kamenogorsk – also 3 worm species - *Opisthioglyphe ranae*, *Rhabdias bufonis* and *Oswaldocruzia filiformis* – were recorded. In the moor frog from Akmola region 4 mature helminthes species – *Opisthioglyphe ranae*, *Haplometra cylindracea*, *Rhabdias bufonis* and *Oswaldocruzia filiformis* – were registered. In the lake frog (*Rana ridibunda*) in Ust-Kamenogorsk 2 worm species - *Opisthioglyphe ranae* and *Oswaldocruzia filiformis*, on the outskirt of Almaty city 3 species - *Skrjabinoeces* sp., larvae *Strigea falconis*, larvae *Alaria alata* – were recorded. In first-year lake frogs from Sorbulak Lake (Almaty region) and Beskaragai district of Eastern-Kazakhstan region helminthes were not recorded. In the green toad (*Bufo viridis*) in Almaty city we found 4 helminthes species parasitized in the mature form - *Acanthocephalus falcatus*, *Rhabdias bufonis*, *Strongyloides* sp., *Cosmocerca commutata*, and larval stage of nematode *Agamospirura magna*. In green toad from Ekibastuz district of Pavlodar region only one parasite species – nematode *Oswaldocruzia filiformis* – was registered. In the common toad (*Bufo bufo*) from Eastern Kazakhstan region two nematode species - *Rhabdias bufonis* and *Oswaldocruzia filiformis* – were registered. By the autopsy of 2 exemplars common toads from Selety River in Pavlodar region helminthes weren't found. Anural amphibians can play the positive role in the improvement of sanitary condition of water biotopes in regard to the helminthes as the direct eliminators of larval stages of nematodes of home hoofed animals of *Strongylata* suborder and the worms' larvae in the intermediate hosts, and also as the definitive hosts of harmless helminthes competing with the parasites of people and home animals.

SY20.P.11

INCIDENCE AND GENETIC CHARACTERISATION OF *TOXOPLASMA GONDII* IN RED FOXES (*VULPES VULPES*) IN SLOVAKIA

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Toxoplasma gondii is an obligate protozoan parasite infecting birds and mammals including humans. Contaminated food of animal origin play an important role in transmission of infection to man, accordinglytoxoplasmosis and agents thereof have to be reported by Member States according to their epidemiological situation (Directive 2003/99/EC).
Monitoring of *T. gondii* infection in free living animal can provide significant data on environmental contamination and circulation of parasite in domestic and natural environment. Thus, the aim of our work was to investigate the infection in red fox (*Vulpes vulpes*) as the most abundant carnivore in Slovakia and genetically characterise its agent.
During 2006-2011 in total 204 foxes were examined. DNA was isolated from muscle tissue and by analyses of TGRIE and B1 genes the presence of parasite was confirmed in 56 specimens (27.45%). For *T. gondii* genotypization, SAG2 locus was used followed by RFLP using restriction endonucleases Sau3 AL and CfoI. Genetic characterisation of *T. gondii* affirmed in foxes the presence of all three genotypes (I, II, III) with domination of G I genotype (39.28%). G II genotype was detected in 25% and G III in 35.72 %.
Results of epidemiological survey point out the foxes being suitable indicator of *T. gondii* infection in natural foci, presence of infected prey, and environmental contamination with oocysts. The obtained data on high prevalence of *T. gondii* in red fox population refer to circulation of this zoonotic agent in wildlife.

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SY20.P.12

MOLECULAR CHARACTERIZATION OF *CONTRACAECUM RUDOLPHII* (NEMATODA: ANISAKIDAE) FROM *PHALACROCORAX CARBO SINENSIS* FROM SICILY

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Specimens of *Contracaecum rudolphii* Hartwich, 1964 (Nematoda: Anisakidae) from *Phalacrocorax carbo sinensis* (Linnaeus, 1758) from Sicily were collected and characterised genetically using PCR-RFLP analysis of the rDNA internal transcribed spacers (ITS-1, 5.8S and ITS-2) and of the small subunit of the mitochondrial rRNA (*rrn*S) (D'Amelio et al, 2007 and 2012).
An individual of *Phalacrocorax carbo sinensis*, the Eurasian subspecies of the Great Cormorant which is usually observed in the winter season also in Sicily, was found and collected by the staff of Wildlife rescue Center of Cattolica Eraclea in province of Agrigento, probably coming from the near Platani river. A total of 92 nematodes, at larval and adult stage, were collected from the stomach at necropsy and analized in the present study. Nematodes were repeatedly washed in physiological saline, stored in 70% ethanol and cleared in glycerine for morphological studies of the anterior and posterior ends for *Contracaecum* by light microscopy (morphology of lips and interlabial tips, length of spicule and morphology of the spicule tip etc) (Abollo et al, 2001). A subsample of nematodes (n=30) was characterized using genetic markers defined previously in the internal transcribed spacers (ITS) of nuclear ribosomal DNA and in the small subunit of the mitochondrial ribosomal RNA gene (*rrn*S). The adult nematodes recovered from *P. carbo sinensis* from Sicily were morphologically identified as *C. rudolphii* (s.l.). The molecular characterization using the PCR-RFLP analysis of the ITS and *rrn*S allowed the identification of the specimens as *C. rudolphii* B. These results have been also confirmed by sequences analysis of representative specimens, BLAST search and aligment with already characterized individuals.
Several studies describe *C. rudolphii* Hartwich, 1964 (s.l.) as a common anisakid of fish-eating birds, with a world-wide distribution: these nematodes have been so far reported in the definitive hosts, mainly cormorants, also in Europe and in Italy were the two cryptic species A and B have been recorder (Mattiucci et al, 2002; Farjallah et al, 2008). From an ecological viewpoint, Mattiucci et al. (2002 Parassitologia) considered *C. rudolphii* A as a species occurring in brackish waters, as is *C. rudolphii* C (D'Amelio et al, 2007), in contrast to *C. rudolphii* B which occurs mostly in freshwater habitats. The possible origin of the definitive host from inland waters in Sicily seems to support such hypothesis. The preliminary results reported provide information regarding the species of *Contracaecum rudolphii* complex parasites of cormorants in this area.

SY25.P.01

A SENSITIVE AND SPECIFIC PCR BASED METHOD FOR IDENTIFICATION OF *CRYPTOSPORIDIUM* SP. USING NEW PRIMERS FROM 18S RIBOSOMAL RNA

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The main goal of the present study was to develop a new sensitive and specific PCR based method for Identification of *Cryptosporidium* sp. using novel primers from 18S ribosomal RNA. Cryptosporidiosis in high-risk host groups particularly in neonates and immuno-compromised individuals may result in death. To the best of our knowledge this is the first study regarding develop a new PCR based method to diagnose the cryptosporidiosis in Iran. A total of 850 human fecal samples from patients clinically suspected to cryptosporidiosis and 100 healthy and diarrheic cattle stool specimens were collected. The simplified formol-ether concentration method was carried out for all samples. They were then examined microscopically by modified Ziehl-Neelsen staining method. Total DNA was extracted by QIA amp DNA stool mini kit was carried out by using designed primers. Twenty nine cases of cryptosporidiosis infection in human and 30 samples from cattle microscopically were positive. The described primary and nested PCR method could detect all *Cryptosporidium* positive samples from human and cattle. Regards to suspected negative samples in primary PCR examination, the Nested PCR could approve two more positive results. Furthermore, Nested PCR analysis was able to detect one more case which was negative in both microscopically examination and primary PCR. Specificity of the test was 100%. Sensitivity of Nested PCR in comparison to our gold standard; microscopy after Ridley concentration modified Ziehl-Neelsen, was 100 %. Our developed PCR based method by using new primers devised from 18S ribosomal RNA revealed the ability for identification of the *Cryptosporidium* species such as *C. parvum* and *C. huminis* with high specificity and sensitivity.

SY25.P.02

SEASONAL PREVALENCE OF GASTROINTESTINAL NEMATODES IN SHEEP AT EL-BEHEIRA PROVINCE, EGYPT: EGGS AND THIRD LARVAL STAGE CHARACTERIZATIONS

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The study was carried to investigate the gastrointestinal nematode genera and seasonal prevalence in sheep at Beheira province, during March 2010 till February 2011. A total of 244 fecal samples of sheep from local breeds were collected and examined using flotation and fecal culture

techniques. Third stages larvae of eight nematode genera were detected as *Haemonchus*, *Ostertagia*, *Trichostrongylus*, *Cooperia*, *Bunostomum*, *Chabertia*, *Oesophagostomum* and *Strongyloides*. Seasonal dynamics of the infestation of sheep with gastrointestinal nematodes revealed that highest infection rate was observed in autumn (97.5%) followed by spring (81%), winter (73.6%) then summer (55%). Morphological descriptions of these larvae were given, and the most prevalent one was Haemonchus. Out of three gastrointestinal tracts from 10 were infested with Haemonchus contortus. Ten gastrointestinal tracts (GIT) of sheep brought from abattoirs of El Delengat, Etay El Barud, and Kom Hamadah at Beheira province; The freshly GIT were examined for nematodes as well as parasitic lesions. The content of abomasums, small and large intestine were washed through sieve for collection of parasites, then identifying the recovered worms. Isolation of eggs from uterus of previously identified female nematode and fecal specimen from each infected slaughtered animal was taken from the rectum. Each specimen was carried out for floatation, fecal culture and isolation of 3rd stage larvae techniques. Biochemical analysis of 70 sera samples from naturally infested sheep with gastrointestinal nematodes, found that decrease total serum protein, serum albumin and albumin globulin ratio, and increase serum globulin. Histopathological examinations of lesions in abomasums of sheep naturally infected with *Haemonchus contortus* showed that goblet cells hyperplasia desquamated of gastric cells, lymphocytic aggregation and edema.

Keywords: Sheep, Gastrointestinal nematodes, *Haemonchus*, *Ostertagia*, *Trichostrongylus*, *Cooperia*, *Bunostomum*, *Chabertia*, *Oesophagostomum* and *Strongyloides*.

SY25.P.03

IDENTIFICATION OF *ECHINOCOCCUS MULTILOCULARIS* IN CANIDS OF IRAN

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Echinococcus multilocularis causes alveolar echinococcosis, a serious zoonotic disease present in many areas of the world. The parasite is maintained in nature through a life cycle in which adult worms in the intestine of carnivores transmit infection to small mammals, predominantly rodents, via eggs in the feces. Humans may accidentally ingest eggs of *E. multilocularis* through contact with the definitive host or by direct ingestion of contaminated water or foods, causing development of a multivesicular cyst in the viscera, especially liver and lung. This investigation assessed the presence of *E. multilocularis* infection in definitive hosts in the Chenaran region of Razavi Khorasan Province, northeastern Iran.

Over a period of two years, from 2009 to 2011, 77 domestic and stray dogs and 16 wild carnivores from Chenaran area were examined using the flotation/ sieving method followed by multiplex PCR of mitochondrial genes.

The intestinal scraping technique (IST) and the sedimentation and counting technique (SCT) revealed adult *Echinococcus* in the intestines of five of 10 jackals and, of the single wolf examined. Three jackals were infected only with *E. multilocularis* but two, and the wolf, were infected with both *E. multilocularis* and *E. granulosus*. Multiplex PCR revealed *E. multilocularis*, *E. granulosus*, and *Taenia* spp. in 19, 24, and 28 fecal samples, respectively. *Echinococcus multilocularis* infection was detected in the faces of all wild carnivores sampled including nine jackals, three foxes, one wolf, one hyena, and five dogs (6.5%). *Echinococcus granulosus* was found in the fecal samples of 16.9% of dogs, 66.7% of jackals, and all the foxes, the wolf, and the hyena. The feces of 16 (21.8%) dogs, 7 of 9 (77.8%) jackals, and all three foxes, one wolf and one hyena were infected with *Taenia* spp.

Base on the present study, Chenaran area, northeastern Iran is an endemic area of *E. multilocularis* infection and red fox, jackal, wolf, hyena, and dog are definitive hosts. Therefore, the

existence of this parasite and definitive hosts show clearly that *E. multilocularis* is an important source of human alveolar echinococcosis in this region.
As a result, the local population and visitors are at risk of infection with alveolar echinococcosis and intensive health initiatives for control of the parasite and diagnosis of this potentially fatal disease in humans, in this area of Iran, are needed.

SY25.P.04

SEROLOGICAL SURVEY OF ANIMAL TOXOPLASMOSIS IN DAKAR AND IN SINE-SALOUM (SENEGAL)

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Toxoplasma gondii is an important zoonotic intracellular protozoan parasite, which can affect all warm-blooded mammals and birds throughout the world, including humans. In recent years, surveys of *Toxoplasma gondii* infection in animals and humans have been reported worldwide. However, little is known about the prevalence of *T. gondii* in animal population in Senegal. The aim of the present study was to focus on the Sine-Saloum region of Senegal in order to explore the extent of toxoplasmosis in the animal population of two villages: Dielmo and Ndiop. 188 serum samples have been collected in the two villages: 56 cattle, 52 goats, 43 sheep, 27 dogs and 10 donkeys during 2011-2012. Additional samples have been collected in Dakar: 27 serum samples of dogs (16 originating from National Gendarmerie and 11 from French Armed Forces) and 64 serum samples of horses (50 from National Gendarmerie and 14 from French Armed Forces). The serum samples have been tested by MAT technique. 11 samples of small insectivore and rodent brains (*Crocidura olivieri, Mastomys erythroleucus, Mastomys huberti, Tatera gambiana*) were collected also in Dielmo for PCR identification of *T.gondii*.
The seroprevalence of *T.gondii* in the two villages of the Sine-Saloum region was variable from 48% in dogs to 23% in ovines, 9 % in goats and 7 % in bovines. None of the donkeys tested present any antibodies against *T.gondii*. In Dakar area, the animals tested show a prevalence of *T.gondii* of 22% in dogs and 3 % in horses. The dilution titers were generally higher in the Sine-Saloum region (maximum 1/12800 in ovines) than in Dakar (maximum 1/ 800). One brain sample *(Mastomys huberti)* revealed positive when tested by PCR.
The present results on *T. gondii* seroprevalence in animals confirm that the zoonotic parasite is widely present in Senegal, as it has been previously shown in other studies (Deconinck, et al., 1996; Pangui et al., 1993). Extensive field investigations in the domestic animal population as well as the wildlife are requested in order to confirm the present results.

SY25.P.05

TOXOPLASMOSIS AND TRICHINELLOSIS: AN EPIDEMIOLOGICAL SURVEY OF PIG POPULATION IN MADAGASCAR

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Besides cysticercosis, scarce information is available about the other meat-born zoonotic parasitosis in the domestic animal population of Madagascar. The present study aimed at investigating the extent of two other major parasitic diseases, namely toxoplasmosis and trichinellosis, within the Malagasy pig population.
Two hundred and fifty pig serum samples were collected during 2010 in the 4 major slaughterhouses of Antananarivo, the Malagasy capital. Sampled pigs were raised in 11 different regions (on the total of 22 regions) and transported by traders before slaughtering. Samples were stored at -80°C and sent for analysis in ANSES (metropolitan France).
Serological investigations were conducted using ELISA technique: ID Screen® Toxoplasmosis Indirect ELISA kit (IdVet, France) and PrioCHECK® Trichinella Ab (Prionics, Switzerland).
Preliminary results show a seroprevalence of 22.8% (57/250) for toxoplasmosis. All regions are endemic for toxoplasmosis with a frequency of positive pigs from 12% (n=8) in the southern Toliara province, 21% in Antananarivo (n=69) or Fianarantsoa (n=79) in central uplands, to 33% (n=69) in Mahajanga on the coastal north-west region; no region of the island can be considered as free of toxoplasmosis. No correlation were found with the sex, breed or age category of pigs.
Regarding *Trichinella,* two pigs presented positive values in ELISA based on excretory/secretory antigens but those results were not confirmed in Western blot. Even if serological test for the detection of *Trichinella* provide a high degree of sensitivity and specificity, the existence of a blind window of time, especially for light to moderate infection, involves the occurrence of false negative results during the early stages of infection. Further investigations needs to be conducted, in correlating serology and muscle analysis to conclude on the *Trichinella* status of Madagascar.
National field investigations in the pig and human populations are requested in order to confirm the present results as well as to correlate the observed prevalences with on-farm risk factors and with the transmission of trichinellosis and toxoplasmosis to Malagasy consumers and especially pregnant women.

SY25.P.06

STUDY OF GASTROINTESTINAL NEMATODES IN SICILIAN SHEEP

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A high incidence of parasitic infections in domestic animals has a big impact on productivity and it leads to severe economic losses. The parasite-infected animals increase their metabolic rate and reduce the amount of metabolic energy used for production, as the parasites use their nutrients, damage some vital organs and cause animals to become more susceptible to other pathogenic agents. We conducted a preliminary study with the aim of setting out the composition of gastrointestinal parasite communities in sheep farms in Sicily, the largest island in the Mediterranean sea. In this study the prevalence of parasitic infections in sheep caused by intestinal

parasites was analysed. The study was carried out in adult sheep randomly chosen from selected farms from January to December 2011. Individual faecal or intestine samples were collected and tested. Almost 60% of the tested animal resulted to be positive to parasites. Co-infection supported by more than a single parasite was observed in almost all the farms; sheep could even harbour three different parasites simultaneously (members of the families of Trichostrongyloidea, Strongyloidea, Anoplocephalidae). Distribution of parasites has been related to spatial location of farms and seasonal changes. The herds studied were located at different altitudes and in different climatic conditions. Eradication of gastrointestinal parasites from the environment is generally impractical; though, infections can be limited, and control programmes should mainly minimise the deriving economic losses. Moreover, in temperate regions with wet and hot climates, like Sicily, it is impossible to organize anthelmintic treatment based on results from other countries, as the winter hypobiosis was not observed in our region.

SY25.P.07

COPROPARASITOLOGICAL INVESTIGATION IN DOGS FROM SOUTHERN ROMANIA, WITH FOCUS ON CESTODES

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Intestinal parasites are common pathogens in dogs, some of them with zoonotic potential. The aim of the present study was to asses the prevalence of intestinal parasitic infections in dogs from southern Romania, with focus on cestodes, especially *Taenia* spp./*Echinococcus* spp., and additionally *Dipylidium caninum*. The investigations were carried out during of October 2010 to March 2012. The coproparasitological examination of 787 faecal samples collected from urban areas (n=544), rural areas (n=109) and livestock guardian dogs (cattle and sheep) (n=134), revealed a prevalence of 71.5% (563/787; 95% CI = 68.24 - 74.67) of intestinal parasites (*Ancylostoma caninum* 36.4%, *Uncinaria stenocephala* 20.8%, *Toxocara canis* 12.8%, *Toxascaris leonina* 2.7%, *Trichocephalus vulpis* 25.2%, *Eucoleus* spp. 0.9%, *Strongyloides stercoralis* 0.4%, *Cystoisospora* spp. 7.6%, *Sarcocystis* spp. 0.4%, *Dipylidium caninum* 6.5%, and *Taenia* spp. 1.1%). Significantly differences (p=0.003) were found within the dog category prevalence: 69.7% (379/544) in dogs from urban areas, 85.3% (93/109) in dogs from rural areas, and 67.9% (91/134) in livestock guardian dogs. Intestinal parasites prevalence was statistically correlated with season (p=0.002). The highest prevalence was observed in autumn (77.5%, 158/204), followed by summer (72.9%, 231/317), spring (69.9%, 128/183), and winter (55.4%, 46/83).
Taenia spp. eggs were identified in 9/787 (1.1%; 95% CI = 0.52 - 2.16) faecal samples. A single cestodes species was certainly identified, i.e. *D. caninum*, in 51/787 (6.5%; 95% CI = 4.86 - 8.44) faecal samples. Both *Taenia* spp. and *D. caninum* predominated in rural areas (4/109, 3.7% versus 15/109, 13.8%), than in urban areas (2/544, 0.4% vs. 30/544, 5.5%) and livestock guardian dogs (3/134, 2.2% vs. 6/134, 4.5%), with significant prevalence differences (p=0.005 and p=0.004) within-dog category.
A total of 123 faecal samples randomly selected and the 9 *Taenia* positive samples were examined by CoproAg-ELISA for *E. granulosus.* The coproantigens were detected in 4/46 (8.7%; 95% CI = 2.42 - 20.8) dogs from rural areas, and in 7/86 (8.1%; 95% CI = 3.33 - 16.06) dogs from urban areas, but the differences were not found to be statistically significant (p>0.05). All of the nine samples containing taeniid eggs were negative for *E. granulosus* antigens.
The findings showed a high infestation with intestinal parasites among dogs from southern Romania, some of them with a high potential risk for public health.

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SY25.P.08

NEOSPORA CANINUM IN DOGS FROM BUCHAREST AREA, ROMANIA: SCREENING FOR SEROCONVERSION BY INDIRECT FLUORESCENT ANTIBODY TEST

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Neospora caninum is an apicomplexan parasite closely related with *Toxoplasma gondii.* The dog and other canids are definitive hosts, but neosporosis is a major cause of reproductive failure with predilection in cattle. To investigate the exposure of dogs to *N. caninum* infection, the specific antibody response was evaluated using 80 serum samples from apparently healthy dogs from different urban and rural places located in Bucharest area, southern Romania. The samples were tested using a commercial indirect fluorescent antibody test (IFAT). *N. caninum* specific antibodies were observed in 17/80 (21.3%; 95% CI=12.89-31.83) dog serum samples. *N. caninum* infection was more common in dogs from rural areas, with 23.8% prevalence (10/42; 95% CI=12.05-39.46), than in dogs from urban areas, with 18.4% prevalence (7/38; 95% CI=7.74-34.33), respectively (p>0.05). Subcategories were also analyzed. The urban dogs from a permanently shelter had a prevalence of 11.1% (1/9; CI=0.28-48.25), while in stray dogs the seroprevalence was higher, up to 20.7% (6/29; 95% CI=7.99-39.73), although the differences were not found to be statistically significant. In dogs from rural areas, the seroprevalence was significantly higher (p=0.03) in cattle farm dogs (8/21; 38.1%; 95% CI=18.1-61.57) than in guard dogs (2/21; 9.5%; 95% CI=1.17-30.38). The seropositivity to *N. caninum* increased significantly with age (p=0.009). Dogs aging over 10 years were 4 times (66.7%, 4/6) more likely to be infected than dogs 1 to 5 years of age (16.7%, 7/42), and 2.3 times more likely to be infected than dogs aging 6 to 9 years (28.6%, 6/21). No dog less than 1 year of age was infected (0/11), suggesting preponderance of post-natal exposure to *N. caninum* infection. No significant differences in *N. caninum* seroprevalence related to gender were observed (p>0.05).
These findings confirm exposure to *N. caninum* infection of dog populations from Bucharest area and revealed an important level of subclinical infection especially in farm dogs, leading to a high potential risk infection for cattle in the area.

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SY25.P.09

MOLECULAR BIOLOGY METHODS FOR DETECTION AND IDENTIFICATION OF *DEMODEX* MITES

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Demodex are microscopic mites present both in animals and man. *Demodex folliculorum* and *Demodex brevis* are two species known to infest humans. These parasites are found in the follicular infundibulum and sebaceous or meibomian glands. Finding small mites can be difficult especially in the scrapings from the face. Molecular analysis can detect mites and also differentiate the species.
For *Demodex* mite presence, 83 patients from 32 to 76 - years old were examined (34 men, 49 women, mean age: 58 years). Each patient was examined by epilation of few eyelashes from each eyelid. Skin scrapings were collected from the patient's face too. To obtain animal mites, skin

scraping from hamster and dogs were performed. Eyelashes were put on the microscopic slide and some eyelashes were taken to DNA isolation. The same with skin scrapings – slide and DNA isolation was made. The concentration and purity of DNA was checked using a spectrophotometer. Molecular analysis was performed with specific primers based on sequences from GenBank characteristic for human, hamster's and dog's *Demodex* mites. The samples were studied under a light microscope. A positive result was recorded if any larval forms, adult, or eggs of *Demodex* mites were found. For molecular diagnosis PCR with specific primers was made.
Among 83 examined patients 47 had positive result (16 men, 31 women). Almost all of the positive results were confirmed both - by molecular and microscopic analysis. Only one positive result was confirmed only by molecular analysis, under the microscope the result was negative. To check if primers for human mites were specific, *Demodex* isolated from the hamster and dog were taken to analyses. There was no product after using *D. folliculorum* and *D. brevis* primers for animal mites. The product of PCR was made with hamster's mites, but using another primers, specific for hamster and not working with human samples.
The mites are often very difficult to find so microscopic examination of skin scraping could be supplemented by molecular biology methods.
PCR can be used for the diagnosis of both human and animal *Demodex* mites.

SY25.P.10

MOLECULAR CHARACTERIZATION OF *BABESIA* PARASITES FROM DOGS IN BANAT REGION USING PCR-RFLP

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The continuous expanding of tick borne diseases, including canine babesiosis, is considered a serious threat for pets' health in all European countries.
The aim of this study was to assess *Babesia* species and subspecies in dogs, with babesiosis from Banat region, Romania using molecular tools, in order to complete a nationwide picture with new data.
Between January 2010 and April 2011, blood samples from 101 dogs, suspected with canine babesiosis, were previously screened for the presence of *Babesia* spp. using Diff-Quik staining technique. The positive samples were selected for polymerase chain reaction (PCR) analyses of the small subunit rRNA gene (18S), followed by restriction fragment length polymorphism analyses (RFLP) with H*in*f I and T*aq* I restriction enzymes.
Overall, the microscopic examination revealed the evidence of a large *Babesia* sp. in 11 (10.9%) blood smears. All microscopic positive samples were suitable for molecular analysis and showed specific restriction patterns for intraerythrocytic piroplasms (410 bp) through PCR analysis. Subsequently, the lack of digestion of PCR amplicons with H*in*fI and T*aq*I restriction enzymes, revealed, in all cases, that *Babesia canis canis* was the only subspecies found in the examined samples.
In accordance with results of other studies, previously conducted in temperate regions of Europe, including Romania, our findings confirm that *Babesia canis canis* is the most common canine *Babesia* subspecies. Further studies, screening a higher number of animals and investigations of tick populations, as vectors for canine babesiosis, are still required.

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SY25.P.11

DETECTION AND MOLECULAR CHARACTERIZATION OF *BABESIA CANIS* AND *BABESIA VOGELI* FROM NATURALLY INFECTED ROMANIAN DOGS

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Canine babesiosis in Romania has become frequent in the last few years, with a wide variety of clinical signs, ranging from mild, nonspecific illness to per acute collapse, and even death. Traditionally, *Babesia* infection in dogs is diagnosed based on the morphologic appearance of the intraerythrocytic piroplasms observed in peripheral blood smears. The present study aimed at molecular detection and genetic characterization of the species of *Babesia* that cause canine babesiosis in Romania. For this purpose, eleven dogs with typical signs of babesiosis (lethargy, anorexia, fever, dark urine and thrombocytopenia) and microscopically proven positive for large piroplasms, and five clinically normal dogs were tested using polymerase chain reaction and subsequent genetic sequence analysis of a fragment of the 18S rRNA gene. Of the 16 samples, 12 (all the symptomatic dogs and one clinically normal dog) were positive in the PCR amplification. All PCR products from the positive samples were sequenced and BLAST analysis of GenBank revealed the presence of two *Babesia* species, namely *B. canis* in the 11 dogs with clinical signs of babesiosis and *B. vogeli* in one clinically normal dog. Alignment of the 478-bp sequences (477 bp for *B. vogeli*) showed that the *B. canis* sequences were all identical to each other and differed in 18 nt positions from the *B. vogeli*. The partial 18S rDNA sequences were submitted to GenBank® (accession numbers: JF461252 – JF461263). These findings provide basic information toward a better understanding of the epidemiology of canine babesiosis in Romania and will help to promote an effective control.

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SY25.P.12

STRONGYLIDS (NEMATODA; STRONGYLIDA) IN EQUIDS AT THE ASKANIA-NOVA BIOSPHERE RESERVE, UKRAINE: ANALYSIS OF BIODIVERSITY OF PARASITE COMMUNITY

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In Ukraine, six species of wild and domestic equids (horses, donkeys, Przewalski's horses, Turkmenian kulans and zebras Grevy and Burchelli) are kept in zoos and natural reserves. In the Askania-Nova Biosphere reserve all 6 equid species are grazed together at big steppe grasslands. That is why studies of biodiversity of parasite communities of these equid species at the Askania-Nova reserve are of special interest from parasitological and ecological points of view.
During 2004–2011 strongylid communities of 92 equids from the Askania-Nova reserve (22 domestic horses and ponies, 29 Przewalski horses, 15 donkeys, 9 kulans and 17 zebras) were examined *in vivo* by the diagnostic deworming technique. Animals were treated with macrocyclic lactone drug "Univerm" (0.2% aversectin, Russia). Faecal sampling (200 g from each animal) was performed at 24, 36, 48 and 60 hours after treatment. All nematodes expelled (totally 72,136 specimens) were collected and identified.
Totally, 33 strongylid species from 12 genera were registered in six equid species at the Askania-Nova reserve – 7 species of subfamily Strongylinae and 26 species of Cyathostominae. In domestic horses and ponies, 27 strongylid species were found: 6 species of Strongylinae and 21 –

of Cyathostominae; from 5 to 23 species (13.4±4.0) parasitized per host. In Przewalski's horses, 31 strongylid species were found: 6 species of Strongylinae and 25 – of Cyathostominae; from 7 to 8 species (14.3±2.8) parasitized per one host. In donkeys, 26 strongylid species were found: 4 species of Strongylinae and 22 – of Cyathostominae; from 6 to 16 species (12.7±2.4) parasitized per one host. In kulans, 21 strongylid species were found: 4 species of Strongylinae and 17 – of Cyathostominae; from 7 to 18 species (12.8±3.4) parasitized per host. In zebras, 21 strongylid species were found: 2 species of Strongylinae and 19 – of Cyathostominae; from 3 to 14 species (8 ±3.2) parasitized per one host. General structures of strongylid communities in all equids examined were multimodal with dominant, subdominant, background and rare species; this type of community structure is typical for equids that are not undergone with regular anthelmintic treatments. Bray-Curtis cluster analysis of biodiversity strongylid communities revealed higher similarity of these communities in zebras and Turkmenian kulans comparing to other species of equids.

SY25.P.13

A STUDY ON PARASITES OF PIGEON (*COLUMBA LIVIA DOMESTICA*) IN URBAN AREA OF MOLDAVA N/BODVOU, EASTERN SLOVAKIA

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Pigeons (order Columbiformes) are commonly occurring birds, found worldwide in urban and rural areas. The co-existence of humans and pigeons in urban and rural areas represents the public health risk caused by pigeon-transmitted pathogens. Many diseases of pigeons are zoonoses. Therefore reducing the number of pigeons becomes necessary in some areas where the populations of pigeons are overgrowing.

Our study was focused on detection of endo- and ecto- parasites in pigeons (*Columba livia domestica*) which were killed within the regulated reduction of urban pigeon population in city Moldava n/Bodvou in Eastern Slovakia during March 2012. In total, 85 adult pigeons were examined, 53 males and 32 females. Ectoparasites were sampled by shaking out the plumage. Each individual was subjected to parasitological dissection and the intestinal tract content was examined under stereomicroscope. Pooled faecal samples were examined for the presence of parasite eggs and coccidian oocysts using standard flotation technique.

Ectoparasites were present on 44.71% of pigeons. Four species of Mallophaga have been distinguished: *Goniocotes hologaster* (60.5%), *Goniodes gigas* (31.6%), *Lipeurus caponis* (23.68%) and *Cuclotogaster heterographus* (7.89%). It appeared that the first two of the above mentioned species showed the highest intensity of occurrence. The most frequent was the mixed invasion of these two species together with *Lipeurus caponis*.

Only one of 85 investigated individuals was found to be infected with intestinal nematode – 1 specimen of *Capillaria* spp. in small intestine. Faeces examination revealed 51% of pigeons being infected with *Eimeria* spp.

Surprisingly, our study confirmed low prevalence of intestinal helminths in urban pigeon population from Moldava n/Bodvou city. However, the presence of coccidian parasites and bird lice evidence the potential role of pigeons as a reservoir and carrier of these parasites to domestic birds. The occurrence of haematozoan parasites in pigeons remains to be investigated.

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SY25.P.14

EVALUATION OF SODIUM ACETATE ACETIC ACID FORMALIN (SAF) VERSUS 10 % FORMALIN AS PRESERVATIVE IN THE DETECTION OF INTESTINAL PARASITES IN STOOL

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Fixatives play an important role in preservation and transportation of human faecal specimens and in the accurate diagnosis of parasitic diseases. They are critical to epidemiological studies, particularly when conditions do not permit immediate processing. We aimed to compare between Sodium Acetate Acetic Acid Formalin (SAF) and 10% formalin preservative in the recovery of intestinal parasites and to evaluate the quality of preservation as well as the suitability of the fixative for long-term conservation. The present study was carried out in two stages: in the first stage, 630 stool samples were collected from primary and preparatory school children.They were divided and well mixed in two containers, one containing 10% formalin, and the other containing SAF. Parasitological examination using wet mount (iodine and Lacto-phenol cotton blue (LPCB)) smears, concentration technique and permanent staining was done for detection of intestinal parasites within one week .In the second stage, the positive stool samples for parasites were reexamined at various intervals (1 month, 6 months and one year) by the same methods formerly used. Examination of preserved stool samples in first stage revealed intestinal parasites in 355 out of 630 (56.3%) stool samples: 201 / 355 (56.6%) were detected in SAF preserved stool while 154 out of 355 (43.3%) in the conventional 10 % formalin . LPCB was found to be a useful stain; since it better stained SAF preserved parasites, it differentiated their internal structures clearly, thereby facilitating their detection and accurate identification even after long term preservation. Consequently SAF provides an excellent alternative instead of the 10% formalin in the routine examination of stool samples for parasites as it had a better fixation and staining abilities even after long-term storage. Moreover LPCB is suggested for routine use in the wet mount preparation of the SAF preserved stool in either field research and parasitology laboratory.

SY25.P.15

THERAPEUTIC APPROACHES IN CANINE DEMODECOSIS

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Demodecosis canine treatment remains a topical issue because individual reactions, favoring factors that are involved in the disease and the therapeutic protocol determine unexpected results sometimes. Some authors argue acaricide treatment, other authors argue herbal treatment of demodecosis. Most authors agree with the importance of imunostimulation and eliminating the factors favoring. The aim of this study was to establish which is the most efficient therapeutic approach in localized canine demodicosis. The study was performed on a 36 dogs were diagnosed with demodecosis – localized and dry form of clinical evolution. The study was conducted between March 2011 - March 2012. Dogs were hospitalized to the Veterinary Hospital of the University Clinics of a Faculty of Veterinary Medicine Timisoara. We formed three groups. Dogs were treated with topical and systemic acaricides (group 1). Dogs in this group were treated locally with 2% Amitraz and Ivermectin s.c., once a week. In group 2 was applied topically solution of honey and vinegar, three times a week. Lot 3 was treated with a solution containing aloe vera, every day. Immunostimulation was performed in groups 2 and 3 with Immunosuport, every day, for one month. There was no secondary reaction to the administration of the three therapeutic protocols. The results showed a clinical and parasitological cure in less time in group 2 compared with results obtained in groups 1 and 3. We support the spontaneous healing occurs in canine demodecosis without a specific treatment with acaricides. We recommend eliminating the factors favoring (parasitism, poor microclimate), the introduction of imunostimulation and application of topical

honey and vinegar solution. We consider this therapeutic approach as a successful and noninvasive protocol in treatment of localized canine demodecosis.

SY25.P.16
CANINE TOXOCARIASIS IN SOUTH EAST OF IRAN

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Toxocariasis is a parasitic zoonosis with worldwide distribution that affects both dogs and cats. *Toxocara canis* is the common roundworm of dogs, and is considered causative agents of human toxocariasis. It is an infection predominantly caused by migration of the roundworm *Toxocara canis* larvae to organs and tissues. The major clinical consequences of prolonged migration of *T. canis* larvae in humans are visceral larva migrants (VLM) and ocular toxocariasis (OT). Humans acquire the infection as a result of the accidental ingestion of the eggs with the second stage larvae of *Toxocara canis*. Children are the social group most vulnerable to the infection because of their frequent contact with the soil. Moreover, between the ages of one and four years geophagy is not uncommon. A cross-sectional survey was undertaken to study the prevalence and intensity of infection with *Toxocara canis* in 100 owned dogs, from May to Novamber 2011 in urban areas of Kerman, southeastern of Iran. A total of 100 fecal samples were evaluated by the fecal sedimentation method. A total of 10 dogs were found to be infected with *T. canis*. The prevalence of *T. canis* was 10% in owned dogs in Kerman. The age distribution of toxocariasis in dogs less than 6 months old had a higher overall prevalence than those dogs over 6 months of age (p<0.05). There was a significant difference in the prevalence between male (13.2%) and female (7%) dogs (p<0.05). The high prevalence of *T.canis* infections among canids and contamination of environment by eggs of *T. canis* may be increase the risk of infection for native people. It is imperative to educate the dog-owning population of the potential risks associated with dog toxocariasis. This will allow for the more effective implementation of strategic control programs or minimize zoonotic transmission.
Keywords: Toxocara canis, dogs, prevalence, Kerman.

SY25.P.17
THE PREVALENCE, ABUNDANCE, AND DISTRIBUTION OF SMALL STRONGYLES (NEMATODA, STONGYLIDAE) IN HORSES FROM WESTERN ROMANIA

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The most important horse parasites are small strongyles (Nematoda, Stongylidae), also known as cyathostomins or cyathostomes. The objective of this study was to collect and identify small strongyle species from the caecum, ventral, and dorsal colons of horses from western Romania and to report their prevalence in this part of country. Forty-seven horses from Arad, Timiş, and Caraş-Severin counties were examined postmortem for cyathostome infections. Samples were collected as described by Ogbourne (1975). Twenty-four species of small strongyles were identified. *Cyathostomum catinatum, Cylicocyclus nassatus,* and *Cylicocyclus insigne* had a prevalence of 100%. The 10 most prevalent species were: the three mentioned above plus *Coronocyclus coronatus, Cylicostephanus calicatus, Cylicostephanus goldi, Cylicostephanus longibursatus Cyathostomum tetracanthum, Cylicostephanus minutus,* and *Gyalocephalus*

capitatus. They comprised 75% of the total adult population. Two rare species were recorded in a higher prevalence and overall abundance: *Cyathostomum tetracanthum* (45% and 3.4%, respectively) and *Cylicocyclus brevicapsulatus* (32% and 3.3%, respectively). The latest one has been reported in the highest prevalence until now. Almost half (49.08%) of the cyathostome species was collected from the ventral colon, 35.9% from the dorsal colon, and 14.92% in the caecum. Two cyathostome species were concentrated in the caecum, 13 in the ventral colon, five in the dorsal colon, and the other four species were distributed uniformly throughout the intestinal contents. All horses were parasitized by at least five species, and two of them were infected with all 24 cyathostome species.

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SY25.P.18

CRYPTOSPORIDIUM SPP. IN FECAL SAMPLES OF CANARIES (*SERINUS CANARIA*) IN BRAZIL

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The genus *Cryptosporidium*, responsible for infect the brush borders of columnar epithelial cells and cause cryptosporidiosis, includes several species and is a significant pathogen of humans and animals. Infections caused by *Cryptosporidium meleagridis*, *Cryptosporidium baileyi* and *Cryptosporidium parvum*, have zoonotic potential and also have been reported in domestic birds. The purpose of this study was to evaluate the occurrence of natural *Cryptosporidium* spp. infection in healthy captive exotic canaries (*Serinus canaria*). The birds came from four Brazilian states, whose owners participated in the Brazilian championship of Ornithology, in 2011. The fecal samples were obtained for seven consecutive days and stored under 4°C. For screening *Cryptosporidium* oocysts, it was used the water - diethyl ether centrifugation method and an aliquot of the sediment was submitted to the modified Ziehl-Neelsen staining. The results showed the presence of Cryptosporidium spp. oocysts in 14, 28% (3/21) of the stools samples analyzed until now. Among the positive birds, two of them were from the same site (Blumenau - S 26 55.12513 W 49 3.96152 - Santa Catarina state) and one belonged to another city (Araraquara - S 21 47.65 W 48 10.45922 - São Paulo state). Molecular assays will be performed to elucidate which species are involved in those parasitized birds. In a canaries breeding colony, the excrements are discarded in common garbage and sometimes in domestic sewage therefore, the captive canaries may play an important role in the dissemination of zoonotic parasitic disease, like cryptosporidiosis, through the environment. Thus the importance of other animals such as domestic birds in the epidemiology of cryptosporidiosis should not be underestimated since this parasite causes an important zoonotic disease to animals and human health.

SY25.P.19

LEUCOSPORIDIUM SPP. A NEW PATHOGEN SPECIES OR A NEW NAME FOR A KNOWN AGENT OF SYSTEMIC CANDIDIASIS IN FARM-REARED RED-LEGGED PARTRIDGE (*ALECTORIS RUFA*)?

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This report describes the results of radiological, pathological and molecular examination of one farm-reared red-legged partridges (*Alectoris rufa*) affected by candidiasis. A juvenile farm-reared red-legged partridges was sent for clinical and pathological investigations. The animals had showed apathy, diarrhea, ruffled plumage and respiratory rattles. Post mortem total body lateral projection radiograph showed an increased perihilar interstitial pattern and air bronchogram signs due to lung edema. At necropsy, carcass showed cachexia; the pericloacal region was soiled by diarrheic fecal material. From the mouth to the intestine, a mucous yellowish fluid was present on a slightly reddish mucosa. At microscope, cytological smears revealed several hyphae, pseudohyphae and blastospores. Histopathology showed slight edema and congestion with different free fungal elements, referable to blastospores, hyphae and pseudohyphae. Molecular exam identified the most similar sequences as belonging to *Leucosporidium scottii*. To our knowledge, this case report describes for the first time this fungal species as a causative agent of candidiasis in birds. *Leucosporidium scottii*, synonymous *Azymocandida scottii*, *Candida scottii*, *Vanrija scottii*, is a fungal species frequently isolated from Antarctic and Italian waters, in terrestrial soil, in algae and decomposing plant, in chilled beef and fish, with high adaptability at medium-low temperatures, being the fungus a relative mesophyle. Different candida species have been reported in birds as commensal yeast and as causative agents of disease. In the last ten years, the application of PCR and the creation of specific data banks for fungi, has allowed to identify and to distinguish fungi morphologically almost similar. Candidiasis in birds is often associated with stress and poor husbandry, as is the case reported here. Thus, it is likely that those factors were the real problem in this bird.

SY25.P.20

MOLECULAR STUDY OF *CRYPTOSPORIDIUM* INFECTION IN CATTLE IN MASHHAD, IRAN

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Cryptosporidium is one of the most common diarrhea-causing parasitic genera in the world .Its emergence as a significant human pathogen and its known zoonotic potential make it a threat to global public health. Unfortunately, relatively little is known about the ecology of *Cryptosporidium* in areas with high human-animal interaction. *Cryptosporidium* is intacellular and extra-cytoplasmic protozoan that belongs to the phylum Apicomplexa. Fecal samples were collected from 800 dairy cattle (under 6,6-18, up to 18 months age), on 10 industrial dairy farms in Mashhad, Iran(from 2010 to 2011 years). The presence of *Cryptosporidium* sp. oocysts was determined by modified cold Ziehl-Neelsen's staining and a polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP) analysis of the small-subunit (SSU) rRNA gene was used to detect and identify *Cryptosporidium* spp. Results of microscopy observation showed that 23 samples (2.87%) were positive. Samples were confirmed by a small subunit rRNA-based nested PCR, which amplified a portion of the rRNA gene (830 bp). *Cryptosporidium* spp. was determined by the banding patterns of restriction digestions of PCR products with SspI, VspI, and DdeI. The RFLP analysis of three PCR products from each sample with restriction enzymes SspI and VspI; these results suggest that these PCR products belonged to either *C. muris* or *Cryptosporidium andersoni*. Further RFLP analysis with DdeI showed that banding patterns identical to

Cryptosporidium andersoni. The results showed that the species involved in all the samples found positive was *Cryptosporidium andersoni*. The results showed no significant difference between healthy and diarrheic groups and age groups cattle.

SY25.P.21

THYSANOSOMA ACTINIOIDES IN SHEEP FROM MENDOZA PROVINCE, ARGENTINA

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Thysanosoma actinioides (Cestoda: Anoplocephalidae) is a fringed tapeworm that parasites the bile ducts and the first portion of the small intestine of wild and domestic herbivores. It has been reported in cattle, sheep, goat, camel, moose, mule deer and wapiti. Its cycle is still controversial, especially those aspects concerning the intermediate host. Its presence can cause obstruction of the bile ducts; which can lead in some cases to maldigestion. The hepatic parenchyma can develop fibrosis and the bile ducts can manifest hyperplasia. This tapeworm has been described in the southern and western United States, South America and recently found in camels of Saudi Arabia. In Argentina *T. actinioides* has been found in the Andean Patagonia and in some regions of Buenos Aires and Corrientes provinces. In Río Negro Province, 100% of sheep examined, were found to be heavily infected, with a mean worm burden of 35.5 specimens per individual. The aim of this study is to report the presence of this parasite in the province of Mendoza, Cuyo region, where there are currently no reports. The sheep studied were from El Sosneado, southern Mendoza, which has a dry climate, characteristic of Patagonian plateau. It is a scantly populated region, dedicated mainly to subsistence sheep and goat farming. At the abattoir, post mortem inspection was undertaken on 26 livers. Cestodes where recovered from 9 of them and identified, according to Schmidt (1986), as *T. actinioides*. Seven of the livers had concomitant infection with the liver fluke *Fasciola hepatica*. This is the first report of *T. actinioides* in sheep from the Cuyo region, thus contributing to the actual knowledge of the distribution of this parasite in Argentina. This broadens the geographic distribution and also highlights its adaptability to diverse ecosystems. From a practical point of view, studies should be done to determine if this parasite has an economical impact on sheep production in the region. Also, it would be important to contribute to the epidemiological knowledge of this parasite which still has many aspects to be resolved.

SY25.P.22

EPIDEMIOLOGICAL INVESTIGATION OF SOME FUNGAL DISEASES IN POULTRY

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Some progress has been made in the control of fungal diseases in birds. Fungal infections, while not the most economically important various poultry diseases, still have an impact on the health of birds. From the fungal diseases most commonly encountered is aspergillosis. Acute aspergillosis is usually characterized by severe outbreaks in young birds and high morbidity and high mortality. Chronic aspergillosis occurs in adult breeder birds or occasionally in birds in an adult flock or aviary. The transmission can be made by egg-borne or during the incubation. The clinical signs are dyspnea, gasping, and accelerated breathing. The existing diversity of drugs and treatment methods are not sufficiently for solving this fungal disease. One of the most important things can be sever decontamination of the growing poultry spaces, systematically laboratory control of the feed, litter, etc.

Our investigation was focused on the establishment on the incidence and the frequency of aspergilliosis in the industrial conditions of poultry growth and the efficiency of some disinfectant solutions which were used for decontamination; thereby, when doing the disinfection it was demonstrated that Multocide-200 (2% solution) is more efficient then Verocid (1% solution). Generally, an effective means of therapy for avian aspergilosis is not available.
Prevention is currently the preferred means of control. This usually involves eliminating the source of the organism, such as moldy feed and litter, and treating the poultry houses and litter with antifungal compounds.
Keywords: aspergilosis, morbidity, diseses, decontamination.

SY25.P.23

REPRODUCTIVE ANALYSIS OF DAIRY COWS IN TERM OF NEOSPORA-ASSOCIATED ABORTIONS

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Neospora caninum is a major cause of prenatal loss in dairy cattle worldwide, with a negative economic impact on their breeding. The serological, molecular and reproductive studies carried out in a dairy herd with a high occurrence of abortions in north-eastern Slovakia are reported. Within epidemiological analysis of neosporosis, blood sera of 117 dairy cows with reproductive disorders and 226 sera of heifers were examined. To determine the dynamics of specific antibodies five seropositive dairy cows were selected and blood samples were taken off regularly, in month intervals. In tested dairy cows also reproductive parameters were analyzed.
In dairy cows post abortion 45.3% and in heifers 33.6% seroprevalence was found. Monitored seropositive cows gave birth to clinically healthy calves at 285th – 290th day of pregnancy. In 5 selected cows, specific antibody response determined by indirect ELISA (ID-VET, France) was relatively high during the first and second trimester, with a slight increase in the third trimester of pregnancy and persistence of antibodies 3 to 5 months after the birth. Two cows showed a slight decrease of anti-*Neospora* antibodies in the second trimester of pregnancy. In a cow monitored during two gestation periods, Western Blot analysis showed different immunodominant antibody pattern in gestation and in post partum period. Molecular analysis of foetus aborted in the fifth month of gravidity confirmed the presence of *Neospora caninum* DNA.
All analysed reproductive parameters overreached the optimal values. Interval between calving and first insemination (ICF), days open (DO) and inter-service interval (ISI) were longer by 33.3% – 107.4% and the number of services per conception (SC) was almost two times higher than in optimal conditions. The pregnancy rate achieved only 54.5% of optimal value. The percentage of fertilization after first insemination in cows (20.2%) was also below optimal values (55 – 60%) and repeated inseminations only moderately increased the percentage of fertilization.
The results of serological analysis revealed the potential risk of endogenous transmission of infection to calves. A transient increase of specific antibody levels in dairy cows during the last trimester of pregnancy and their persistence to almost 5th month after the birth indicates the activation of the parasite during pregnancy in chronically infected animals. Results confirm the worsening of reproductive parameters compared to the optimum, reflecting the increased incidence of fertility disorders in the herd. Our findings confirmed that *N. caninum* infection in herd is maintained over several bovine generations primarily by vertical transmission.

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SY25.P.24

EPIDEMIOLOGICAL OVERVIEW OF THE CATTLE CRYPTOSPORIDIOSIS IN THE REGIONS OF TIZI OUZOU AND BOUIRA (ALGERIA)

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The aim of our study was to evaluate the prevalence of bovine cryptosporidiosis, predisposing factors its appearance and the means of preventing this infection in areas of Tizi Ouzou and Bouira. To do this we conducted a field survey in the form of questionnaire, in 50 veterinary surgeons installed in the study area. Our results show that 33.33% of parasitic diseases that affect cattle were caused by cryptosporidae. 94% of veterinarians surveyed found that race factors (local or imported) and sex (male or female) do not influence the sensitivity to cryptosporidiosis. We noted that 75% of veterinarians encounter the disease at the age of 1day to one week and 69% between them were found between the ages of one week until weaning. Concerning the season we found that most of the responses were in favour of winter and spring (72% and 54% respectively). The responses obtained for the rate of prevalence of cryptosporidiosis according to the type of exploitation were 100% for traditional farms and 12% for the modern. According to veterinary practitioners, hygiene farm tops the list (with 100% of responses) of the factors predisposing the appearance of the disease followed the type of farming (54%). These results indicate the high economic impact of cryptosporidiosis in cattle from which we must focus on hygiene, the separation of calves and early administration of colostrum in sufficient quantity.
Keywords: epidemiology, cryptosporidiosis, cattle, Algeria.

SY06.P.01

FREQUENCY OF *ENTAMOEBA HISTOLYTICA* AND *ENTAMOEBA DISPAR* PREVALENCE AMONG PATIENTS WITH GASTROINTESTINAL COMPLAINTS IN CHELGERD CITY, SOUTHWEST OF IRAN

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Differentiation between *Entamoeba histolytica* and *Entamoeba dispar* is very important for both clinical therapy and epidemiological studies. Although these two species are morphologically identical, they have differences in genetic, chemical specifications and pathogenicity. This study was carried out to differentiate *E. histolytica* from *E. dispar* and also to find out frequency of the two species.
Fecal samples were collected three times from 655 patients with gastrointestinal complaints (47.3% male and 52.7% female), who were referred to the primary health care centres of Chelgerd, Chaharmahal and Bakhtiary province.
Samples were examined microscopically with direct smear, formalin-ethyl-acetate concentration and trichrom staining methods to distinguish *E. histolytica* from *E. dispar* complex and differentiate them from non-pathogenic intestinal amoeba. Genomic DNA was extracted from microscopy positive isolates and polymerase chain reaction (PCR) was carried out to different the two morphologically identical *Entamoeba* isolates.
Among the 655 recruited patients, eleven subjects with *E. histolytica* / *E. dispar* isolates (1.7%) were identified by microscopy methods. Ten of the positive isolates (90.9%) were identified as *E. histolytica* by PCR and one isolate (9.09 %) was positive for *E*. *dispar*.

This study revealed that *E. histolytica* was more prevalent than *E. dispar* in the studied area. This result was different from the previously reported data in other parts of Iran.
Keywords: Gastrointestinal Complaints, *Entamoeba histolytica*, *Entamoeba dispar*, Polymerase Chain Reaction, Iran.

SY09.P.01
SEROPREVALENCE OF HUMAN CYSTICERCOSIS AMONG BLOOD DONORS IN UAE

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Taenia solium infection in humans via ingestion of eggs can cause cysticercosis and neurcysticercosis. The prevalence of cysticercosis in the United Arab Emirates (UAE) is not known for lack of reliable epidemiological data. The present study was carried out to estimate the prevalence of cysticercosis as measured by serum anti-cysticercal antibodies among healthy blood donors residing in UAE. Screening for cysticercosis was carried out among 1100 blood donors seen at Blood Transfusion and Research Center, Sharjah - UAE. Serum samples were tested for the presence of anti-*Cysticercus* antibodies (AB) using enzyme-linked immunosorbent assay (ELISA) and Western blot analysis as a confirmatory test. Data regarding age, gender, nationality were included. A total of 32 blood donors (2.9%), 11 females (1%) and 21 males (1.9 %) with a mean of age 36.6 ± 7.07 in females and 34.9 ± 7.6 in males were found to be positive by the AB-ELISA test. These included 9 Indians, 3 Pakistani, 2 Egyptians, 3 Syrian, 5 Lebanese, 6 Jordanian, 2 Iranian and 2 Sudanese individuals. Of the 32 positives, 20 (1.8%) were confirmed by western blot. Conclusion: Determination of serum antibody against *Cysticercus* antigen(s) is a useful tool in estimating prevalence of cysticercosis in the community.

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SY12.P.01
DETECTION OF *DIROFILARIA IMMITIS* IN DOGS FROM MENDOZA PROVINCE, ARGENTINA

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Dirofilaria immitis, a mosquito-borne filarial nematode, is the etiologic agent of canine heartworm disease, important from a veterinary point of view and with public health implications due to its zoonotic nature. It is distributed worldwide in tropical, subtropical and temperate regions. It has been described in dogs of temperate and subtropical regions of eastern and northern Argentina and human cases have been reported. *Aedes aegypti* and *Culex pipiens* have been shown to carry larvae of *D. immitis* in urban areas of Buenos Aires. To our knowledge, there have been no reports in the Cuyo provinces of Midwestern Argentina until the year 2008 when the first autochthonous case of canine heartworm was reported and later identified by molecular techniques. Due to the confirmation of the presence of *D. immitis* in the region, our objective was to evaluate its prevalence in the dogs of Mendoza province in western Argentina. Blood samples submitted for hemograms by veterinary practitioners to a private lab were screened for microfilaria by the hematocrit method. Whole blood collected in EDTA was processed the same day, after centrifugation of the microhematocrit tube during 5 min the buffy coat interface was examined for microfilaria. Positive samples were processed by the modified Knott's technique and microfilaria identified according to distinct morphological features (size, shape of anterior and posterior end, presence of cephalic hook) Between September 2011 and April 2012, 850 dog samples from the

province of Mendoza were processed, 3 (0.35%) of them had a microfilaria positive hematocrit test and after concentration by modified Knott's technique, were identified as *D. immitis*. All three dogs were from the province of Mendoza and had not travelled. Previous works determined that the region of Cuyo would be of low risk for transmission of *D. immitis*. Mendoza province, with its mountainous and arid climate, is drier and colder than the endemic regions for Argentina reported up to now. Our findings suggest that there may be microhabitats that sustain the presence of the vector and focal transmission of *D. immitis*. Since there are no previous reports, and a survey done in the year 2001 did not find any positive animals, this disease could be emerging in the region. Further work should be done to assess its epidemiology and monitor its distribution.

SY12.P.02
CANINE FILARIOSES IN CENTRAL POLAND

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In recent years the canine filarioses has became a problem for European veterinarians. In Poland, dogs infected with *Dirofilaria repens* were identified in several locations close to the capital (21°00'E 52°20'N**).** The objective of our research was to estimate the frequency of infection with selected filarial diseases in a population of dogs living in central Poland between 2010 and 2012. Screening of 300 dogs for *D. repens*, *D. immitis*, and *Acanthocheilonema* spp. microfilariae was performed by PCR and Real Time PCR. *D. repens* and *A. reconditum* infections were detected. The frequency of infection with *D. repens* was lower among domestic dogs animals then those from rescue shelters. *D. repens* is a new parasite in central Poland, results from our study showed that cases of canine infection are frequent. To stop further dissemination of the disease preventive measures should be introduced.

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SY12.P.03
HUMAN *DIROFILARIA REPENS* INFECTION IN POLAND

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Dirofilaria repens is a subcutaneous parasite of dogs and other carnivorous animals. Humans may act as incidental hosts. The aim of this work was to analyze the epidemiological situation of human *D. repens* infection in Poland between 2007 and 2012. From the nineteen cases reported, three were confirmed to be autochthonous for the first time in Poland. *D. repens* was found in various parts of the body of the infected people: in the form of subcutaneous nodules containing single nematodes surrounded by granulation tissue. In three patients the worms were localized subconjuctivally. The autochthonous infection cases were identified in the area were canine dirofilariosis was present and infected *Culex* sp. mosquitoes were found. The collected data shows that *D. repens* infection should be considered novel a zoonosis in Poland.

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SY13.P.01

SCREENING FOR IMMUNOSTIMULANT ACTIVITY OF CHITOSAN DURING *TRICHINELLA SPIRALIS* INFECTION IN MICE

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Vaccination is proposed as one of the strategies to control parasitic infections but in case of many diseases it is still an issue. In order to induce stabile and intense protection, new adjuvants are searched for that will overcome parasite evoked immunosuppression and assert immunity. Chitosan, a natural polysaccharide derived from chitin, seems to be a substance with such potency. It has proven ability to enhance antibody responses to vaccine antigens and elicits Th2-type responses that are protective against parasitic infections. Our study attempted to provoke Th2 related immune reaction with chitosan during *Trichinella spiralis* infection and establish whether it affects the level of infection and immunity to the parasite. Male BALB/c mice were given chitosan intraperitoneally, 500μg each second day, 5 days prior to and 5 days after infection with 400 L1 larvae of *T. spiralis*. At 32 dpi, during muscle phase of infection, mice were sacrificed and peritoneal fluid and sera were obtained. In peritoneal fluid eosinophils were identified and macrophages were isolated. In sera, level of specific IgG1 was measured, while IgA was detected in peritoneal fluid. Isolated macrophages were cultured for 48h in presence of LPS (2μg/ml), L1 somatic extract (10μg/ml) or chitosan (10μg/ml) and then assayed for nitric oxide (NO) production and arginase activity. Level of infection was measured as number of larvae isolated through artificial digestion of skeletal muscles. We observed that chitosan administration during enteral phase of infection reduces the number of cells in peritoneal fluid and abolishes local eosinophilia. But at the same time, isolated macrophages produced more NO in culture with simultaneous diminished arginase activity. Mean levels of specific antibodies were not changed but chitosan altered pattern of their distribution. During control infection, IgG1 production negatively correlated with the number of larvae, while chitosan treatment abolished this dependence and slightly switched it to negative IgA/larvae correlation. Despite these changes in immune response the level of infection was not altered, mean numbers of larvae recovered from muscles were comparable in all experimental groups. We showed that chitosan administration alters host immunity to *T. spiralis* but it does not affect level of infection. Neither enhancement of macrophage response in the enteral phase of infection nor reduction of IgG1 level did not affect the number of muscle larvae. Further studies, aiming at recognition of chitosan immunomodulatory action during parasitic infections, are in progress.

SY13.P.02

A NEW DRIVER - DEVELOPMENT A SPECIFIC ALGORITHM OF SEVERITY WITH CLINICAL AND BIOLOGICAL CHARACTER IN TRICHINOSIS

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Parasitic infections are still a constant threat to the health of more than 80% of the world's population. Although trichinosis is investigated by many specialists both in the country and abroad, progress in terms knowledge from etiological point agent, diagnosis and treatment of this disease it is still very slow. The solution in to establish a correct diagnosis is a complex diagnosis.
To achieve this aim ,which is justify in all aspects, often it be up technical difficulties, for risk management of parasite is still unsolved in our country, but no internationally, we develop a specific diagnostic algorithm of severity with clinical and biological character in trichinellosis. Were monitored and analyzed on devices and systems, impairment of human trichinellosis in both clinical, biological and immunological, neuro-psychiatric, renal, respiratory, liver, heart and immunological, allergic and mucous cutaneo manifestations; with the establishment of statistical

bases, well-documented scientific criteria for severity. Analysis of the clinical picture of symptoms and constant growth in laboratory and in relation to the progressive clinical forms of trichinosis, ensure scientific validity some useful conclusions and the effect expected will be the formulation of a specific diagnostic algorithm clinico-biological diagnosis of severity to human trichinosis.

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SY13.P.03

COMPARATIVE ULTRASTRUCTURAL STUDIES OF THE ALTERATIONS TO MOUSE LUNG PARENCHYMA DURING *TRICHINELLA SPIRALIS* OR *TOXOCARA CANIS* INFECTION

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Trichinella spiralis and *Toxocara canis* have tissue dwelling larvae stage and are important causes of disease. Larvae have developed complex and unique strategies to escape from immune reactions and they are able to persist in the host tissue for many years. Both *T. spiralis* and *T. canis* larvae migrated through the lung and induced many alterations in the lung parenchyma. These changes resulted from mechanical damage or from local inflammatory reactions provoked by these larvae.
The purpose of this report was to describe the pattern of changes in the lung parenchyma at the electron microscopic level of mice experimentally infected with *T. spiralis* or *T. canis*. These changes was studied for *T. spiralis* between 6 - 12 days post infection (DPI) of mice, which were orally infected with 400 or 800 *T. spiralis* larvae and for *T. canis* between 21-28 DPI – infected with 1000 *T. canis* eggs. These times were chosen because the morphological changes in the lung parenchyma are well-developed and the most specific for *T. spiralis* or *T. canis* infection.
Mice were killed under anesthesia and the fragments about 2 x 2 mm of the lung parenchyma were isolated and processed for electron microscopy studies. The tissue samples were fixed with a mixture of 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) for 20 hrs and postfixed in 1% OsO_4 and 0.8% K_4FeCN_6. After dehydration in ethanol and infiltration with propylene oxide, the lung parenchyma samples were embedded in Spurr resin. Ultrathin sections (~ 50 nm) were examined using a JEM 1200 EX transmission electron microscope (TEM).
The ultrastructural studies demonstrated that the tissue-migratory *T. spiralis* larval stage evoked mainly destruction of type I epithelial cells, destruction of lamellar bodies of epithelial cells or extracellular alveolar lining layer. The severity of these changes was dependent on the number of infective *T. spiralis* larvae) and possibly the result of mechanical damage in the lung parenchyma. They could be dangerous for the hosts evoking periorbital oedema, especially in mice infected with a higher dose e.g. 800 of *T. spiralis* infective larvae. In contrast, infection with *T. canis* larvae initiated mainly eosinophilic pervasculitis and vasculitis as well as macrophage accumulation in the lung, which were additionally impacted by numerous crystalloid inclusions in macrophages.
T spiralis larvae and *T. canis* larvae induced different pathological changes in the lungs of infected mice.

SY13.P.04

EFFECT OF *TRICHINELLA SPIRALIS* ANTIGENS ON ITS WORM STAGES AND INFECTION PHASES

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T. spiralis presents the host with a complex antigenic stimulus, evoking a powerful immune response in the host. *T. spiralis* mouse models were used to investigate the effect of immunisation with *T. spiralis* Adult Antigen (TAA) and Larval Antigen (TLA) on the adult and larval stages in both phases of infection at the microscopic and ultra structural level. Three groups of laboratory mice were immunised using (TAA) and (TLA), Group 1 (G_1): *T. spiralis* infected mice and none immunized, Group 2(G_2): *T. spiralis* infected mice and immunized with (TLA) and Group 3(G_3): *T. spiralis* infected mice and immunized with (TAA). Adult worms and larvae recovered from the three groups were counted and examined by both light and scanning electron microscopy (SEM). In the immunized groups, Adult *Trichinella* worms were early expulsed from the intestine, the recovered number was significantly reduced, correspondingly isolated muscles larvae were significantly reduced too, compared to G1 .Light microscopic examination of adult and larva revealed no obvious differences between the 3 studied groups in contrast to SEM changes seen in the immunized groups. SEM examination of G2 isolated larvae show the loss of the normal curvature of the body (lacy appearance) and smoothening of the anterior end with loss of demarcation between the cephalic dome and the rest of the body, along with loss of cuticle integrity which appeared as mottling of electron dense deposits on the larval surface, which may represent an antigen-antibody complex. Moreover, the lateral surface shows flattening of the annular folds corrugations and cuticular ridges. While larvae recovered from G3 show slight distortion in the normal curvature of the body, the transverse folds are slightly flattened with attenuated demarcation between the cephalic dome and the rest of the body. The lateral surface appeared flattened with poor cuticular ridges and shallow grooves. The study reaches that TLA and TAA antigens had affected both phase of *Trichinella* infection. They operate directly on the intestinal mucosa through triggering the intestine to early expulse the adult worms, without affecting their structural integrity as observed by SEM examination. While in the muscle phase the TLA had a more prominent accomplishment as it decreased the magnitude of newly born larvae invasion to the host muscles, besides targeting the larval structure which reflect an immunological reaction. TLA bring into being a future promising tool in the *Trichinella spiralis* prevention and treatment by targeting its worm stages in both phases.

SY13.P.05

A NOVEL MICROSATELLITE WITHIN LSRDNA EXPANSION SEGMENT V OF *TRICHINELLA BRITOVI* AND *TRICHINELLA NATIVE*

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Expansion segment V '127 bp fragment' of *Trichinella* taxa, has been investigated for the last 20 years using electrophoresis and direct Sanger sequencing. Ribo HRM, a single-tube PCR and high resolution melting (HRM) assay was developed for differentiation of the expansion segment V of *Trichinella* spp. Presence of polymorphisms at the level of distinct *T. britovi* and *T. nativa* isolates was revealed by comparison of the HRM curves of PCR amplification products. Cloning and sequencing of the PCR products showed that several variants of the amplified genomic fragments, differing in either length or nucleotide sequence or both, were present in single *T. britovi* or *T. nativa* isolates. A novel microsatellite region was identified and the first *T. britovi* and *T. nativa* '127 bp fragments' sequences were deposited in GenBank. The results showed that the diversity level of large subunit ribosomal DNA sequences in *T. britovi* and *T. nativa* is higher than expected.

SY13.P.06

REGULATION OF THE INTESTINAL IMMUNE RESPONSE BY THE HYPHOPHYSIS DURING *TRICHINELLA SPIRALIS* INFECTION IN THE GOLDEN HAMSTER

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The influence of anterior pituitary hormones on the gastrointestinal tract of humans and animals has been described. Hypophysectomy (HYPOX) in the rat causes atrophy of the intestinal mucosa, reduction of gastric secretion and intestinal absorption, as well as increased susceptibility to viral and bacterial infections. However, to our knowledge, no studies exist concerning the immune response following HYPOX during worm infection, particularly referring to that caused by the nematode *Trichinella spiralis*. Thus, the aim of this work was to analyze the effects of complete or partial HYPOX on the establishment of *Trichinella spiralis* in the intestinal lumen, together with duodenal and spleen cytokine expression. Our results indicate that 5 days post infection, only neurointermediate pituitary lobectomy (NIL) surgery reduces the number of intestinally recovered *T. spiralis* larvae. Using semiquantitative inmunofluorescent laser confocal microscopy, we observed that the mean intensity of all tested Th1 cytokines was dramatically diminished, even in the case of duodenum sections from infected controls, in contrast to a high level of expression of these cytokines in the NIL infected hamsters. Likewise, a significant decrease in the fluorescence intensity of Th2 cytokines (with the exception of IL-4) was observed in the duodenum of control and sham infected hamsters, compared to animals with NIL surgeries, which manifested an increase in the expression of IL-5 and IL-13. Histological condition of duodenal mucosa from NIL hamsters presented an exacerbated inflammatory infiltrate located along the lamina propria, which evidently related to the presence of the parasite. We conclude that hormones from each pituitary lobe affect the gastrointestinal immune responses to *T. spiralis* by a variety of mechanisms.

SY13.P.07

PRELIMINARY STUDIES ON INTRA-SPECIFIC VARIABILITY OF POLISH, SLOVAK AND CZECH *TRICHINELLA* ISOLATES BY ISSR-PCR

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Since Owen first described *Trichinella* as a human pathogen in 1835, the number of organisms comprising this genus has grown dramatically. In three neighbouring countries: Poland, Slovakia and Czech Republic two *Trichinella* species: *T. spiralis and T. britovi* have been described as the main etiological agent in domestic and/or sylvatic animals.
Because both of nematode species may occur in various definitive hosts, the question exists if any intraspecific variation in nematode genotype may affect preference in the choice of the host species. In total, 30 isolates were studied: 26 of *T. britovi* (14 from Poland, 11 from Slovakia, 1 from Czech Republic) and 4 isolates of *T. spiralis* from Poland. *Trichinella* larvae were identified at species level by multiplex polymerase chain reaction (multiplex PCR) according to Zarlenga et al. (1999). Inter-simple sequence repeat polymerase chain reaction (ISSR-PCR), was performed as described by Perteguer et al. (2009). The molecular weight of separate bands was calculated using KODAK 1D™ System. Jaccard's similarity index (*J*) was used to consider the similarity between isolates (Real, 1999).
Trichinella britovi isolates analyzes by ISSR-PCR, revealed that all samples presented very similar pattern. However some additional bands were observed. *J* values calculated for Slovak and Czech isolates ranged from 0.05 to 0.85 and revealed significant similarities for the majority of Slovak *T.*

britovi isolates obtained from wild boars and red foxes ($p \leq 0.01$). The lack of relationship was found between the Slovak *T. britovi* isolated from marten (High Tatras Mountains) and the other isolates. Additionally, there were no similarities between Slovak isolate from wild boar and all other isolates and *J* values for these isolates ranged from 0.05 to 0.26. Polish *T. spiralis* isolates collected from 1 domestic pig and 3 wild boars revealed very similar pattern visible in ISSR-PCR analysis. Calculated *J* values ranged from 0.53 to 0.8 ($p \leq 0.01$). The statistical analyses of results obtained from other Polish isolates are in progress. However, ISSR-PCR analysis showed high similarity between isolates, the molecular weights of separate bands pointed out some intraspecific varabilities in both species.
These preliminary results revealed the polymorphism within *T. britovi* isolates and may suggest the differentiation of isolates in relation to the locality and the host origin.

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SY13.P.08

CLINICAL FEATURES OF HUMAN TRICHINELLOSIS AND BIOLOGICAL CHARACTERISTICS OF *TRICHINELLA* ISOLATES

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Trichinellosis is a disease in which the differential diagnosis process often is long, due to the diversity of the clinical data. The lack of etiological treatment leads to severe course of disease and complications. The identification of the *Trichinella* species isolates is of importance to the medical and veterinary experts with regard to the implementation of efficient measures in the prevention and therapy of trichinellosis. The species identification of three *Trichinella* isolates from the region of Sofia- district following epidemic outbreaks has been determined. Diagnosed and treated are 71 patients from tree regions in and near the capital town Sofia. The beginning of the disease is acute with a picture of grippe, with high fever to 40°C, face oedema, myalgia and skin rash more than a month of the initial symptoms. Patients have been treated with antibiotics without effect. The diagnosis was made based on clinical symptoms, haematologycal (eosinophilia, leucocytosis, high level of creatine phosphokinase), serologycal (ELISA and haemagglutination test) and epidemiological data. After repetition of differential counting with a microscope, it was found in all patients hipereosinophilia. The treatment was made with Albendazole (Zentel) 10 mg/kg for 7 to 10 days and there was marked clinical improvement and a trend towards normalization of laboratory data. The results obtained from the cross – breeding and PSR multiplex performed with the three isolates from two domestic pigs and from rat, show that they belong to the *T. spiralis* and *T. britovi* species.
Keywords: Trichinellosis, diagnosis, eosinophilia, Albendazole, domestic pigs, rat, *T. spiralis*, *T. britovi*, PCR, cross-breeding.

SY13.P.09

TS CARD PORK - USEFUL BUT NEGLECTED TEST FOR THE DETECTION OF *TRICHINELLA* ANTIBODIES IN SWINE

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Developed for fast detection of *Trichinella* antibodies in swine more than one decade ago, TS Card Pork lateral flow test (IVD, USA and ARTE. SRL. Romania) gave us hope in its wide and easy application for multipurpose use. As a screening test, it could be a foundation of on-farm or field based inspection system to significantly improve food safety in countries with a high prevalence of *Trichinella* in pigs, such as Serbia. Based on the results from our very extensive study (on the whole blood and sera samples) done at that time in Romania, this test proved to be almost as specific and sensitive as ELISA, but easier to use. Since the test was designed for the whole or dried blood, serum, or tissue fluid samples, here we are reporting comparative analyses on the results obtained by application of this test and ELISA for *Trichinella* antibody detection in meat juice from pig carcasses in Serbia. For final judgment on test results parasitological findings were used (trichinelloscopy and artificial digestion). In this study it was established that very low level infections with *Trichinella* (0.12 LPG of muscle) can be detected using TS Card Pork test. Infection of 0.04 LPG was below the sensitivity of either this test or self developed ELISA test. The presented results are promising for application of the test in an on-line laboratory based inspection system. Confidential level of the TS Card Pork test and pooled digestion method of 1 g meat sample is 100%. Therefore this test could be useful not only for on-farm or field based inspection but also in case that animals died or were slaughtered and serum samples could not be collected. Muscle juice can be collected from fresh, cool and frozen meat and used as a substitute sample for detecting anti-*Trichinella* antibodies. Its application in epizootiology of *Trichinella* infection especially in wildlife may also have a value. The main reason that the test is unfairly overlooked is economic. In Serbia, that has a problem with *Trichinella* and trichinellosis, legislation which request serological testing of pigs exists, but the lack of interest for test implementation is due to the shortage of financial resources in the country and at the individual level.

SY15.P.01

THE EFFECT OF *ANISAKIS SIMPLEX* LARVAL PRODUCTS ON MURINE DENDRITIC CELLS

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Chronic parasite infections are associated with a regulatory network protecting of the highly expressed Th2 phenotype. In *Anisakis* patients a broad immunologic stimulation involving Th2 and Th1 lymphocyte responses is produced, as has also been demonstrated in experimental *Anisakis* simplex infections. A. simplex is the first parasite known to be associated with a high prevalence of acute allergic disorders. This has been postulated to be due to humans not being a natural host for this parasite, where parasitism is only acute and therefore lacking immuno-regulatory features typical of chronic helminthosis. In this work, the immunological effects of larval crude extract (CE) and excretory-secretory (ES) products were studied on antigen presenting cells. Bone marrow derived dendritic cells (BMDDCs) were obtained from C57BL/6 mice. Cell activation was determined by evaluating membrane marker (MHC-I/MHC-II) and co-stimulatory molecule (CD80/CD86) expression. BMDDCs were cultured in the presence of GM-CSF and collected six-nine days after culture, then plated, and CE or ES antigens added into wells. The toll-like receptor

(TLR)-4 agonist LPS, or the TLR-9 agonist CpGDNA were also added to the wells in the presence of A. simplex CE or ES. The expression of activation markers was increased in CD11c+ BMDDCs in the presence of A. simplex CE and ES products; co-stimulatory molecules CD80 and CD86 showed the greatest increase in expression. The inflammatory stimuli caused by the A. simplex CE and ES antigens were increased by the administration of the TLR agonists LPS and CpGDNA. Intracellular staining for the cytokines IL-12 and IL-10 was also determined in BMDDCs stimulated with A. simplex CE or ES larval products for 16 h. IL-12 expression was slightly increased following antigenic stimulation, although the higher expression was seen after the co-stimulation with the antigens and both TLR-agonists with higher values than the observed using LPS and CpGDNA alone. In contrast, IL-10 expression was increased by priming with both antigens when compared to the RPMI-treated control. These results indicate that in vitro treatment of bone marrow DCs with A. simplex larval CE and ES products increased their ability to produce anti-inflammatory cytokines such as IL-10.

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SY15.P.02

CYTOKINE RESPONSE OF MICE TO HEAVY METAL INTOXICATION AND *ASCARIS SUUM* INFECTION

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T-helper cytokine polarization is determining in the immune response to parasites. Th1 cells play a key role in cell-mediated reactions and Th2 cells are involved in humoral response of the host against a parasite. Heavy metals affect the Th1/Th2 polarization. Host cytokine response after heavy metal intoxication and subsequent *Ascaris suum* infection was studied. The Th2 cytokine production (IL-5, IL-10) after heavy metal intoxication (lead-Pb, cadmium-Cd, or mercury-Hg) was nonsignificantly increased from week 3 or 2, whereby Hg intoxication significantly (p<0.01) increased the IL-10 production. A significant (p<0.01) stimulation of the IL-5 synthesis induced by *A.suum* infection in week 2 post infection (p.i.) was not affected with metal intoxication. *A.suum* infection increased the IL-10 production with a progressive rise till week 2 p.i. (p<0.01). The increased IL-10 synthesis was found in mice intoxicated with Pb or Cd and subsequently *A.suum* infected, but it did not achieve the concentrations in mice only infected (without intoxication). The significant (p<0.01) high IL-10 values in mice intoxicated with Hg were not influenced with the parasite infection. TNF-α; response (Th1 type) was suppressed from week 2 of Pb intoxication. Cd stimulated the TNF-α; production, Hg inhibited this cytokine for the first 2 weeks, then it significantly (p<0.05) increased the TNF-α; generation till the end of the experiment. *A.suum* infection of intoxicated mice reduced TNF-α; under the control. Pro-inflammatory IFN-γ; was suppressed by Pb intoxication, Cd significantly (p<0.05) increased and Hg slightly increased the cytokine production after 3 week of Hg intoxication. *A.suum* infection increased the IFNγ; production in mice without intoxication at the first week p.i. Low IFNγ; concentrations were found in mice intoxicated with Pb or Hg and subsequently infected, only Cd intoxicated mice stimulated the IFN-γ; production also after the infection (p<0.05). Heavy metals Pb and Hg modulated the immune response into the Th2 type, that was dominant also after *A.suum* infection and allow the more intensive development of the infection in mice intoxicated with Pb. The numbers of larvae were low in mice intoxicated with Hg, what probably does not relate to the immune response but to the bad host condition caused by a high level of pro-cachectic cytokine TNF-α;. Cd intoxication stimulated the IFN-γ; production, that conduced to the larval destruction in the liver and their reduced numbers.

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SY15.P.03

EFFECT OF HEAVY METALS ON *ASCARIS SUUM* INFECTION AND MACROPHAGE ACTIVITY IN MICE

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Heavy metals can induce important changes in cell physiology and are able to modulate functions of immune system. Exposure of organisms to heavy metals can impair their immunocompetence and increase the susceptibility to parasite infections. Mice intoxicated with lead (Pb), cadmium (Cd), or mercury (Hg) were infected with *Ascaris suum* eggs. In comparison with infected mice without intoxication, the parasite burden was increased in mice intoxicated with Pb, almost 2-fold reduced number of *A. suum* larvae in the liver of mice intoxicated with Cd was observed, and the lowest number of larvae were found in the liver and the lungs of mice intoxicated with Hg. Mice intoxicated with Hg were extremely cachectic, mice intoxicated with Pb showed slight weight loss, and mice intoxicated with Cd were in good condition and and increased in body weight in comparison to control. Metabolic activity of peritoneal macrophages was evaluated by in vitro production of superoxide anion (O2-). Pb intoxication induced a suppression of the free oxygen radical and the subsequent *A. suum* infection significantly inhibited its production. The generation of O2- was stimulated throughout Cd intoxication and the subsequent *A. suum* infection did not change macrophages's metabolic activity. Intoxication with Hg had a dichotomic effect on the O2- production. A reductive effect of Hg lasted for 2 weeks of intoxication and then it was changed by a stimulation of macrophages. High metabolic activity of macrophages of mice intoxicated with Hg for a long time probably balanced a decreased number of these cells. However, the subsequent *A. suum* infection of mice intoxicated with Hg reduced the O2- production significantly lower as control values for the first 7 days after the infection. In mice intoxicated with heavy metals and subsequently infected with *A. suum* the differences in intensity of the parasite infection were found in dependence on a type of heavy metal. Found differences in parasite numbers might be explained by a different immunotoxic effect of heavy metals on effector cells of the immune response – macrophages.

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SY15.P.04

GLUCAN IMMUNOMODULATOR CAN PROTECT THE HOST TO MIGRATION OF *ASCARIS SUUM* LARVAE

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The *Ascaris suum* infection induces elevated immunoglobulins IgE, eosinophilia and mastocytosis in the host due to the development of Th2 immune responses, particularly in the intestinal phase. The Th1 response present during the larval development of the parasite is inhibited by products of the Th2 cells. The study examined the effect of glucan immunomodulator in mice with experimental ascariasis. Glucans are b-(1,3)-D polymers of glucose present as a basic component of cellular wall of bacteria, fungi or yeasts. In comparison with infected mice without glucan treatment, the parasite burden was reduced in mice with glucan. The numbers of *A. suum* larvae in the liver were significantly decreased (about 36%) on day 4 post infection (p.i.) and also pulmonary larval migration was reduced on days 7 and 14 p.i. (about 38% and 17.6%, respectively). Glucan immunomodulator increased the proliferative activity of splenic T lymphocytes within 2 weeks of the treatment and subsequent *A. suum* infection stimulated the proliferative activity even more for next 2 weeks. The similar effect of glucan treatment was found in the numbers of CD4+ T helper cells, with the maximum on week 3 of the immunomodulation. *A. suum* infection prolonged this stimulation of CD4+ T cells for next week, i.e., week 2 p.i. Cytokine response of mice with glucan

treatment was directed into Th1 type, production of IFN-γ; was increased and subsequent *A. suum* infection did not reduced the cytokine synthesis. Production of IL-5 (Th2 cytokine) was increasing after *A. suum* infection but in mice treated with glucan the IL-5 concentration was lower. Also generation of IL-10 and IL-4 cytokines was significantly decreased in mice immunomodulated with glucan. Th1 cytokine response activated macrophage's metabolism. Production of superoxide anion (O2-) in peritoneal macrophages was stimulated on week 2 after the glucan treatment and the peak of the metabolic activity was recorded on week 1 after *A. suum* infection. Production of O2- in pulmonary macrophages was stimulated with maximum in glucan immunomodulated mice after *A. suum* infection on weeks 1 and 2 p.i. The results suggest the glucan immunomodulation positively influenced the prime effectors of cell-mediated immunity; it stimulated synthesis of IFN-γ; and metabolic activity of macrophages that could contribute to a significant reduction of the parasitic infection in host.

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SY15.P.05

SEROTONIN AND NEUROPEPTIDE IMMUNOREACTIVITIES IN METACERCARIAE OF SOME TREMATODES

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Trematodes have a complex life cycle with change of generations and hosts and include the parasitic and free-living stages. Despite of the fact that the neuromuscular system of parasitic flatworms was studied intensively in the recent years, appropriate data concerned to the larval stages of life cycle is still scarce. There are a limited number of papers which shows the presence of catecholaminergic, serotoninergic and peptidergic components in the nervous system of metacercariae of several species. The aim of present study was to investigate the serotoninergic (5-HT) and neuropeptidergic (FMRFamidergic) components in the nervous system of metacercariae from different families of trematodes - Opecoelidae (*Helicometra fasciata* Rudolphi, 1819 from *Palaemon elegans*), Microphallidae (*Microphallus piriformis* Odhner, 1905 from snails *Littorina saxatilis*), Strigeidae (*Cotylurus* sp. from snails *Radix ampla*), Leucochloridiomorphidae (*Leucochloridiomorpha lutea* von Baer, 1826 from snails *Viviparus contectus*) using immunocytochemical methods and confocal scanning laser microscopy. The presence of serotonin and neuropeptide (FMRFamide) was revealed in the central and peripheral nervous systems of metacercariae. The FMRFamidergic nerve structures were more extended than serotoninergic elements. The general plan of nerve system in investigated metacercariae is the same, but the number and size of serotoninergic nerve cells vary in metacercartiae of different families. The received results come to agreement with literature data about localization of investigated neurotransmitters in metacercariae from families - Bucephalidae (Steward et al., 2003), Echinostomatidae (Šebelová et al., 2004), Opisthorchidae (Terenina et al., 2008), Diplostomidae (Barton et al., 1993), as well as with the biochemical data about presence of serotonin in tissue homogenates of metacercariae of *Codonocephalus urnigerus* Rudolphi, 1819 (Diplostomidae) from body cavity of frog *Rana ridibunda* (Terenina, Gustafsson, 2003).

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SY15.P.06

IN VITRO CYTOKINE RESPONSE OF THP-1 CELL LINE TREATED WITH SELECTED *ANCYLOSTOMA CEYLANICUM* SECRETED PROTEINS (ASPS)

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Ancylostoma ceylanicum is a member of parasite nematodes which inhabits hosts small intestines and feeds on blood. As many as 740 million people worldwide suffer from hookworm infections. Increasing drug resistance and often re-infections are the reason for an extensive search of an effective antigen for vaccine construction.
One of the groups of antigens currently studied are *Ancylosotoma* secreted proteins (ASP) also known as Venom allergen/ASP-like proteins (VAL). Despite 16 years of studies on ASPs and more than 17 members of this group already described among *Ancylostomatidae* family their role is still unknown. Studies conducted on the structure of ASPs and vaccine trails showing a reduction of the intensity of hookworm infection suggest their immunological activity and in particular an important role in immunomodulation.
In this study human monocyte cell line THP-1 was used to define *in vitro* cytokine response to selected ASPs stimulation. The experiment consists of two groups: cells stimulated and non-stimulated with lipopoysaccharide from *E. coli*. THP-1 were differentiated into macrophages with phorbol 12-myristate 13-acetate (PMA) and the recombinant proteins were produced in prokaryotic pET System. Both groups were treated with various concentrations of selected recombinant ASPs. The levels of IL-1β, IL-6, IL-10, IL-12 p40, IFNγ, TNFα secreted by treated cells were determined in culture medium using ELISA tests. Changes in cytokine levels have been found which suggests that ASPs can influence the macrophage response.

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SY15.P.07

CELL AND MOLECULAR ASPECTS OF IMMUNE RESPONSE IN PERITONEAL CAVITY OF MICE WITH *MESOCESTOIDES VOGAE* INFECTION

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Modulation and suppression of immune response is one of the main phenomenons during infection with larval stage of *Mesocestoides vogae* (Cestoda). Thus, the larval long-term survival and their asexual reproduction are present in the peritoneal cavity and in the liver of intermediate hosts while causing a serious damage. In the present *in vivo* study, regulation of immune responses in the peritoneal cavity of mice infected with larvae (tetrathyridium) of *M. vogae* was examined by means of evaluation of the changes in the dynamics of inflammatory cells number, changes in macrophage and monocyte phenotypes, regulation of their efector functions and alternation of gene expression of selected Th1 and Th2 cytokines, which are directly involved in the immunomodulation. In addition, the effects of excretory-secretory antigens of *M. vogae* on the selected parameters of cells were examined *in vitro.*
Mice were orally infected with 60 larvae and peritoneal exudate cells (PEC) were obtained from peritoneal cavities within two months post infection. Infection triggered massive accumulation of inflammatory cells, of which macrophages and eosinophils were dominant. Macrophages comprised different phenotypes, i.e classical mature macrophages, intermediate types (alternative) and large multinucleated giant cells. Induction of alternative macrophage activation correlated with the increase of expression of IL-4, IL-5 and IL-13 cytokines of Th2 immune response, and decrease of INF-γ and TNF-α expression, what is typical for helminth infections. With progressing infection the nonspecific efector functions of macrophages (respiratory burst, phagocytosis,

adherence) were suppressed and no cytotoxic effect and larval reduction was recorded. As these functions are characteristic for mature macrophages, the shift from Th1 to Th2 type of immune response correlated with the reduced numbers of classically activated mature forms of macrophages in a favor of alternatively activated macrophages and giant cells. In vitro experiments indicated the main role of selected larval excretory/secretory antigens in suppression of efector functions and antigen presentation by macrophages for T cells.
Our data indicate that down-regulation of macrophage functions and dominance of alternatively activated forms and giant cells likely play a role in prevention of massive inflammation and induction of host-parasite tolerance.

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SY15.P.08

LOCALISATION OF *TOXOCARA CANIS* LARVAE AND EXCRETORY/SECRETORY ANTIGENS AND APOPTOTIC CELLS IN HOSTS TISSUES DURING THE ACUTE AND CHRONIC PHASE OF EXPERIMENTAL LARVAL TOXOCARIASIS

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Human toxocariasis is a zoonotic disease caused by the infective larvae of nematode *Toxocara canis* or *Toxocara cati*, which are released from ingested parasitic eggs in the small intestine. Clinical signs include cough, fever, wheezing, headache, rash and abdominal pains and these are attributed to the migration of larvae and pathological reactions to larval excretory and secretory antigens (TES) or their remnants in the tissues.
In the experiments inbred male mice of $C_{57}BL_6$ strain were inoculated orally with a single dose of 500 embryonated eggs *T. canis*. Collection of the liver, skeletal muscles, brain and lungs from infected mice was performed on days 1, 2, 5, 14, 28, 56 and 72 post infection (p.i.) for larval isolation. TES antigens were prepared after *in vitro* cultivation of larvae and anti-TES polyclonal sera were raised in rabbits. Cells undergoing apoptosis were identified on the sections from the liver, lungs and brain tissues using modified TUNEL technique and antigen expression was evaluated by western blot analysis.
The first larvae appeared in the livers on day 1 p.i. and on day 3 p.i. in the lungs. Larvae accumulated in the brains and carcas with the maximum recorded after 3 months p.i. In the liver and lungs migrating *T. canis* larvae caused pathological response accompanied with the small vascular leakage and the foci of inflammatory cells co-localized with TES antigens. In the brains inflammatory lesions were not seen except of a few eosinophils and TES were localized mainly in the white matter. Apoptotic cells were predominantly immune cells and no apoptotic cells were detected in the brains. TES shed from the surface of larvae persisted within 3 months p.i. and stimulated strong humoral immune response represented mainly by IgG antibodies to TES antigens of molecular weight 32 kDa, 55kDa, 70kDa, 120 kDa, 132 kDa and 400 kDa.
Present study demonstrated accumulation of *T. canis* larvae in the brains and muscles of mice and correlation between TES expression, tissue localisation and induction of apoptosis in immune cells indicating the role of individual TES antigens in programmed immunosuppression.

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SY15.P.09

MOLECULAR CLONING AND IMMUNOMODULATORY EFFECT OF SERINE PROTEASES FROM *HYPODERMA DIANA*

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Hypodermosis is a common parasitic disease affecting livestock and wild ruminants. The disease causes huge economic losses in animal production due to its effect on meat, milk, and the leather industry. It can also inflict general health dysfunctions, mostly affecting the immune system of infected animals.
The first stage larvae (L1) of *H. diana* penetrate the skin and migrate within the host body through the conjunctive tissue, and later instars form nodules with a breathing hole under the skin of the animal's back. After leaving the host larvae pupate on the ground. The adult stage – a fly, lasts only up to five days.
Attempts to identify vaccine antigens have led to a group of serine proteases (hypodermins) which play a key role in parasite feeding, migration through host tissues, and immune evasion.
The cDNAs of the two serine proteases from *H. diana* were cloned using the RACE-PCR method. Computational analysis showed that the cDNAs of serine proteases 2 and 3 encode 257 and 264 amino acid proteins with a molecular mass of 27 and 29 kDa, that show high homology to serine proteases from related species. The recombinant proteins were expressed in *E. coli* followed by purification and mouse immunisation. Using raised anti-r*Hd*-HYP-2 and anti-r*Hd*-HYP-3 (anti-recombinant-*Hypoderma diana*-hypodermin 2 and 3) serum the presence of both hypodermins in the larval stage of *H. diana* was confirmed.
In order to study the immunomodulatory effect of the parasite on the host, the mononuclear cells isolated from peripheral blood of an adult calf (BOMA cells) were treated with purified recombinant hypodermins 2 and 3 and the homogenised larvae of *H. diana*. The level of TNF-α secreted by bovine macrophages was measured. It has been demonstrated that there is a decrease of TNF- α production in the cells stimulated by lipopolysaccharide. It can prove the suppression of an inflammation during the parasitic infection which can contribute to the survival of the parasite during the migration through the host tissue.

This study was supported by the Polish Ministry of Science and Higher Education (grant no. N N308 573640).

SY15.P.10

LYMPHOPROLIFERATIVE RESPONSE, PRE AND POST-INFECTION, IN BALB/C MICE IMMUNIZED WITH THE RECOMBINANT CHIMERIC PROTEIN L25A-HSP70M1 OF *LEISHMANIA BRAZILIENSIS*

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The aim of this study was the analysis of the lymphoproliferative response of BALB/c mice immunized with the recombinant chimeric protein, consisting of the N-terminus of the protein L25 (L25a, 1-68aa) and amino domain fragment of HSP70 of *L. braziliensis* (HSP70M1, 109-245aa), named L25a-HSP70M1 and cloned into the expression vector pQE30 (Qiagen). The protein expressions were performed in *E. coli* Topp3 and purified in native conditions. Three groups of 10 mice/group were immunized 3 times: a group with 5 μg/dose, another with 20 μg/dose and a control group with PBS, subcutaneously without adjuvants. We performed the study of the lymphoproliferative response at different times of the assay against each protein (L25a, HSP70M1

and L25a-HSP70M1) through stimulation of splenocytes from BALB/c mice immunized, before and after infection with 10^3 metacyclic promastigotes of *L. amazonensis*. The results showed that the lymphoproliferative response before the infection induced a high stimulation index (SI) against proteins, where HSP70M1 showed the major SI. At 4 and 6 months post-infection, the SI of infected mice were similar to the uninfected group, but remained slightly higher.

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SY16.P.01

A BAYESIAN KRIGING MODEL WITH COVARIATES TO ESTIMATE THE PROBABILITY OF PARASITIC INFECTION

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The aim of this work is to develop a Bayesian Kriging model with covariates to obtain prediction of infection probabilities for each farm of the Campania Region.
We take advantage of a cross-sectional study carried out in the years 2004 - 2005. All the sheep farms of the region were geo-referenced. A grid of 10 x 10 km was overlaid on the region for a total of 135 equal cells. For each cell the farm closest to the centroid was selected. Only pastured farms with more than 50 animals were included in the final sample. Out of the total 135 quadrants, 121 were investigated. Faecal samples were collected and the FLOTAC technique was employed for coprological examinations of 23 different helminths. A GIS was constructed using environmental data layer. Data on each of these layers were then extracted for pasturing areas of the 121 farms, previously digitalized on aerial images. The choice of the covariate to include in the prediction process is not an easy task, in particular for these parasites for which the life cycle is not very clear. Thus, a first step Bayesian variable selection has been performed in order to select the relevant covariates for each one of the parasite.
A Bernoulli Likelihood on the presence/absence of infection in the 121 investigated farms was assumed. A Bayesian Gaussian spatial exponential model was specified on random terms in the linear predictor of a probit function of the probability of infection. The hyper-parameters of the correlation matrix have been chosen in such a way that the correlation between points be 0.97 at minimum distance (1,42 Km) and 0.01 at maximum distance (202,23 Km). A Bayesian Kriging was performed to predict the probability of infection in 1500 unknown points which represent the centroid of the cells of a regular grid of 3×3 Km on the region. GIS covariates at pasture farms were used to characterize the spatial trend of parasitic infection. Within the model, the regression coefficients were then used to make predictions at each grid point.
The 23 different responses were very different in term of geographical pattern and prevalence of infection. We show posterior probability maps for two selected parasites (*Fasciola hepatica, Dicrocoelium dendriticum*).
Probabilities of infections can be easily understood by non expert, differently from the results of a test statistics. Posterior probabilities (and related uncertainty) of infection can be used to tailor future sampling strategy for parasitological surveillance.

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SY18.P.01

LIGHT AND ELECTRON MICROSCOPY OBSERVATIONS OF EMBRYOGENESIS AND EGG DEVELOPMENT IN THE HUMAN LIVER FLUKE, *OPISTHORCHIS VIVERRINI* (PLATYHELMINTHES, DIGENEA)

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Eggs of most species digenean flukes hatch in the external environment to liberate larvae that seek and penetrate a snail intermediate host. Those of the human liver flukes, *Opisthorchis viverrini*, hatch within the gastro-intestinal canal of their snail hosts. While adults parasites are primarily responsible for the pathology in cases of human opisthorchiasis, their eggs also contribute by inducing granulomata and in serving as nidi for gallstone formation. In view of the peculiar biology of *O. viverrini* eggs and their contribution to pathology, we investigated embryogenesis in this species by light and transmission electron microscopy. Egg development was traced from earliest stages of coalescence in the ootype until full embryonation in the distal region of the uterus. Fully mature eggs were generally impermeable to resin and could not be examined by conventional electron microscopy methods. However, the use of high pressure freezing and freeze-substitution fixation of previously-fixed eggs enabled the internal structure of mature eggs, particularly the sub-shell envelopes to be elucidated. Fertilization occurs in the ootype, and the large zygote is seen therein with a single spermatozoon wrapped around its plasma membrane. As the zygote begins to divide, the spent vitellocytes are pushed to the periphery of the eggs, where they progressively degrade. The early eggshell is formed in the ootype by coalescing egg-shell precursor material released by approximately 6 vitelline cells. The early eggs have a thinner eggshell, are larger than, but lack the characteristic shape of, mature eggs. Characteristic shell ornamentation, the “muskmelon” appearance of eggs, appears after eggshell polymerization in the ootype. Pores are not present in the shell of *O. viverrini* eggs. The inner and outer envelopes are poorly formed in this species, with the outer envelope evident beneath the eggshell at the opercular pole of the mature egg. The miracidium has a conical anterior end that lacks the distinctive lamellar appearance of the terebratorium of other digeneans, such as the schistosomes. The miracidium is richly glandular, containing an apical gland in the anterior end, large cephalic gland and posterior secretory glands. Each gland contains a secretory product with different structure. The paucity of vitelline cells associating with eggs, the reduced size of eggs and reduced complexity of the extra-embryonic envelopes are interpreted as adaptations to the peculiar hatching biology of the miracidia.

SY18.P.02

LIGHT AND SCANNING ELECTRON MICROSCOPIC OBSERVATIONS ON *GRILLOTIA ERINACEUS* (VAN BENEDEN, 1858) (CESTODA: TRYPANORHYNCHA) PLEROCERCOIDS IN THE BLACK SEA WHITING, *MERLANGIUS MERLANGUS* L, 1758

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The genus *Grillotia* Guiart, 1927 is cosmopolitan in its distribution, with metacestodes occurring in a variety of teleosts and adults developing in orectolobiform, carchariniform, hexanchiform, squaliform and rajiform elasmobranchs. This genus is characterised by paired bothridia, a heteroacanthous armature, a longitudinal band of hooklets on the external surface of the tentacle and postovarian testes. The type-species, *G. erinaceus* (Van Beneden, 1858), has been relatively well described. However, in the present study, the specimens of *Grillotia erinaceus* were obtained for the first time in Turkey from the mesenteries of whiting caught by commercial fishing vessels off

Sinop. Fish were examined during 2011 - 2012. Standard parasitological investigation methods were applied standard indices of infection were also calculated. Prevalence (P) and mean intensity (MI) values in 217 fish specimens collected from Sinop coasts of the Black Sea were 22.6% and 1.82 ± 0.17 parasites per infected fish, respectively. *G. erinaceus* larvae were also registered in whiting off Balaklava, Crimean coasts of the Black Sea (P=3.3% and MI=1.0; 126 fishes were studied). Morphological diagnostic features of whole parasite, bothridium, scolex, tentacular armatures and tentacles were studied in detail using light and Scanning Electron microscope (SEM). Plerocercoids of this parasite had a total length of 13 mm in average. Specimens for light microscopy were studied fresh in Olympus BX53 fitted with digital camera attachment, while specimens for Scanning Electron Microscopy were subjected to standard protocols and viewed with a Jeol JSM-6510LV at an accelerating voltage of 10kV; photomicrographs of each parts of the parasite are presented. This study presented detailed morphological features of *Grillotia erinaceus* from the Black Sea whiting, *Merlangius merlangus*.

Keywords: *Grillotia erinaceus*, whiting, Black Sea, Sinop, Balaklava.

SY22.P.01

STUDY OF ENTOMOPATHOGENIC NEMATODES FROM GENERA *STEINERNEMA* AND *HETERORHABDITIS* IN UKRAINE

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An integral part of biology of entomopathogenic nematodes (EPN) from genera Steinernema and Heterorhabditis is their symbiosis with bacteria Enterobacteriaceae. Penetrating into the insect bodies, nematodes inoculate them with pathogenic bacteria causing their death within 24-48 hours. Therefore, they can be used as agents of biological control. Despite the wide distribution of *Steinernema* and *Heterorhabditis* EPN in many ecosystems of more than 50 countries, information on their location in Ukraine is scanty. The aim of our study is to identify EPN in different biocenoses of Ukraine with determination of their specific names and efficacy in control of hidden insects.

Nematodes were taken from soil samples with the aid of caterpillars of wax moth *Galleria melonella* used as “live traps”. Efficacy of local of EPN strains was tested in laboratory and field conditions.

In 2007-2010, during examination the field and orchard agrocenoses in different regions of Ukraine, 344 samples of soil were analyzed, of them 69 samples (20%) were contaminated with EPN. Samples from agrocoenoses were the most infected (25%), the percentage of infected samples from orchards was 22%, while pine nurseries and nut plantations were uncontaminated. Crimean biocenoses were infected far less: of 493 samples, only 27 (5.47 %) contained EPN. In plantations of ornamental plants, in meadow and forest (201 samples) infection rate was 4.57%, in orchards (168 samples) - 8.3%, vineyards (60 samples) - 6.7%. Soil samples from fields of sunflower, sorghum, maize were free from EPN. Nematodes isolated were identified as belonging to three species: *Steinernema feltiae, S. carpocapsae* and *Heterorhabditis bacteriphora*.

While cultured on wax moth, in nematodes *S. feltiae* and *H. bacteriphora* mutual and unique features of their biology were found. They have the same duration of the life cycle (21 days), and two generations. The differences are in the sexual structure of generations and the numbers of infective larvae leaving the dead insects. There were no males of *Heterorhabditis* in the first generation, they appeared in the second generation only; *Steinernema* males were present in both generations. The number of *Steinernema* infective larvae was much less then that of *Heterorhabditis* (6,000 to 29,000).

When tested in laboratory and field, both revealed EPN isolates and industrial biological preparations caused high mortality in larvae of beet root weevil (100%), western corn rootworm (100%), Colorado potato beetle (80-100%), dark-winged fungus gnats (100%), oriental fruit moth (88 -97%), codling moth (89-100%). In vegetation experiments on use EPN against cockchafer

larvae on strawberries, and European mole cricket on white cabbage, according to the dose of infective larvae, technical efficiency was 47.6-100 and 10-60%, respectively.

SY22.P.02
PREVALENCE OF *NOSEMA APIS* AND *NOSEMA CERANAE* IN SLOVAK REPUBLIC

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The *Nosema apis* was the only diagnosed microsporidian intracellular parasite of the honey bee (*Apis mellifera*) in Slovakia up to 2008. In Slovakian honeybee colonies the causative agent *Nosema ceranae* was confirmed in 2008. The aim of our study was to monitor the prevalence of mono infection and co-infection of both species *N. apis* and *N. ceranae* by using polymerase chain reaction (PCR). The analyses were performed on 72 samples of dead bees, which were collected from bee colonies representing all regions of Slovakia. Prior PCR analyses positive samples were selected by microscopic examination, confirming presence of *Nosema spp.* spores.
In the year 2009, were examined a total of 29 samples. *N. apis* mono infection was diagnosed overall in one sample only. The *N. ceranae* mono infection was diagnosed in 16 samples, while *N. apis* / *N. ceranae* co-infection was diagnosed in 2 samples. In Three samples causative agent was not identified by differential diagnostics, they considered as *Nosema spp.* positive. The ascertained proportion *N. apis* / *N. ceranae* was 14.3% / 85.7%.
In the year 2010, 43 samples were included in the testing and the *N. apis* mono infection was not diagnosed, *N. ceranae* mono infection was diagnosed in 27 samples, *N. apis* / *N. ceranae* co-infection was confirmed in 3 samples. The ascertained proportion *N. apis* / *N. ceranae* was 9.1% / 90.9%.
In a period of two years, we recorded a gradual increase in the prevalence of *Nosema ceranae* and decrease in the prevalence of *Nosema apis* using polymerase chain reaction (PCR) analysis of bees (*Apis mellifera*) in Slovakia. It is necessary to monitor the prevalence of both species of *Nosema* in Slovakia also in the forthcoming bee-keeping seasons.

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SY24.P.01
CYTOKINE ORODUCTION IN GASTRO-ALLERGIC ANISAKIOSIS AND ASSOCIATED CHRONIC URTICARIA

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Anisakis simplex is a ubiquitous fish parasite that has been associated with acute urticaria in gastro-allergic anisakiosis (GAA) and with chronic urticaria (CU), when associated with sensitization (CU+). In previous works, using *A. simplex* recombinant major allergens, we observed that sensitization against this nematode is due to previous parasite infection in most patients with CU+. Further, different urticaria phenotypes were associated with distinct serum cytokine levels. In the present work we study 18 GAA and 22 CU+ patients that were compared with 27 patients

diagnosed of CU without sensitization against *A*. *simplex* (CU-). Cytokine production was measured in supernatants after stimulation of peripheral blood mononuclear cells (PBMC) stimulated with *A*. *simplex* antigen or Concanavalin A (BD TM Cytometric Bead Array (CBA) Human Th1/Th2/Th17 Cytokine kit). Expectedly, PMBC from GAA and CU+ patients produced overall higher cytokine amounts than CU- patients after parasite larval antigen stimulation. When comparing GAA and CU+, significantly higher levels of IL-4 and IL-10 were detected in GAA. In GAA, we observed higher levels of IL-6, IL-10, IL-17, TNF-α; and IFN-γ; after mitogen stimulation, compared to levels after *A*. *simplex* stimulation. On the contrary, significantly higher concentrations of IL-2 were measured when PBMC of these patients were stimulated with parasite antigen. Similar results were observed in the case of PBMC stimulation of CU+ and CU- patients with again higher cytokine levels after mitogen stimulation with significant differences for IL-4, IL-6, IL-10, IL-17, TNF-α and IFN-γ. As a final conclusion, the IL-2, IL-4 and IL-6 have been increased after stimulation with *A*. *simplex* antigens in GAA and CU+. The anti-inflammatory IL-10 production was higher in GAA than CU+, whereas the pro-inflammatory IFN-γ production was higher in CU+ than GAA. In other words, the phenotype GAA produces a more anti-inflammatory response than CU+, which produces more pro-inflammatory cytokines.

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SY24.P.02

DUST MITE ALLERGENS: A MAJOR RISK FACTOR IN DEVELOPMENT OF ALLERGIES IN DUBAI

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The prevalence of allergic diseases, especially asthma and allergic rhinitis, has dramatically increased during the last decades. Mite and cockroach, which are the most common allergens in house dust, are the major indoor allergens in asthmatic and allergic rhinitis patients. This study was designed to (1) evaluate the dust mite allergen level and its consequences among 120 municipality workers in two different seasons in the city of Dubai (UAE) (2) Compare the sensitivity of Ventia rapid test and *Dermatophagoides farina* major allergen group 2 (DERF2) ELISA test. A questionnaire was designed to include variables as age, sex, nationality, frequency of exposure to dust, vacuuming, previous history of atopic dermatitis, allergic rhinitis or asthma, residence characteristics as kind of carpeting and floor type. Blood samples were collected from the workers to measure the specific immunoglobulin E level. The dust samples were collected in summer and winter in the residence of the workers, from carpets, curtains, window sills, AC filters, chairs and sofas in their residence. Dust samples were analyzed using dust mite allergen detection venita rapid test and DERF2 allergen was determined using ELISA. Allergen levels were found to be higher in summer than winter. The highest level of allergens was detected in carpets (≥ 1 µg /gm of dust) followed by AC filters and curtains (0.2 µg /gm of dust). Allergens were below detectable range in chairs, sofas and window sills. There was a high significant association between vacuuming, floor type and the occurrence of atopic dermatitis, allergic rhinitis or asthma p= 0.001.Venita test was proven to be sensitive and specific method for evaluating allergen level compared to the quantitative monoclonal antibody ELISA. The variation of specific immunoglobulin E levels in allergic individuals was found insignificant in the two seasons.

SY26.P.01

ASSESSMENT OF THE EFFECT OF NEW ETHYL AND METHYL CARBAMATES ON *RHIPICEPHALUS MICROPLUS* RESISTANT TO CONVENTIONAL ACARICIDES

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Rhipicephalus microplus is the most important tick of tropical and subtropical areas of many countries of the word and it causes enormous economic loss to the livestock industry. During many years, the strategy that has been mostly used for its control has been the use of chemical acaricides; nevertheless, the high selection pressure caused by the exaggerated use of these products has promoted the phenomenon of resistance to commercial acaricides. This obligates the development of new pharmaceutical alternatives for the control of these ticks. Among these alternatives is the development of new molecules for which ticks have not developed resistance. Therefore, in this study, the effects of eight carbamates designed and synthesized in FES-Cuautitlán-UNAM were evaluated; six carbamates on engorged female ticks (adult immersion test), and two carbamates on larvae from conventional acaricide resistant strains of *Rhipicephalus microplus* (San Alfonso and La Mora) and from an isolate obtained in the field. These carbamates were previously effective on tick strains that were susceptible to commercial acaricides; they are totally synthetic and belong to a chemical group different from any of the current commercial acaricides. The six carbamates used on the engorged females inhibited oviposition ($p<0.05$) up to 65.4% and inhibited egg hatching up to 100%. Products that had inhibition of larvae hatching at a lower concentration for each strain were: San Alfonso: LQM914=0.427mg/ml and LQM996=0.589 mg/ml; La Mora: LQM904=0.428 mg/ml and LQM996=0.452 mg/ml; field ticks: LQM914=0.498 mg/ml and LQM996=0.477 mg/ml. Eggs produced by treated females had a darker, drier and opaque appearance. Also, it was observed that there was a loss of adherence between them. The carbamates identified as LQM 934 and LQM 938 had an effect on larvae mortality ($p<0.05$). Lethal concentrations 99% for each product/strain were: San Alfonso: LQM934= 1.188% and LQM938=0.395%, La Mora: LQM934 = 1.373% and LQM938=3.548%, field ticks: LQM934= 1.188% and LQM938=0.395%. Data suggests that the first carbamates act as inhibitors of the biological cycle of R. microplus and the second group has a neurotoxic activity on the larvae.

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SY26.P.02

FLEA AND TICK RESISTANCE IN DOGS AND CATS TO PARAKILL PRODUCT (FIPRONIL)

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Cats and dogs have always been subject to tick and flea infestations; however in recent years the diseases transmitted by these parasites (known as vector-borne diseases) have become more widespread, posing a very real threat to both pets and humans. These changes have come about as a result of a complex interplay between many factors. Animals used in experiments to test the resistance of the fleas and ticks to Parakill, were from clinics of Veterinary Faculty from Cluj-Napoca, respectively pet owners from the county of Cluj, Bistrița Năsăud and Mureş. It was selected 4 groups (n = 24), two experimental (n = 12) and two controls (n = 12) of dogs and cats naturally infected with fleas and / or ticks. Animals group were composed from males and females of various breeds, with aged more than six weeks, with clinically confirmed health. Statistical interpretation of results was performed by quantifying the percentage of reduction of the number of

post-therapeutic fleas and ticks compared with the pre-treatment, and the data obtained were processed statistically to determine the upper and lower limit 95% confidence interval. The evolutionary dynamics of AT and PT up to 28 days extensivity of the flea and tick number and infected animals from the groups C1 and P1 were observed the following: Fleas and ticks on dogs skin disappeared after 72 hours of PT; animals are not reinfected over the 28 days of clinical observation; Ticks from the skin of cats have disappeared after 24 hours PT and fleas within 72 hours; animals are not reinfected over the 28 days of clinical observation; Statistical interpretation of data obtained from dogs group infested with ticks showed a rate of 100% reduction at 72 hours PT; from dogs group infested with fleas showed a rate of 100% reduction at 72 hours PT with an upper limit value of 95% confidence interval 97% to 24 or PT; Statistical interpretation of data from cats group infected with ticks showed a rate of 100% reduction at 24 hours PT; from cats group infested with fleas showed a rate of 100% reduction at 72 hours PT with a lower upper limit of the confidence interval 95% - 98% at 24 hours PT; Testing of resistance to the fipronil, of the fleas and ticks in dogs and cats in groups treated with therapeutic doses, showed no adaptive phenomena of parasites to this drug substance.

SY26.P.03

IS LARVAL DEVELOPMENT TEST RELIABLE ENOUGH TO DETECT ANTHELMINTIC RESISTANCE IN THE FIELD?

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To detect anthelmintic resistance (AR), *in vivo* and *in vitro* methods may be used. Most of these methods, though, have drawbacks in cost, applicability, interpretation, or reproducibility. Larval development tests are the most widely used in vitro method for the detection of AR in ovine nematodes.
Lambs were infected with susceptible and anthelmintic resistant isolates of *Haemonchus contortus*. We have used the micro agar larval development test (MALDT) which was performed on 96-well microtitre plates. Different proportions of resistant and susceptible eggs were incubated. To diagnose benzimidazole (BZ) resistance - thiabendazole and ivermectin resistance ivermectin-aglycone drugs were prepared. The percentage of resistant eggs ranged from 2% to 20.0% of all eggs in the wells.
The MALDT was able to clearly indicate the presence of approximately 4% of BZ resistant worms amongst a susceptible background population. The probability to positively diagnose only 1–2% of BZ resistant worms within the population was approximately 50%. In all cases, the MALDT was able detect the presence of a minimum of 10% of ivermectin resistant worms amongst a susceptible background population. The probability was approximately 87% of positively diagnosing a proportion of ivermectin resistant worms of only 2-4% within the population. The current study indicates that ivermectin aglycone at concentrations of 10.8 ng/ml could be considered as threshold discriminating doses for ivermectin resistance and 0.02 mg/ml of thiabendazole could be used as a threshold discriminating dose for BZ resistance in *H. contortus*.
MALDT showed comparable and reliable results for the detection of BZ and ivermectin resistance in *H. contortus*. Additionally, the test was able to reveal a relatively small proportion of resistant worms in the population, a sensitivity that should have potential in determining resistance in field tests.

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SY26.P.04

THE FIELD SURVEY OF IVERMECTIN RESISTANCE IN SHEEP PARASITE IN THE SLOVAK REPUBLIC

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Gastrointestinal nematode parasites in sheep cause severe economic losses. The broad spectrum anthelmintics are the most commonly used drugs for prophylaxis and therapy of parasitic worms - helminths. The extensive use of anthelmintics for the control of helminth infections has resulted in the development of resistance that has become a major practical problem in many countries. The problem of resistance includes all of commercial available anthelmintics in several generas and classes of helminths. The resistance in the world is probably more severe than some documents present it up to now. In some countries the benzimidazole anthelmintics have not used for decades because of high occurrence of resistance and the farmers are forced to use anthelmintics with different mechanism of action which ultimately enables the onset of resistance against the last available anthelmintics – macrocyclic lactones.
In vitro and *in vivo* tests are used to detect the anthelmintic resistance. From three tested *in vitro* methods we have chosen the micro agar larval development test. This method was performed as described by Coles et al. (2006). Micro agar larval development test is suitable, sensitive and reproducible method for detection of macrocyclic lactones resistance in sheep parasites and with this method we were able to detect 2 – 4% resistant parasites within the tested population. Consequently, we used this test for monitoring of prevalence of ivermectin resistance in nematode parasites of sheep in the Slovak Republic. 49 farms from 17 districts of Slovakia were examined. On 2 farms (4.35%) high occurrence of resistant parasites has been detected. Resistance to ivermectin was low on 12 farms (26.07%) and 32 farms (69.56%) were declared without ivermectin resistant worms. The situation in Slovakia is similar to the situation in European countries and refers to initial onset of ivermectin resistance in Slovak sheep farms.

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SY26.P.05

EVALUATION OF CYDECTIN POUR-ON AGAINST LICE ON NATURALLY INFESTED CATTLE WHEN TREATED AT THE START OF THE HOUSING PERIOD

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The objective of the present study was to evaluate the efficacy of Cydectin®Pour-on (CPO) for Cattle (Pfizer Animal Health) at the recommended dose rate of 0.5 mg/kg bodyweight against natural infestations of lice when treated before or in the first 4 weeks of the housing period. It is known that CPO is effective against *Bovicola* (*Damalinia*) *bovis*, *Linognathus vituli*, *Solenopotes capillatus* and *Haematopinus eurysternus*. The current study aimed to evaluate if cattle on a farm will remain lice-free when all in-contact animals are treated on the same day, no new cattle are introduced to the treated group, all treated animals remain in the same enclosure throughout the winter housing period, and the treated group does not have contact with any other cattle. To this end, 2 beef farms in the Burgundy region of France were selected, each with approximately 200 animals. Both farms had recurring problems of lice infestations in previous years. All animals were treated and on each farm 35 animals were selected for follow-up based on high pre-treatment lice counts. The efficacy of the treatment was monitored by monthly evaluation of the lice burden up to the end of the housing period. On both farms, animals were housed and treated in December 2011, and animals were released on pasture in the first two weeks of April 2012. Prior to treatment,

all animals were infested with *L. vituli* (both farms) and/or *B. bovis* (1 farm). Several animals were also infected with *H. eurysternus*. On both farms, none of the animals displayed any clinical signs suggestive of re-infestation with lice. On one farm, all 35 animals remained free of lice during the entire housing period. On the other farm, one animal was infected with a total of 3 lice (2 *B. bovis* and 1 *L. vituli*) at the third and fourth monthly evaluation after treatment. All other animals were negative throughout the winter, and none of the animals displayed any clinical signals. The results of this study demonstrate that a single treatment with CPO at the start of the housing period resulted in a lice-free status of cattle on these farms during the entire housing period when used with appropriate husbandry to reduce the risk of introducing new infection.

SY26.P.06

EVALUATION OF THE EFFICACY OF MOXIDECTIN PLUS TRICLABENDAZOLE POUR-ON AGAINST LICE ON CATTLE

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Moxidectin is a macrocyclic lactone (ML) - milbemycin with broad-spectrum efficacy against a wide range of parasites in cattle, including chewing and sucking lice. Cydectin®Triclamox Pour-on for Cattle (CTPO) (Pfizer) contains 0.5% w/v moxidectin and 20% w/v triclabendazole. The dose rate of moxidectin (0.5 mg/kg) in CTPO is the same as that for Cydectin® 0.5% Pour-on for Cattle (CPO) (Pfizer). It would thus be reasonable to expect that CTPO would exhibit similar efficacy to CPO against lice in cattle. The efficacy of CTPO against lice on cattle was evaluated in accordance with WAAVP guidelines. In brief, 20 animals naturally infected with *Linognathus vituli* and/or *Bovicola bovis* were included. On day 0, animals were weighed and treated either with vegetable oil or CTPO. Post treatment lice counts were conducted weekly from day 7 to day 56. The efficacy of CTPO against *L. vituli* was high: in 6 out of the 8 post-treatment observations the efficacy based on arithmetic mean was ≥ 95%; the efficacy was < 94.5% on day 7 and 85.7% on day 28. This lower efficacy on day 28 was probably due to the simultaneous hatching of eggs, in this case mainly on 1 animal on which a large number of newly hatched lice were observed. Noteworthy is the high efficacy (≥97.4%) of CTPO in the subsequent counts (days 35-56). A similar efficacy pattern was found for *B. bovis* despite low infection levels, with a short relapse around day 28-35, and a high efficacy (100%) thereafter. As such, the efficacy reported in the present study for CTPO is consistent with previous studies using CPO in cattle.

SY26.P.07

EVALUATION OF THE PERIOD OF PERSISTENT EFFICACY OF MOXIDECTIN PLUS TRICLABENDAZOLE POUR-ON SOLUTION AGAINST *OSTERTAGIA OSTERTAGI* AND *DICTYOCAULUS VIVIPARUS* IN CATTLE

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To evaluate the period of persistent efficacy of a moxidectin (0.5% w/v)/triclabendazole (20% w/v) pour-on solution (Cydectin®Triclamox Pour-On for Cattle, Pfizer [CTPO]) against selected nematode parasites in cattle, 2 experimental studies were conducted. Efficacy was determined against challenges of *Ostertagia ostertagi* and *Dictyocaulus viviparus* at 4 weeks, 5 weeks and 6 weeks after treatment. Efficacy was determined versus non-treated controls. In each study, 32 parasite naïve, male calves between 4 to 5 months of age were enrolled. Animals were randomly allocated into 4 treatment groups of 8 animals each: one group remained untreated, each of the other groups was treated with CTPO either 4, 5 or 6 weeks prior to experimental infection. All animals were infected with approximately 2,000 *D. viviparus* and 20,000 *O. ostertagi* infective larvae. All animals were euthanised 28-29 days after infection, in order to retrieve the abomasum and lungs for *O. ostertagi* and *D. viviparus* worm counts respectively. The primary variable for the

persistent efficacy was the percent reduction in worm counts relative to the non-treated controls. The worm count data were analysed using a mixed linear model. Data were log transformed prior to analysis. The total *O. ostertagi* worm counts were calculated based on the adult and L4 stage larval counts in the abomasum. The majority of the worms were adult and only a small percentage was L4 larvae. Treatment resulted in significant reductions in worm counts for both species relative to controls ($p < 0.05$) for all challenges. The persistent efficacy against *O. ostertagi* was >90% for 6 weeks in both studies. The persistent efficacy against *D. viviparus* was >90% for 5 weeks in the first study and 6 weeks in the second study. Efficacy in the first study was 88.9% at 6 weeks. The shorter duration of persistent efficacy against *D. viviparus* in the first study is attributable to low worm counts in the untreated group. As such, the persistent efficacy reported in the present study for CTPO is consistent with previous studies using Cydectin® 0.5% Pour-On in cattle.

SY26.P.08

INSECTICIDE SUSCEPTIBILITY OF WILD-CAUGHT SAND FLY POPULATIONS COLLECTED FROM CERTAIN DISTRICTS OF AEGEAN REGION

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Sandfly-borne diseases like visceral, cutaneous leishmaniasis and phlebovirus infections are seen endemically in Turkey. However, no study was performed about the susceptibility/resistance in phlebotomine sand flies against pyrethroids in Turkey. For this reason, in our study, we aimed to evaluate the pyrethroid group insecticide susceptibility/resistance in phlebotomines using WHO standard protocols. The Standard WHO testing procedure “Tube Test” has applied for pyrethroid susceptibility by using 0.05% deltamethrin and 0.75% permethrin impregnated papers. The tests were repeated 5 times including control ones. The sand fly specimens were collected from several localities; la province where insecticides regularly applied and one village of Aydin province where no insecticide application. Following the standards of WHO testing procedures, all specimens were dissected, mounted and identified according to the standard species key charts of the Mediterranean region. A total of 230 and 246 sand fly specimens collected from Mugla and Aydin provinces were used as 15-20 specimens in each tube, respectively. The 90% and 93.3% susceptibility was detected among the sand fly specimens collected in Mugla against deltamethrin and permethrin after 24 hours of exposure, respectively. Because of the mortality rates are below the 5%, no correction was made with the Abbotts formula. The resistance was detected in these specimens according to WHO standards. The 99% and 100% susceptibility was detected among sand fly specimens collected in Aydin against deltamethrin and permethrin after 24 hours of exposure, respectively. Because of the mortality rates were found as 15%, the correction was made using Abbotts formula according to WHO standards. No resistance was found in these specimens. Sand fly fauna of the study areas and species spectrum used in the study were as follows; in Mugla province, we found three (64% *P. tobbi*, 30% *P. papatasi*, 5% *P. neglectus/syriacus*) and one (1% *S. minuta*) species belonging to Phlebotomus and Sergentomyia genera while four (79% *P. tobbi*, 9% *P. neglectus/syriacus*, 6% *P. papatasi*, 2% *P. alexandri*) and two (2% *S. minuta*, 2% *S. dentata*) species belonging to Phlebotomus and Sergentomyia genera were found in Aydin province, respectively. In conclusion, the resistance against deltamethrin and permethrin were detected in the areas where insecticide applications have been applied for long time while no resistance were found in the insecticide free area. We also showed the presence of vector sand fly species for *L. infantum* in the study areas. These results clearly pointed out the more attention are needed by the authorities involved in control programs for sand fly-borne diseases.

SY26.P.09

EFFICACY OF GASTRO-RESISTANT CAPS OF RONIDAZOLE AGAINST FELINE *TRITRICHOMONAS FOETUS* INFECTION

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Ronidazole (RDZ) is an effective treatment for feline *Tritrichomonas foetus* infection, but neurotoxicity adverse effects have been observed in some cats. The ENVA recently developed a new formulation of gastro-resistant RDZ caps in order to reduce adverse effects with the use of RDZ.
A randomized double-blind clinical trial was initiated to assess the efficacy and safety of this new RDZ treatment against feline *T. foetus* infection. This trial included 64 cats naturally infected by *T. foetus*. Cats were randomly allocated to placebo (n=31) or RDZ treatment (n=33) group. RDZ or placebo was administered to cats at 30 mg/kg orally once a day for 15 days (D15).
Feces swab were examined for *T. foetus* by a commercially available system "In PouchTM TF test" (BioMed Diagnostics, Oregon USA), or a polymerase chain reaction (PCR) testing at D15.
At D15, *T. foetus* was undetectable by In PouchTM TF testing in 94% and 55% of cats who received RDZ and placebo, respectively (p<0.01). Using the more sensitive PCR testing, these percentages were 84% and 20% for RDZ and placebo, respectively (p<0.01). None of the cats receiving RDZ developed adverse reactions.
Oral administration of gastro-resistant caps of RDZ at 30 mg/kg one a day for 15 days eradicates infection in most cases and can be considered as safe regarding classical adverse effects of ronidazole.

SY26.P.10

ALLOZYME PROFILES OF *HAEMONCHUS CONTORTUS* RESISTANT AND SUSCEPTIBLE TO ANTHELMINTICS, WITH AN INDICATION OF DIPEPTIDASES LINKED WITH RESISTANCE

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Due to the widespread use of anthelmintics, a common trichostrongylid nematode *Haemonchus contortus* has developed resistance to the major classes of drugs used for its control. The purpose of the study was to characterize allozymic changes in selected strains of *H. contortus* that occur as resistance develops.
Four anthelmintic-resistant and three susceptible strains of *H. contortus* were used for the electrophoretic experiments (isoelectrofocusing). The BZ-resistant isolates MHco13 and MHco12 were recently isolated from Swiss farms. The MHco4 (the White River strain, WRS) strain was originally isolated from the South Africa and is now maintained as a multidrug-drug resistant strain (BZ, IVM, CLOS, RAF-resistant). MHco10 (CAVR) was characterized as resistant to IVM and BZs. The susceptible strain MHco3 (ISE) was inbred from a heterogeneous outbred population that had been maintained at the laboratory since the 1950s but which may have originated in Kenya. MHco9 and MHco6 were isolated as susceptible isolates of sheep from Germany and Kenya, respectively.
From nine candidate gene-enzyme systems, two enzymes (MPI, PEP-D) had polymorphic electrophoretic patterns. In MPI two alleles were alternating in examined strains, but Pearson's chi-

squared test did not indicate any significant differences in genotypic or allelic frequencies of MPI between the resistant and susceptible strains (all *p* values >0.05). The profiles of PEP-D (dipeptidase enzyme), though, showed striking differences between the two groups of strains. Three electromorphs generated by the hybridization of two loci with forming heterodimers (isoenzymes 80, 90, 100) were seen in susceptible isolates; whereas the resistant isolates expressed only one active locus with the isoenzyme of mobility 100 (shared with susceptible isolates). This electromorph was detected in 23 resistant worms. One resistant worm from strain MHco13 also manifested isoenzyme 90 indicating that a minor part of susceptible alleles can also be present in resistant lines.
Peptidases function in the turnover of proline-rich collagen in nematodes. Collagen is used extensively in nematodes for the periodic synthesis of cuticle during moulting, and recycling of the cuticular components requires peptidases capable of degrading proline-rich proteins This protein is also a key component of the basement membranes in nematodes, and peptidases are therefore likely to be involved in the hydrolysis of collagen-derived peptides. The observed differences in the PEP-D patterns may be associated with altered membrane-bound proteins that play an important role in drug transport and efflux from cells and may thus influence the development of resistance in *H. contortus*.

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SS01.P.01

USE OF THE MONOCLONAL ANTIBODY MM3 FOR DETECTION OF COPROANTIGENS OF *FASCIOLA HEPATICA* IN VACCINATED AND NON-VACCINATED GOATS

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The aim of this study was evaluate the use of the MM3 antibody for detection of coproantigens of *Fasciola hepatica* in vaccinated and non-vaccinated goats as an early immunodiagnostic method.
Twenty one goats were randomly allocated into 3 groups of 7 animals. Quil A was used as adjuvant and was combined with FhCL1 and FhGST for preparation of vaccines. Each immunisation dose was prepared as follows: 100 µg of antigen was diluted in 1 ml of sterile PBS and added to 1 ml of Quil A (1 mg). For immunisation of adjuvant-only control animals, 1 ml of sterile PBS was mixed with 1 ml of Quil A (1 mg). Animals from group A and B were immunised with FhCL1 and FhGST respectively, goats from group C remained as adjuvant-control group. All animals were immunised on two occasions at week 1 and 4, experimentally challenged with 100 metacercaria of *Fasciola hepatica* at week 10 and slaughtered at week 25 of the trial.
After challenge, faecal samples were obtained at intervals of 10 days. A capture ELISA using a polyclonal antibody and the MM3 monoclonal antibody was performed. A flotation method was carried out to assess egg output.
To complete the study, faecal samples from 50 goats from 5 different extensive and semi-intensive farming systems were analysed.
Detection of coproantigens from animals of the vaccination trial commenced at 50 days post-infection (dpi) and increased until the end of the trial, reaching maximum level between 70-80 dpi. The analyses of faecal egg output showed that eggs first appeared at 70 dpi in all infected animals, reaching maximum levels at 90 dpi. The mean liver fluke burden per goat for group A, B and C was 55.8, 59 and 49.2, respectively. No significant differences were detected between groups.

In goats from the farming system, the analyses of faecal samples showed neither coproantigens nor *Fasciola hepatica* eggs, thought *Eimeria* spp oocysts and eggs from nematodes of the superfamily Trichsotrongyloidea were observed in all animals.
These results indicates that detection of coproantigens using the MM3 antibody is an efficacious and reliable method for detecting *Fasciola hepatica* infection and permits an early diagnostic. The level of coproantigens detected in all animals correlated with the fluke burden and no cross-reaction was observed between *Fasciola hepatica* and *Eimeria* spp and Trichostrongyloidea spp.

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SS01.P.02

STUDY OF THE LOCAL IMMUNE RESPONSE AT THE EARLY STAGE OF *FASCIOLA HEPATICA* INFECTION IN GOATS IMMUNISED WITH CATHEPSIN L1 (FHCL1)

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The immunological protective mechanisms elicited during vaccination trials against Fasciolosis play a key role in controlling the disease. In laboratory animal models, these mechanisms have been located in the intestine, peritoneum and liver at the early stage of the infection, though little is known about the early immune response in goat fasciolosis.
The aim of this study was to analyse the local cellular immune response in the intestine, peritoneum and liver at the early stage of infection with *Fasciola hepatica* in goats vaccinated with FhCL1.
Twenty five goats were allocated into 3 groups: group A (n=10) was immunized with 100 µg of FhCL1 diluted in 1 ml of sterile PBS and added to 1 ml of Quil A (1 mg), group B (n=10) received 1 ml of Quil A (1 mg) diluted in 1 ml of sterile PBS, group C (n=5) remained as non-infected control. Animals were immunised on two occasions at week 1 and 4 and challenged with 100 metacercaria of *Fasciola hepatica* at week 10. Three animals from each group were slaughtered between 7-9 dpi (early stage) and the remaining goats were slaughtered at week 25.
After necropsy, fluke burden was obtained and immunohistochemical techniques were carried out to analyse the cellular immune response of the animals killed at 7-9 dpi.
The mean fluke burden in animals harbouring late stage infection was 55.8 and 49.2 for group A and B, respectively. Two of the animals from group A showed 19 and 25 worms. No significant differences were detected between groups.
At the early stage of the infection, the analyses of the liver parenchyma in two of the goats from group A showed abundant and well-marked eosinoiphilic infiltration around the migratory juvenile larva, and granulomas with multinucleated cells and scarce necrotic migratory tracts. It was also detected a higher number of eosinophils and expression of iNOS in the peritoneal macrophages. By contrast, little or no eosinophilic infiltrate was observed around the migratory larva in animals immunized only with Quil A.
The fluke burden indicates that immunisation with FhCL1 did not induce a protective immune response. However, the low fluke burden observed in two of the immunised goats suggest that a partial immune response might have occurred and that the effector mechanism may be related to expression of iNOS in the peritoneal macrophages and to the eosinophils in peritoneum and liver.

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SS01.P.03

ORAL ADMINISTRATION OF *CURCUBITA MOSCHATA* SEEDS AS A THERAPEUTIC ALTERNATIVE IN GOATS' GASTROINTESTINAL NEMATODES CONTROL

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One of the main limiting factors for the goat industry in Northeastern Brazil is the gastrointestinal nematodes (GIN). This study aimed to evaluate the anthelmintic potential of the *C. moschata* (pumpkin) seeds in an in vivo system. Two therapeutic schemes were utilized (10 animals per group) one applying the drug ivermectine® 13.5% - 1ml/50kg of living animal (IVG) and other with 10% of the pumpkin seed flour added to the commercial goat food Guabi® offered ad libitum (PSG). The control group ate only goat food and was not vermifugated. The goats weighed 14 to 17 kg and were naturally infected. The animals were observed for 60 days, and have been subjected to parasitological, clinical and hematological evaluations every 15 days. SAS version 7 was utilized for analyzing the results. The control group was statistically different from the treated groups (p < 0, 02175). No statistical difference concerning the eggs reduction per gram of feces (EPG) was verified in the treated groups (p > 0, 05). The mean concentration of EPG of the PSG showed a 70.73% (±15. 17) reduction, statistically similar to the results of the IVG which presented reduction of 83.82% (±8, 67). The control group showed a 181, 67 % (± 208, 75) increases in the mean of EPG. The final weight was higher for the PSG (34, 86%), while the increase in the animals' total length was significantly different between the control and PSG (p = 0,044) the curves referring to these variables showed homogeny only for the PSG. During clinical evaluation, 30 % of the animals from the control group presented respiratory manifestations, a natural consequence attributed to the nematodes life cycle after passing through the host's lungs. Moreover, 30% of goats of the control and IVG groups, had diarrhea and liberated proglottids of *Moniezia* sp. in their feces. In relation to the *Famacha*, the three groups did not present statistical differences among themselves, (p = 0,726). The hematological evaluation indicated a slight hypochromic macrocytic anemia in the control group, which possibly occurred due to the parasitic load. The seeds of *C. moschata* appear to be a therapeutic alternative to helminthic control in in vivo goat systems, reducing the quantity of EPG in the superfamilies' Trichinelloidea, Strongylloidea and Trichostrongylidea. The utilization of the seed of C. moschata may favor the control of the parasites, avoiding the application of the ivermectine and its residual effects in goat's meat.
Keywords: Goats; Gastrointestinal; Nematodes; C. moschata SEBRAE/SE CAPES.

SS01.P.04

INTESTINAL PARASITES IN GOATS IN SERBIA

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The number of goats in Serbia is estimated to below 100.000, but is currently expanding due to the increasing use of both goat meat and milk / milk products. Although parasites are generally prevalent and affect animal health and quality of products, no data have been published on the parasitic fauna in goats in Serbia. Thus, a survey of parasitic fauna in goats was initiated in January 2011. To date, seven farms from five (out of 12) epizootiological regions throughout

Serbia were examined, of which only two had an intensive raising system. These two were examined at the owners' request although no symptoms/signs of parasitic infection were observed, whereas in all others signs of infection, including respiratory and digestive symptoms, were noted. From all farms, at least 10% of all animals were examined, bringing the total of examined goats to 68. Of the examined goats, 12 were kids, 23 between 1 and 2 years of age, and 34 were above 3 years of age. Three stool samples were examined from each animal by standard coprological methods, and infection intensity was determined according to McMaster. Of the seven farms, one large farm was parasite-free, whereas the other large one, though free from all other parasites, was 100% infected with *Fasciola hepatica*. In the small farms, the findings included oocysts of *Eimeria* (*E. arloingi*, *E. caprina*, *E. hirci*), eggs of Anaplocephalidae, larvae of protostrongylids (genus *Mullerius*, *Protostrongylus*, *Cystocaulus*, *Neostrongylus*, *Dictiocaulus*) and nematode eggs (genus *Haemonchus*, *Ostertagia*, *Trichostrongylus*, *Cooperia*, *Nematodirus, Strongyloides*). The presented data indicate that the parasitic fauna in goats in Serbia is variable and depends on animal age, race, husbandry practices, area etc. Whereas a level of infestation is naturally expected in extensive raising, it is of note that a high level of infection with Fasciola hepatica has been found in intensive raising, indicating use of contaminated food obtained from endemic fasciolosis areas. Since upon delivery of results on parasite findings dehelminthization has been performed in the examined farms, the study will be continued by control examination of the same flocks after a suitable period of time. It is expected that such activities will help improve both the areas of animal health and economy as well as of public health (goat products as a source of human infection).

SS01.P.05

PHENOTYPIC ANALYSIS OF EGGS OF *FASCIOLA HEPATICA* RECOVERED FROM CREOLE GOATS, IN WESTERN ARGENTINA

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Fascioliasis is a zoonotic disease that is spreading worldwide. The disease is caused by both *Fasciola hepatica*, of cosmopolitan distribution, and *Fasciola gigantica*, from tropical areas of Africa and Asia. The main reservoirs of *F. hepatica* are considered to be cattle and sheep, but parasitism is also frequent in goats, horses, wild ungulates and even humans. Each trematode species has its own egg shape, and the length and width of the eggs are generally within a specific range. Furthermore, the final host species decisively influences the size of *F. hepatica* eggs. Egg's morphology has been effective in the differentiation between *F. gigantica* and *F. hepatica*. The aim is to compare the eggs of *Fasciola hepatica* recovered from Creole goats from different geographical areas. Feces samples were collected from Creole goats from range and andean regions (Mendoza and La Rioja provinces, respectively), in Western Argentina. *F. hepatica* eggs were recovered with Lumbreras' sedimentation technique and 140µm filtration. Measures taken on 56 eggs from each region: Length (EL µm), width (EW µm), size =EL*EW (ES µm2) and ratio =EL/EW (ER) (=1 in round eggs, >1 in elliptical eggs). Kruskal-Wallis non-parametric test was used for statistical comparison ($P < 0.05$). Measures obtained (range, mean +/-S.D.) from i) Range region: EL 108.14-150.45, 128.5 +/-8.10, EW 59.73-76.47, 67.67 +/-3.73, ES 6691.82-11124.57, 8707.57 +/-766.06, ER 1.5-2.3, 1.9 +/-0.15; ii) Andean region: EL 113.12-147.74, 132.13 +/-6.14, EW 62.67-79-41, 69.95 +/-3.71, ES 7561.58-11282.94, 9243.80 +/-665.89, ER 1.62-2.18, 1.89 +/-0.13. EL and EW showed statistical differences, and thus ES, being smaller the eggs from range region (Mendoza). It should be remarked that ES from both regions are smaller than previously reported for goats from Patagonian region (mean 10202.7 µm2). Meanwhile, eggs were characterized as elliptical and no statistical difference was found between ER. Differences observed (EL, EW and ES) could be attributed to relationship with geographical origin, but ER should also be expected to differ. On the other hand, crowding effect, reflected in a decreased adult development when parasite burden is high, may explain the results obtained. Smaller size of the eggs may be related to the reduced uterus development as a consequence of the crowding

effect and diminished adult size. Since recent results had demonstrated no apparent relationship between the shape of fasciolid adults and altitude or geographical origin in Peru, crowding effect seems a more feasible explanation.

SS01.P.06

FASCIOLA HEPATICA INFECTION AND ASSOCIATION WITH GASTROINTESTINAL PARASITES IN CREOLE GOATS, IN PLATEAU AND ANDEAN REGIONS OF WESTERN ARGENTINA

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Goats, frequently referred to as “the cow of the poor”, are an example of a sustainable production fully integrated within the local rural development. Being of great importance in small farming systems, is the domestic livestock species with the most significant population growth world-wide in recent years. Frequently disregarded, gastrointestinal parasitism constitutes one of the main constraints to outdoor and extensive breeding in temperate and tropical countries. Despite a Creole goat population of nearly 4 million heads, local reports of parasitological prevalence in the species are scarce, and, while F. hepatica infection is spread all over Argentina, this ruminant species is usually neglected as a reservoir and economic losses are not considered. Fecal samples from 663 Creole goats from plateau and Andean regions from western Argentina were collected and analyzed by means of coproparasitological techniques (Sheater, Formo-ether and Lumbreras). Statistix® 7.0 and SPSS® 17.0 were used for statistical analysis, comparison of categorical variables and chi-square test. Values of $p < 0.05$ were taken as significant and Odds Ratio (OR) was calculated. Almost 85% of the animals (562/663) sampled were found to harbour one or more parasite species. 217 (32.73%) of the examined animals were positive for *F. hepatica*, 344 (51.88%) were positive for nematodes, while 422 (64.72%) were positive for *Eimeria* sp. Considering presence of parasite types, the most common was single presence (38.61%), with *Eimeria* sp. as the predominant type (21.27%), followed by double presence (31.82%). The most frequent combinations were *Eimeria* sp. + *Nematodirus* sp. (15.38%) and *Fasciola hepatica* + *Eimeria* sp. (11.01%). *F. hepatica* mainly occurred as a coinfection with another parasite, while only 22.12% (48) of the cases occurred as monoparasitism. It was most frequently combined with *Eimeria* sp. (11.01%), followed by the duet *F. hepatica* + *Nematodirus* sp. (2.11%), and lastly combined with Strongyle eggs (0.90%). Significant positive associations were detected between *F. hepatica* and Strongyle eggs (6.11, p= 0.013, OR= 1.96), *Eimeria* sp. and *Nematodirus* sp. (7.91, p= 0.005, OR= 1.61), and *Nematodirus* sp. and *T. ovis* (9.89, p= 0.002, OR= 6.09). Further studies are required to define whether these associations are causal or not, and their relevance in the epidemiology of the parasites implicated. *F. hepatica* is rarely considered in the country as a parasite of goats, but a stunning 33% prevalence poses an interrogation about the role of goats on the transmission and dissemination of this zoonotic trematode.

SY01.P.01

PROPOSE OF A STANDARD DETECTION METHOD FOR RECOVERING *GIARDIA* SPP. CYSTS FROM SOIL SAMPLES

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The soil is an important vehicle for the transmission of the protozoan *Giardia duodenalis*, which causes giardiasis, a gastroenteric disease of variable severity. The objective of this study was to propose the development of a standard detection method for recovery of *Giardia* spp. cysts from

soil samples. The following variables and treatments were evaluated: vortex Homogenization for 2 minutes, magnetic stirrer for 10 minutes, manual shaking for 5 minutes, homogenization in rotary mixer for 30 minutes; Dispersion Solutions such as tween 40, 1M glycine, 0.1M tetrasodiumPPi and 1% ICN 7X; Purifying Solutions such as saturated sodium chloride, sucrose, polyethylene glycol 60% and immunomagnetic separation. Four replicates were performed for each assay in 10g of artificially contaminated soil with a known number of *Giardia duodenalis* cysts (103 – 5 x 103). For Homogenization, the highest recovery was achieved with manual shaking (44.2% for 103 cysts and 49.6% for 5 x 102) and with rotary mixer homogenization (54% for 103 cysts and 70.4% for 5 x 102). Duncan's Multiple Range Test showed no significant differences between both protocols. Rotary mixer was considered the best homogenization method due to greater replicates reproducibility. Regarding Dispersion Solutions, the treatments composed by 1% of Tween 40 (69.4 % for 103 cysts and 28% for 5 x 102), 1% ICN 7X (33.2% for 103 cysts and 37.6% for 5 x 102) and 1M glycine solution (46.4% for 103 cysts and 23.2% for 5 x 102) presented the highest recovery rates. The ICN 7X was considered the best dispersion solution, because presented high recovery rates for both parasites concentrations. For Purifying Solutions, the immunomagnetic separation and the sucrose solution, were the only ones that presented expressive recovery rates (33.2% for 103 cysts and 37.6% for 5 x 102) and (1.6% for 103 cysts and 1.2% for 5 x 102) respectively. Therefore, the standard methodology consisted in 10 g moisten soil with ICN 7X homogenized in rotary mixer for 30 minutes. The samples are left to rest for 5 minutes in order to allow sedimentation of coarser particles and their separation from finest particles and the supernatant. These last two ones are centrifuged at 1050 x g for 10 minutes and submitted to immunomagnetic separation followed by direct immunofluorescence assay. The usage of this effective methodology may help a better understanding of giardiasis distribution through the environment and its control.

Keywords: Detection, Soil, *Giardia* spp., Giardiasis.

SY01.P.02

ENVIRONMENTAL DISTRIBUTION OF *CRYPTOSPORIDIUM* AND *GIARDIA DUODENALIS* IN ESTUARINE AND SHELLFISH HARVESTING AREAS FROM BRAZIL

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Cryptosporidiosis and giardiasis represent an important public health problem in developing and developed countries. In South America, data concerning its distribution and molecular epidemiology in estuarine areas and in shellfish are scarce. This research aimed to evaluate the contamination of *Cryptosporidium* oocysts and *Giardia* cysts in seawater, brackish and river waters and oysters from three estuaries of Southern and Southeastern areas of Brazil and perform molecular characterization of *Giardia*. The first estuarine area (E1), in north coast of São Paulo state harbors bivalves that live at the confluence Point of Ipiranga River and seawater. The second estuarine area (E2) in South coast of São Paulo is an oyster production area, which uses the UV depuration procedure prior to human consumption. In the third estuarine area (E3) located in Santa Catarina state two sites were analyzed: site one, a sporadic oyster's cultivation area and site two, a heavily polluted estuary. The following samples were harvested: E1: 20 water samples (10 for river / 10 for seawater), 10 oysters' samples; E2: 12 water samples (filtered water followed by UV), 11 oyster's samples, collected only after depuration; E3: two water and oysters samples from each site once. The membrane filtration was used for protozoa concentration from water samples. Searching for protozoa were performed in oysters tissues: E1: gills and gastrointestinal tract previously homogenized; E2 and E3: innerwater from each animal and a gill wash pool per dozen of bivalves. Analysis consisted of immunomagnetic separation followed by direct immunofluorescence assay for both protozoa and molecular detection - PCR gene amplification,

Nested PCR to amplify the glutamate dehydrogenase gene and sequencing reactions for *Giardia*. *Cryptosporidium oocysts* were detected in 10% and 40% of river and seawater from E1 respectively and *Giardia* in 60% of river and 20% of seawater samples. Both protozoa were found once in oysters. Molecular assays have shown that all samples were contaminated by *Giardia duodenalis* (assemblage D). In E2, two samples of water were positive for oocysts and three for cysts. *Giardia duodenalis* was found in 54.5% of depurated oysters. In E3, oocysts were detected in gill wash pool from oysters (site one) and cysts in water of site two (genotype A). These results reflect the widespread distribution of both protozoa through Brazilian coastal areas and they denote that the contamination by both zoonotic protozoa have human and animal fecal origin.
Keywords: Cryptosporidium, *Giardia duodenalis*, Estuary, PCR.

SY01.P.03

CLINICAL AND EPIDEMIOLOGICAL SURVEY OF GIARDIOSIS IN THE CLINICAL HOSPITAL OF INFECTIOUS DISEASES BETWEEN 2006 AND 2010

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Giardiasis, produced by *Giardia* spp., forms a parasitosis of great epidemiological and clinical importance due to its high prevalence and pathogenicity amongst animals as well as amongst humans, especially within infant population. Giardiasis is a digestive zoonosis, produced by *Giardia* type protozoan, most commonly involved species being *Giardia duodenalis*. The present study has been carried out between 01st January 2006 and 31st December 2010, at the Infectious Diseases Clinic of Oradea, recorded cases being part of a retrospective study, based on medical documentation (observation sheets). A number of 556 of patients diagnosed with giardiasis have been studied from the total of 22.804 interned at the Clinic of Infectious Diseases of Oradea, Bihor County, during the mentioned period. During the study period, incidence of *Giardia* spp. infection has been 2.44% and annual prevalence of giardiasis has fluctuated (3.43% - 0.95%). Evolution of *Giardia* spp. prevalence amongst the interned patients during the 5 year period has had a decreasing trend (from 3.43% to 0.95%), the highest value being reached in 2006 (3.39%) and in 2007 (3.43%), and the lowest in 2010 (0.95%). During the 5 years period over 20% of cases have been recorded in February and July (10.25%, namely 10.43%). During the given period of 5 years, over 60% of the *Giardia* spp. infected patients have been women (59.7%), women/men ration being: 1.5:1. Along the study this ratio has been between 1.2:1 in 2009 (54% versus 46 %) and 1.7:1 in 2006 (63.1% versus 36.9%). 61.2% of cases are originate from urban environment, resulting an urban/rural ratio of 1.6:1. This ratio takes values between 1.2:1 in 2009 (54% versus 46%) and 1.9:1 in 2006 (65% and 35%). The most frequent biliary-digestive symptoms have been: abdominal pain, especially diffuse, followed by loss of apetite and pain on palpation, especially in the right hypochondrium. Amongst neuro-physical symptoms, on giardiasis affected patients, most frequent has been asthenia, followed by headaches, dizziness, restlessness. Nettle rash has been the most common allergic symptom, especially on children less than 1 year old.

SY01.P.04

GIARDIA LAMBLIA: CORRELATION BETWEEN SUB-ASSEMBLAGES AND SYMPTOMS

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In this study we aimed at determining the genetic variability of *Giardia lamblia* isolated from Romanian human patients. Multilocus genotyping was used as an investigating tool for *Giardia* regarding the relationship between different subtypes detected and some clinical symptoms. Fecal samples from 80 patients (68% children) were diagnosed with *Giardia lamblia* by light microscopy and ELISA for the antigen detection, and then they were subjected to DNA extraction. Genotyping of DNA isolates was performed by PCR-RFLP and sequencing analyses using two molecular markers: glutamate dehydrogenase (*gdh*), and triose phosphate isomerase (*tpi*). By combining the two typing systems, we identified 19 patients infected with assemblage subgroup AII, 1 with assemblage subgroup AI, 14 with assemblage subgroup BIII, 9 with assemblage subgroup BIV, and 38 with mixed assemblage subgroups AII + BIII. Diarrhea was observed only in infections or co-infections with assemblage B subgroups and it was frequently associated with high parasitic loads. 77% of patients with co-infection (AII + BIII) tended to have a slightly longer duration of diarrhea and showed chronic fatigue, loss of appetite, epigastric pain, nausea more significant than in patients infected with one assemblage of *Giardia lamblia*. Assemblage A subgroup -II was predominantly found in asymptomatic patients. Assemblage B subgroups (BIII and BIV) compared to subgroup AII showed a relatively high pathogencity. Weight loss was substantial in patients infected with assemblage B subgroups. Assemblage B subgroup -IV showed a higher virulence than BIII and it is often associated with a syndrome of severe dehydration induced by the large number of vomiting and diarrhea. This study shows that assemblage A subgroup -II has relatively low virulence and it is often associated with asymptomatic infections. Assemblage B alone or dual infection with both assemblage A and B was more common in patients expressing a diarrheal syndrome. Multilocus genotyping for detection of assemblage subgroups of *Giardia lamblia* can be a good approach to public health, mainly in outbreak situations.

SY21.P.01

MOLECULAR CHARACTERIZATION OF *ANISAKIS* LARVAE FROM MARINE FISH CAUGHT OFF SICILY

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Larval forms of the genus *Anisakis* are parasitic nematodes transmitted by marine fish and cephalopods: the accidental consumption of infected raw or poorly cooked fish may cause a clinical disease in humans (anisakiasis). The accurate identification of anisakid nematodes at any life cycle stage is important both to deepen the knowledge on their taxonomy, ecology and epidemiology and for diagnosis and control. The aim of the present work was to investigate, using RFLP genetic, the occurrence, species variability and host preferences of *Anisakis* spp larvae in commercial fish. The fish species, collected from marine fishes at different sites off the Sicilian coasts, comprised *Engraulis encrasicolus*, *Lepidopus caudatus*, *Scomber scombrus*, *Merluccius merluccius*, *Trachurus trachurus, Lophius piscatorius.*

A total of 250 *Anisakis* larvae, recognised as belonging to Type I and Type II larvae (*sensu* Berland 1961), were identified by PCR-RFLP of the ITS region (ITS-1, ITS-2 and the 5.8S subunit) using a previously established molecular key (D'Amelio et al 2000; Pontes et al 2005). The larvae were identified as belonging to *A. pegreffii*, *A. simplex* s. str. (Type I) and *A. physeteris* (Type II). Specimens with a heterozygote restriction pattern between *A. pegreffii* and *A. simplex* s.str with *Hinf*I digestion, were also observed and analyzed by sequencing of the entire ITS region, to confirm the presence of both nucleotides (C/T) in the correct position (Abollo et al. 2003).
Anisakis pegreffii was most frequently observed from different sites off the Sicilian coasts. *A. pegreffii* is the dominant species of *Anisakis* in the Mediterranean Sea and it is presently the most important anisakid nematode in several pelagic and demersal fish species because of the occurrence of various dolphin species as definitive hosts. *A. simplex* s.str occurred in *Scomber scombrus* collected from the west and south coasts of Sicily. This specie occurred frequently in *S. scombrus* in the Tunisian coasts (Farjallah et al. 2008). All the larval forms of *Anisakis spp* identified as *A. physeteris* (definitive host: sperm whale) were collected from teleost fish in the Ionian Sea from the Strait of Messina, an important site of migration of Cetacea, inclused *Physeter macrocephalus.* In the present work new records of the presence of the heterozygote genotype are reported: this pattern was previously identified in larval nematodes collected off the Iberian, Tunisian, Moroccan and Mauritanian waters (Abollo et al. 2003; Farjallah et al. 2008) and more recently in Sardinia waters (Meloni et al. 2011).

SY21.P.02

ANISAKID INFECTIONS IN 8 SPECIES OF LANTERN FISH (MYCTOPHIDAE) FROM THE WESTERN MEDITERRANEAN

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Lantern fishes of the family *Myctophidae* are a major component of the oceanic nekton community in the meso- and upper bathypelagic zones. Most of these species undergo daily vertical migrations to feed on zooplankton and are important prey, not only for piscivorous fish and squid, but also for cetaceans and oceanic birds. Parasitological surveys on myctophids in the Atlantic suggest that some species can play a significant role in the life cycle of *Anisakis* spp. However, quantitative information about the role of myctophids as intermediate / paratenic hosts of heminth parasites in the Mediterranean Sea is not available. We examined for parasites 726 individuals of 8 species of Myctophidae collected in the western Mediterranean, i.e. *Ceratoscopelus maderensis* (n= 388), *Lampanyctus crocodilus* (n= 121), *Notoscopelus elongatus* (n= 112), *Benthosema glaciale* (n= 70), *Myctophum punctatum* (n= 14), *Lobianchia dofleini* (n= 9), *Diaphus holti* (n= 8) and *Hygophum benoiti* (n= 4). Sampling was carried out on the continental slope of the Spanish Mediterranean from Valencia to Málaga, within a range 320 – 800 m depth during November, 2010, and November and May, 2011. Based on morphological traits, L3 of two anisakid taxa were identified. Individuals of *Contracaecum* sp. were found in *N. elongatus* (Prevalence [95% Confidence Interval]): 16.1% [10.1-24.0%] and *L. crocodilus* (1.7 [0.3-6.0]). Single specimens of *Anisakis* sp. were found only in *N. elongatus* (7.1% [3.4-13.7%]). There was statistical evidence that infections of both anisakids in *N. elongatus* were significantly higher than those from the other three myctophid species with n>50 individuals (p<0.025 in all comparisons). Substantial differences in infection levels of anisakids had also been observed between myctophids from the Central Atlantic. In our system, it is unlikely that these differences are accounted for by host specificity: both *L. crocodilus* and *N. elongatus* could have more opportunities for parasite accumulation just because of their larger size (mean total length (TL): 13.1 and 12.1 cm, respectively, compared with a mean TL from 4.15 to 6.4 cm in the remaining species). Our data suggest that myctophids could act as an ecological bridge to infect oceanic cetaceans (in the case of *Anisakis*) and birds (in the case of *Contracaeum*) in the Spanish Mediterranean. Infection levels

of *Anisakis* sp. were remarkably higher in some myctophid species from the Central Atlantic, but no infections with *Contracaecum* spp. were reported there.

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SY21.P.03

DISENTANGLING THE TAXONOMY AND GEOGRAPHY OF *LEPEOPHTHEIRUS* SPECIES OF LITTORAL FISH SPECIES FROM SOUTH-EASTERN PACIFIC COAST

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Caligid parasites (*Caligus* spp. and Lepeohtheirus spp.) present a wide range of hosts. Along the Chilean coasts, 21 species belonging to *Caligidae* family have been recorded. Of these, 11 species belong to genus Caligus and 10 species belong to genus Lepeophtheirus: *L. chilensis* Wilson, 1905; *L. dissimulatus* Wilson, 1905; *L. zbigniewi* Castro & Baeza, 1981; *L. frecuens* Casto & Baeza, 1984; *L. mugiloides* Villalba & Durán, 1985; *L. edwardsi* Wilson, 1905; *L. yañezi* Stuardo & Fagetti, 1961; *L. selkirki* Atria, 1969; *L. interitus* Villalba & Durán, 1985; *L. nordmanni* Milne-Edwards, 1840; the last four species were described of oceanic fish species. *L. edwardsi* has only been recorded in fish of family Paralichthydae and *L. yañezi* in fish of family Ophidiidae, inhabiting deep waters. The resting five species have been recorded on several littoral fish species. *L. chilensis* has been reported on several fish families from 24º S to 39º S; *L. frecuens* has been reported on several fish species from northern coast (24ºS), whereas *L. mugiloides* has been recorded on two fish species from south and central Chilean coast (30ºS- 44ºS). Therefore, it is thought that some *Lepeophtheirus* species are distributed only in the northern coast, whereas others are distributed only in the central and southern coast. In this study, the taxonomy and geography of *Lepeophtheirus* spp. infesting several littoral fish species from different latitudes along the south-eastern Pacific coast are evaluated using molecular analyses (18S rRNA gene and 28S gene, respectively). Since March, 2010 to January 2012, 200 fish from nine fish species were captured from different latitudes and examined for parasites. The recovered copepods were sorted, counted, and identified following to Stuardo & Fagetti (1961); Castro & Baeza (1981, 1984); Villalba & Durán (1985). Sequences were edited using ProSeq v 3.0 beta and aligned with Clustal 2. The similitude tree reconstruction for each gene was done with Mega 5 using algorithm Neighbor-Joining (NJ) and Maximum Likelihood (ML). The nodes were statistically evaluated by 1000 bootstrap resamplings. As outgroups species, we used *Caligus spp.* obtained from the Genbank database. Molecular analyses showed that *L. frecuens* and *L. mugiloides* are present along the entire Chilean coast (20ºS-45ºS).

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SY21.P.04

FIRST RECORD OF GENUS *MACVICARIA* (DIGENEA: OPECOELIDAE) IN FISH FROM CHILEAN COAST

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Macvicaria Gibson & Bray, 1982 is a large genus of marine opecoelids that are widely distributed around the world. This genus was erected by Gibson & Bray (1982) for marine species of *Plagioporus* Stafford, 1904 (sensu lato), with a ventro-lateral genital pore and an excretory vesicle reaching at least to the level of the anterior testis. The other main diagnostic features of Macvicaria are an unlobed ovary, relatively large eggs and fields of vitelline follicles that extend into the forebody and are confluent dorsally and ventrally in post-testicular area and usually dorsally in the forebody. In a study that evaluated the changes of the parasites composition of parasites assemblages of serranid Paralabrax humeralis (n=124) from four sites in northern Chile, we found a digenean opecoelid morphologically different from those previously reported in Serranids from Chilean coast. The prevalence of this species reached up to 21.88% in one of the four localities sampled. In this work, we present a preliminary advance of morphological and genetic identification of this species. Morphological identification was based on criteria given by Cribb (2005) and Gibson et al. (2005). Additionally, we used molecular markers (mitochondrial COI gene and V4 region of the 18S rRNA gene), and the sequences of this species were compared with sequences published in Genbank. The main morphological characters were: cirrus sac in position pre-acetabulum, pharynx large and very muscular, blind caeca and previously bifurcated, the excretory vesicle extending well anteriorly beyond the posterior testis, the genital pore sinistral in the mid-forebody, the ovary entire and two testis in tandem and the vitellines follicles entering the forebody and extending posteriorly beyond the testes to the posterior end of the body. The molecular analyses show that this Opecoelid is grouped together with other species belonging to Plagioporinae subfamily. Considering that this is the first report of one member of this genus in fishes from the Chilean coast, it is very likely that this species be one new species.

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SY21.P.05

FIRST REPORT OF ADULT OF GENUS *NEOBOTHRIOCEPHALUS* (CESTODA: BOTHRIOCEPHALIDEA) PARASITIZING *SEBASTES OCULATUS* IN THE COAST OF NORTHERN CHILE

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In the Pacific Ocean, the genus *Neobothriocephalus* is commonly reported in pelagic fish *Seriolella* spp., where they reach the adult stage. In the Chilean coast, *Neobothriocephalus* has been reported in *Seriolella violacea* with a 100% of prevalence, and also it has been reported in *Hippoglossina macrops*, a demersal fish with a 6% of prevalence. Previous studies of parasites, carried out in littoral fish *Sebastes oculatus* from Chilean coast, had never recorded adults of Cestoda. However, in a recent sampling of fish from the northern Chilean coast, 47 specimens of *S. oculatus* were examined during two consecutive summers (2010-2011) in two localities from northern Chile, recording a prevalence of 2.17% of cestod adults. Morphological identification was

made following to Khalil et al. (1994) and Kuchta et al. (2008). Additionally, molecular markers: 28S gene (Brabec et al., 2006) and V4 region of the 18S rRNA (Hall et al., 1999) were used and the sequences were compared with those recorded in genbank. Morphology of these cestods were concordant with genus *Neobothriocephalus* (Family Echinophallidae), characterized by absence of segmentation along the mid-line of the strobila, strobila with craspedote segments with enlarged posterolateral margin, lanceolate unarmed scolex, shallow bothria, absent neck, testes in the two lateral fields that confluent posteriorly, structure of the cirrus-sac (large thick-walled and oriented obliquely, with conspicuous spherical swelling in the proximal part near the anterior margin of the segment, surrounded by gland-cells) with an unarmed cirrus, sublateral genital pore, reniform and lobulated ovary. Vagina posterior to the cirrus-sac, with a ring-like sphincter. Vitelline follicles largely cortical, with some follicles entering the medulla, forming two lateral fields separated medially, not reaching the lateral margins of segments. Sinuous uterine duct with an elongation of the gravid segments. Uterus oval to spherical with a submedian uterine pore. Operculate and unembryonated eggs. The molecular analyses showed that this tapeworm is clustering together with other species belonging to the Echinophallidae and Bothriocephalidae families.

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SY21.P.06

PARASITES OF FISH IN KAZAKHSTAN IRTYSH RIVER AREA

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Taxanomic parasites variety of the Irtysh river basin fishes within the limits of Kazakhstan is submitted by 8 types, 11 classes, 25 groups, 44 families, 68 genus, 143 species, including Protozoa - 38 species, Monogenea - 34, Cestoda - 16, Trematoda - 33, Nematoda - 10, Acanthocephala - 3, Hyrudinea - 3, Crustacea- 6. Parasites with a direct development cycle make 60,1% (86 species) of the total fauna, parasites with a complex cycle – 39.9% (57 species). Endoparasites make 57.3% of the whole parasitofauna (82 species), and the quantity of parasites with strip localizations is twice bigger than parasites (64.6%), with fabric one. The main factors of parasites societies' formation are adaptations to the environment conditions and host organism with living in other parasites and simbionts. There are main principles of parasites societies' formation, which are compartmentalization, vikariat interaction or replacement, compensation, system, integrity, resources usage to the definite minimum, which defines the society stability. The quantitative and qualitative helminthofauna structure depends on a reservoir mode. Large lakes with the big amount of different rivers flowing in (including the Zaisan Lake, where 62 parasites were defined) differ by greater specific variety. The parasitofauna of such lakes is special and is formed by parasites of those fishes which get into lake from the rivers running into it and form their own community. In particular, there were 37 parasites species found out in the Irtysh River. In the rivers, especially with the fast flow, pauperization of trematoda species and low parameters of contamination by them in view of the abundance mollusks reduction are marked. The repeated increase in parameters of fish contamination by various larval forms in reservoirs with anthropogenous influence is a signal of influence of the factor resulting in parasites concentration, and this is always a result of balance shift on one of the trophic circuit part. Componential fish parasites communities in reservoirs with anthropogenous influence are usually richer in specific structure; therefore domination of separate parasites kinds is reduced.

SY21.P.07

STUDY OF THE SPECIES COMPOSITION OF FISH PARASITES OF SEVAN LAKE

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The high-mountain Lake Sevan is the largest in Caucasus and has very important economic value. The helminthological investigations of the fish of Sevan Lake were carried out since the beginning of the 20-th century. But the lake was under the influence of anthropogenous factors for several decades. In relation to this, the study of the parasite fauna of aboriginal and introduced species of fish of Lake Sevan is of scientific interest.

The objective of this research was the detection of fauna of fish parasites of Sevan Lake, the study of the species composition of fish parasites under the influence of anthropogenic factors on the lake ecosystem.

110 samples of 4 fish species from Sevan Lake have been studied in 2011: *Carassius auratus gibelio* Bloch, 1782, *Capoeta capoeta sevangi* Filippi, 1865, *Barbus goktschaicus* Kessler, 1877 (Cyprinydae) and *Coregonus lavaretus* Linnaeus, 1758 (Coregonidae). The collections of the fish parasites served as material for the research. The parasites were detected in the abdomen, intestine, heart, crystalline lenses, gills and fins of the fish. The handling with the parasites is carried out by common methods. The identification of the parasites has been conducted by "Determiner of the parasites of freshwater fish of USSR fauna" (1984, 1985, 1987) and by A. Shigin (1986).

The research has shown quite high level of fish invasion by parasites – 45% of fish were invased. The total number of 12 species of fish parasites identified. They are: *Monogenea* - 1 (*Dactylogyrus goktschaicus), Trematoda* – 7 *(Diplostomum spathaceum, D. paraspathaceum, D. rutili, D. paracaudum, D.gobiorum, D.mergi, Ichthyocotylurus erraticus), Cestoda* – 1 *(Ligula intestinalis), Nematoda* – 2 *(Rhabdochona fortunatovi, Rh. macrostoma), Crustacea* – 1 *(Tracheliastes* sp*.).* The extensiveness and intensity of invasion by parasites have been revealed.

Most spread fish parasites of Sevan Lake are the biohelminthes - metacercariae of trematodes of the *Genus Diplostomum* and cestode *Ligula intestinalis.* They were detected among all the species of fish (except of *Coregonus lavaretus).*

The fauna of parasites of fishes of the lake is dominated by parasites with complex life cycle – ten species, and two species - with direct cycle (*Dactylogyrus goktschaicus* and *Tracheliastes sp*.). It is mainly composed of endoparasites – 11 species, and 1 – ectoparasite (*Tracheliastes* sp.). 8 species of fish parasites are generalists (except *Dactylogyrus goktschaicus, Rh. fortunatovi, Rh. macrostoma* and *Tracheliastes* sp*.).*

Most part of the fauna of fish parasites are allogenic species, reaching sexual maturity beyond the aquatic environment, in birds (trematodes of *Genus Diplostomum, Ichthyocotylurus erraticus, Ligula intestinalis*), a smaller part - autogenous species (*Dactylogyrus goktschaicus, Rh. fortunatovi, Rh. macrostoma* and *Tracheliastes* sp*.).*

The parasite fauna of fish of Sevan Lake does not have a rich diversity, as well as the species composition of ichthyofauna. Due to anthropogenic changes that took place in the lake during the last decades, the fauna of parasites became poorer.

The changes in the ecosystem of the lake led to a reduction in the number of some species (trematodes of *G. Diplostomum* and *I. erraticus*), as well as a change of hosts. In recent years there has been a tendency to change the species composition of fish parasites in the lake.

SY21.P.08

MOLECULAR AND MORPHOLOGICAL EVIDENCE FOR A CRYPTIC SPECIES OF THE *RHABDIAS BUFONIS* (HARTWICH, 1972) S.L. SPECIES COMPLEX (NEMATODA: RHABDIASIDAE) FROM THE GREEN FROGS OF *RANA ESCULENTA* SPECIES COMPLEX IN ITALY, AND GENETIC DIFFERENTIATION FROM ITS CONGENERS IN FROGS AND TOADS

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The nematodes of the genus *Rhabdias* Stiles & Hassal, 1905 are common lung parasites of amphibian and reptile species throughout the world. The molecular/genetic approach recently applied to nematodes of this genus has revealed that several parasite species are indeed complexes of morphologically similar species. The resolution of some of the taxonomic issues relating to *Rhabdias* has been achieved by a parallel analysis of morphological traits and genetic characterisation of these nematodes collected from various localities of Italy. Several specimens of *Rhabdias* collected from the green frogs of the *Rana esculenta* species complex (i.e. *R. lessonae* Camerano, and *R. esculenta* Linnaeus, identified genetically by diagnostic allozyme loci) and common toad *Bufo bufo* Linnaeus, were analysed, based on DNA sequence analysis at multiple loci (i.e. mtDNA *cox-1*, 12S rRNA, ITS-1 and partial ITS-2 regions of the nuclear rDNA) and by morphometrical analysis. A concatenated phylogenetic sequences analysis of multiple genes (combined mtDNA *cox-1* and 12S rRNA) was used in order to maximize the power of the phylogenetic inference. Three different taxa were identified in the survey: a new cryptic species, *Rhabdias* n. sp., *Rh. bufonis* (*sensu* Hartwich, 1972), and *Rh. sphaerocephala* Goodey, 1924. The new taxon, *Rhabdias* n. sp., resulted to be different from the other species of *Rhabdias* previously sequenced and deposited in GenBank. Maximum Parsimony (MP) and Maximum Likelihood (ML) were congruent in depicting *Rhabdias* n. sp. as forming a distinct and highly supported clade from the sympatric species *Rh. bufonis* and *Rh. sphaerocephala*. The morphological differential diagnosis of specimens of *Rhabdias* n. sp. has revealed differences in several characters in comparison with the type-species, *Rh. bufonis.* The results achieved in the present study suggest that *Rh. bufonis* could be a complex of cryptic species. *Rhabdias* n. sp. is genetically closely related to *Rh. bufonis* in all of the phylogenetic trees, even if it is distinct from the lineage formed by specimens of *Rh. bufonis*. This seems to indicate that *Rhabdias* n. sp. represents a sister species of *Rh. bufonis*. This result adds to our knowledge the occurrence of *Rhabdias* spp. in amphibians in Italy and it represents the first genetic/molecular characterization of *Rh. bufonis* (*sensu* Hartwich 1972). The data so far collected appear to indicate a remarkable host-preference of *Rh. esculentarum* for the frogs *R. lessonae* and *R. esculenta*.

SY21.P.09

GASTRIC HELMINTHS IN THE SWORDFISH *XIPHIAS GLADIUS* COLLECTED OFF THE COAST OF CENTRAL-SOUTH OF CHILE

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The swordfish *Xiphias gladius* is a large pelagic migratory fish with a wide distribution around the world. There are several parasitological studies in this species, which have showed that trematodes, nematodes and cestodes are common helminths. However, there are a few records of parasites of this fish collected from the open sea of South American Pacific coasts. Therefore, this

study aims to record and identify the parasites found in the stomach of five individuals of swordfish, collected off the central-south coast of Chile. The stomachs were dissected and parasites were separated and preserved in 10% formalin. A total of 3,936 parasites was found, most of them (n = 3,922) were nematodes (*Hyterothylacium* spp.) and a few (n = 14) were cestodes (*Tentacularia coryphaenae* Bosc, 1797). The nematodes were morphologically similar to species from the genus *Maricostula* Bruce & Canon 1989, which latter was placed within the genus *Hysterothylacium*. These parasitological findings are not new, however, the great abundance, the conditions of parasites (different developmental stages), and the fact that several nematode larvae were attached to muscles and scales of the prey, allow us to propose a potential life cycle of these nematodes. The common prey, recorded in the five swordfish specimens, were fish belonging to the genus *Cubiceps* and the cephalopod *Dosidicus gigas.* The *Cubiceps* species are oceanic fish, distributed from temperate to tropical waters. Particularly this prey had larval nematodes in muscles, even crossing the scales, while the adult nematodes were disperse in the swordfish stomachs. The cephalopods might transmit larval cestode to the swordfish.

SY21.P.10

EUMETAZOAN PARASITE COMMUNITIES OF LABRISOMID FISH FROM CENTRAL CHILE

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Parasite communities, based on load and composition, use to be similar in host species which are phylogenetically related, especially if these hosts are in sympatry. This study compares the parasite communities of four fish species that belong to the family Labrisomidae; *Aucheionchus microcirrhis*, *A. variolosus, A. crinitus* and *Calliclinus geniguttatus*, which are sympatric in the intertidal zone of central Chile (33°S, 71°W). A total of 182 specimens were collected during low tide, between 2006 and 2009, from two close localities of central Chile (33°27' S - 33°29'S). Most fish (77% of the sample) belong to *A. microcirrhis*. No less than 75% of the each fish species sample was parasitized with at least one parasite species. Twenty-one parasite taxa were found in the whole fish sample; three parasite species were common in the four fish; an undetermined leech Piscicolidae gen. sp., the copepod *Holobomolochus chilensis* and the digenean *Helicometrina nimia*. These three parasite species were also the most prevalent or abundant parasites. The greatest parasite load was found in *A. variolosus*, which was associated to the largest body length that this fish species had in comparison to the other fish. In contrast, *C. geniguttatus* had the lowest parasite load and the smallest body length. *A. microcirrhis* and *A. crinitus* had the major percentage of similarity in parasite communities (69.1% and 61.1% of Bray-Curtis similarity, based on abundance and prevalence of the parasite species, respectively), whereas *C. geniguttatus* was only similar in 38.5% in parasite abundance and 15.2% in prevalence in comparison to *Auchenionchus* spp. Therefore, congeneric fish (Auchneionchus) were similar in parasite communities, considering composition and parasite loads, although most of parasite taxa found in these fish have been already recorded in other intertidal fish species. The variation in populations and communities of parasites among labrisomid species could be related to fish body size and stochastic environmental variables.

SY21.P.11

METACERCARIAL INFECTIONS OF FRESHWATER FISHES AT PASAK CHOLASID RESERVIOR, THAILAND

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Trematode infections are important public health problems that have been reported over a wide range of areas in Asia. It is well known that freshwater fishes are not only a major protein source for humans but also common intermediate hosts of many kinds of trematode parasites, such as liver flukes and small intestinal flukes. Humans acquire the infections from eating raw or unproperly cooked freshwater fishes. Cyprinoid fishes have been reported important intermediate host of these trematodes. The present study was, therefore, aimed to determining the status of trematode infections in various species of freshwater fish at Pasak Cholasid Reservoir in Thailand. The fishes were collected at fish markets between April 2001 and July 2011. As a first step, the fishes were sorted according to their characteristics, measured, recorded and identified. Larval stage of trematodes, metacercariae, was examined by compression method. The fish samples were investigated trematode cysts from dorsal fins, pectoral fins, ventral fins, gills and scales. Encysted metacercariae were dissected from the fins and flesh under a stereomicroscope. The metacercariae were identified based on their morphology. Then polymerase chain reaction of internal transcribed spacer I and II were performed. Based on size of PCR products, metacercariae species would be indentified. A total of 385 fishes were classified into 15 species. Nine species were found trematode infections. They were *Barbonymus schwanenfeldii, Hypsibarbus wetmorei, Cyclocheilichthys enoplos, Dangila spilopleura, Epalzeorhynchos frenatus, Heincorhynchus siamensis, Hampala dispar, Puntioplites protozysron* and *Osteochilus hasseltii*. The infection rates were 2.08% (8/385), 8.31% (32/385), 2.60% (10/385), 11.43% (44/385), 4.94% (19/385), 0.78% (3/385), 0.52% (2/385), 3.38% (13/385) and 2.08% (8/385), respectively. Based on morphology and molecular results, the trematodes were categorized into two species, there were *Haplorchis pumilio* and *Centrocestus* sp. Interestingly, *C. enoplos*, which was cyprinoid species having high infection of human intestinal flukes *H. pumilio*.
Keywords: infection, trematode, metacercaria, freshwater fish, Pasak Cholasid Reservior.

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SY21.P.12

ACCUMULATION OF DIVERSE CLONES OF THE DIGENEAN *PROCTOECES CF. LINTONI* IN THE GASTROPODS *FISSURELLA* SPP. IN NORTHERN CHILE

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Digenean trematode shows a complex life cycle, alternating sexual and asexual stages. Sexual stages occur in the definitive host, whereas asexual reproduction occurs in the first intermediate host (FIH normally a mollusk). The completion of the life cycle depend largely on the ability of the short lived and free swimming cercaria to reach the second intermediate host (SIH). Because cercarial stages are the product of asexual reproduction, they are considered as clones with identical genotype. Definitive host can concentrate many isolated parasite genotypes, ensuring genetic variability. Highly mobile SIH can be parasitized by a broad range of cercarial genotypes,

but when FIH and SIH shows reduced motility or are sessile and using the same habitat, we can expect a high accumulation of the same genotypes in the SIH. The keyhole limpet *Fissurella* spp. are host for progenetic *Proctoeces* cf. *lintoni* in Northern Chile. We sampled a total of 29 keyhole limpets from rocky intertidal/subtidal habitat in Isla Santa María, northern Chile. 660 specimens of P. cf. lintoni were collected and genotypified for 9 microsatellite loci. We found that seven of the keyhole limpet contained genetically identical progenetic metacercarie (7 lineages). Metacercariae of the same clone were found in three different keyhole limpets (3 lineages). Only one of eight intertidal keyhole limpet (*F. crassa*) harbored two copies of the same clone, whereas each one of three specimens of *F. cumingi* harbored 2 copies of different lineages. In three occasions two different host specimens shows identical clones (three lineages) and one specimen of the subtidal *F. cumingi* were parasitized by copies of three different clones. Our results confirm that, even relatively sessile second intermediate hosts, can accumulate a high diversity of genotypes. But the presence of clones, in the same host individuals, is more common than indicated in the literature.

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SY21.P.13

THE MOST FREQUENT PARASITIC FISH FAUNA OF YOUNG CARP IN THE CYPRINID FISH PONDS IN SERBIA

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Intensive carp farming increases the possibility of spreading of diseases with different etiology, including parasitoses. All categories of fish are subject to various ilnesses throughout the entire breeding cycle, but young fish are particulary susceptible.
Spreading of numerous parasites is facilitated by any change of abiotic environmental factors, as well as by high density of hatching fish and failure to implement preventive measures. Pathological effects of parasites are based on their mechanical and toxic action or their role as carriers of bacteria or viruses.
During study in 2011, we have examined 559 samples of one-month old carp (0+), yearlings, and two-year old carp, sampled from the 21 fish farms located in different regions of Serbia.
The following parasites were most frequently recorded in young carp: *Trichodina domerguei* was present in 47.04%of the examined fish; *Gyrodactilus sp*. in 30.41%; *Ichtyphthirius multifilis* in 20.39%; *Dactylogyrus sp*. in 19.41%; *Chilodonella cyprini* in 17.17%; *Argulus foliaceus* in 14.31; *Bothriocephalus acheilognathi* in 13.21%; *Caryophyllaeus fimbriceps* in 12.52%; *Diplostomum spathaceum* in 11.27%; *Khawia sinensis* in 7.51%; *Lernea cyprinacea* in 5%; *Thelohanellus nikolskii* in 3.93% and *Ichthyobodo necatrix* in 1.78%.
Pathological effect of infestation depended on the species of parasite determined, physical characteristics and chemical parameters of the water, zoohygienic conditions in ponds, the age category of fish and the intensity of infestation.
Keywords: fish parasites, young carp, cyprinids.

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SY21.P.14

PHENOTYPIC PLASTICITY IN HAPTORAL STRUCTURES OF *LIGOPHORUS CEPHALI* (MONOGENEA: DACTYLOGYRIDAE) ON THE GILLS OF *MUGIL CEPHALUS* (TELEOSTEI: MUGILIDAE) FROM THE ALBUFERA LAKE, SPAIN: A GEOMETRIC MORPHOMETRIC APPROACH

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A geometric morphometric analysis was carried out to assess the morphological variation of shape and size of sclerotized haptoral structures of *Ligophorus cephali*. The aims of this study were (i) to investigate the phenotypic plasticity of the ventral and dorsal anchors according the site selection in the gills, and (ii) examine to what extent the size and shape of these structures covary. We examined 267 *L. cephali* from *Mugil cephalus*, collected in the Albufera, Valencia, Spain. Variation in shape was examined using geometric morphometrics, which is a statistical analysis of shape based on Cartesian coordinates, after separating shape from the overall size, position and orientation of the landmark configurations. The significance of interspecific variation in the shape of haptoral structures was analyzed by a permutational analysis of variance (PERMANOVA) on the matrix of variable shape. A PCA analysis reflected significant visualization shape changes in their attachment apparatus in *L. cephali* with the increase in size and linear change in shape of the selected landmarks. A Canonical Variate Analysis revealed significant morphological differences within *L. cephali* and the relation with gill arch and side of the gill in the dorsal anchor, and with section, gill arch and side of the gill for the ventral anchor. The PERMANOVA showed a significant association between dorsal anchor morphology and gill-arch-section and with respect to ventral anchor the gill-arch interaction in an arch-section-area design was significant. Thus, our results indicate that morphological variation in *L. cephali* is due to differences in shape in the specific site selection and likely are influenced by gill morphology at the site of attachment. Therefore *L. cephali* offers an interesting model to investigate to what extent the phenotypic variability of the attachment organ within species is related to environmental variables or host specificity.

SY21.P.15

FIRST STEPS IN THE SEARCH OF VACCINE CANDIDATES AGAINST FISH ECTOPARASITES: *SPARICOTYLE CHRYSOPHRII* AND *LEPEOPHTHEIRUS SALMONIS*

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Ectoparasitoses are a major problem in European aquaculture, causing mortality and serious economic losses in fish farms. Currently, control of the parasites principally relies on chemical treatments, but this practice comes with several problems (mainly related with environment, welfare and human health). Alternative solutions, such as vaccination or natural oral anti-parasitics, are hence urgently needed. This former strategy however, requires that suitable parasite antigens can be identified and used in the vaccines. In the present study, we show the preliminary results from a search for antigens in two ectoparasites: the monogenean *Sparicotyle chrysophrii*, infecting gilthead seabream in the Mediterranean, and the salmon louse, *Lepeophtheirus salmonis*, infecting Atlantic salmon. Our strategy has been to look for 'weak points' in the digestive system of the parasites. Both parasites are blood feeders and, hence, need mechanisms to protect themselves against harmful substances in the ingested host blood, such as immunoglobulins or complement factors. Using several techniques, including parasite survival and haemolytic assays, and 'phage display' technique, we have detected possible inhibitors of fish immune factors. The 'phage display' technique is used to isolate/characterize those molecules that protect the parasites. The technique

involves the preparation of a parasite protein library displayed on the surface of T7 bacteriophages. The recombinant T7 phages in the phage display library are then tested for binding to fish blood proteins such as complement factors and immunoglobulin. Parasite survival assays showed that both parasites can survive up to 30 hours in diluted fish serum, and the haemolytic assays (fish plasma + rabbit erythrocytes + parasite proteins) showed that parasite proteins are able to inhibit erythrocyte lysis by inhibiting the alternative pathway of the complement system. Using 'phage display', we have identified a putative specific inhibitor in salmon louse, i.e., a parasite protein that binds to complement factor C3. Sequence analysis of this candidate has been completed, and functional analyses are now in progress using: a) *in vivo* RNAi, and b) haemolysis inhibition testing.

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SY21.P.16

SPECIES OF *OSWALDOCRUZIA TRAVASSOS*, 1917 (NEMATODA: MOLINEIDAE) PARASITIZING AMPHIBIANS FROM THE TERRITORY OF UKRAINE

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Nematodes of the genus *Oswaldocruzia* Travassos, 1917 are worldwide distributed intestine parasites of amphibians and reptiles. Presently, more than 80 species are assigned to the genus (Schotthoefer et al., 2009).

Most species of *Oswaldocruzia* from Western Palaearctic are morphologically similar and were often considered as one species *Oswaldocruzia filiformis* Goeze, 1782 parasitizing a wide range of amphibian and reptilian hosts (Anderson, 2000).

Recently five European species were described using the differences in structure of synlophe and spicules: *O. duboisi* and *O. guyetanti* from France and Bulgaria; *O. hispanica* and *O. galeanoae* from Spain; *O. bialata* from Italy and Bulgaria (Ben Slimane et al., 1993; Ben Slimane et al., 1995).

Of 10 *Oswaldocruzia* species known in Europe, 5 were previously reported from Ukraine: *O. filiformis*, *O. ukrainae*, O. *ivanizkii*, *O. fulleborni*, and *O. problematica* (Skrjabin et al., 1954; Ryzhikov et al., 1980).

During the investigation of the helminthological material stored in the collection of the Department of Parasitology of the Institute of Zoology, NAS of Ukraine we found 4 *Oswaldocruzia* species from amphibian hosts: *O. filiformis*, *O. bialata*, *O. duboisi*, *O. ukrainae*, and 2 forms clearly different from all known specis, *O. sp. 1* and *O. sp. 2*. *O. duboisi* and *O. bialata* are first reported from the territory of Ukraine.

All studied species differed by structure of synlophe: number of cuticular crests and shape of cervical alae. Most species had "idiomorphic" spicules and caudal bursa of type II (classification after Durette-Desset, 1985) except for *O. ukrainae* having "non-idiomorphic" spicules and *O. sp. 1* with caudal bursa of type III. Additionally, the latter species had cervical alae similar to those of *O. duboisi* but with more developed crests on dorsal side of each ala, and extra branches on ray 10 of the caudal bursa. *O. sp. 2* had a specific structure of cervical alae consisting of two large crests and one smaller on dorsal side of each ala.

O. duboisi had the widest host range parasitizing *Pelophylax spp*., *Lissotriton vulgaris*, *L. montadoni*, *Triturus criststus*, *Mesotriton alpestris*, *Hyla arborea* (the latter 4 species are new host records). *O. filiformis* was found only in *Bufo bufo*; *O. bialata* parasitized frogs *Rana temporaria* and *R. arvalis*; *O. ukrainae* parasitized *B. viridis*; *O. sp. 1* and *O. sp. 2* were found in material from *Pelobates fuscus* and *H. arborea*, respectively.

O. filiformis has small cervical alae consisted of two increased crests and one small between them; *O. bialata* has huge cervical alae rounded-triangular in shape; *O. duboisi* has cervical alae consisted of one big triangular crest and two small crests on dorsal side of it; *O. ukrainae* hasn't

cervical alae; *O. sp. 1* has cervical alae similar to *O. duboisi* but with more developed crests on dorsal side of each ala; *O. sp. 2* has cervical alae consisted of two increased crests and one small on dorsal side.
Only *O. duboisi* is a polyhostal species. In our opinion observed specificity is an example of ecological fitting since its hosts from the both groups commonly share the same fresh-water habitats (Shcherbak and Shcherban, 1980).

SY21.P.17
METAZOAN PARASITE FAUNA OF VIMBA (*VIMBA VIMBA* L, 1758), COLLECTED FROM FISH LAKES IN LOWER KIZILIRMAK DELTA, TURKEY

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Vimba vimba is distributed in fresh waters and in brackish estuaries of rivers draining to the Caspian Sea, Black Sea and Baltic Sea. However, vimba populations collapsed in the late 20th century in most part of its existed basins due to damming of rivers and dredging of rapids destroyed a large part of the spawning grounds for vimba and the habitats of larvae. In this research study, the presence of metazoan parasites were investigated in 26 vimba (*Vimba vimba* L, 1758) specimens collected from fish lakes in Lower Kızılırmak Delta, a natural conservation area, in Turkey. Standard parasitological investigation methods were used and standard indices of infection (prevalence (%) and mean intensity MI) were applied. The total length of fish was measured and the numerical distributions of parasites at their infection sites were recorded. A total of 13 parasite species including *Dactylogyrus extensus* Mueller & Van Cleave, 1932, *Dactylogyrus chalcalburni* Dogiel & Bychowsky, 1934, *Paradiplozoon homoion* Bychowsky & Nagibina, 1959, *Echinochasmus* sp., *Diplostomum spathaceum* (Rudolphi, 1819), *Tylodelphys clavata* (Nordmann, 1832), *Tetracoctyle* sp., *Neascus* type metacercaria, *Bothriocephalus acheilognathi* Yamaguti, 1934, *Neoechinorhynchus* sp., *Spiroxys contortus* (Rudolphi, 1819), *Contraceacum* sp., *Eustrongylides* sp. were identified. The overall infection prevalence (%) and mean intensity were 84.62% and 33.50 ± 8.68 per infected fish, respectively. *Tylodelphys clavata* had maximum infection prevalence (50%) and mean intensity values (45.23 ± 12.830 per infected fish). Photomicrographs of all identified parasites are provided and the infection data were illustrated in tables and figures. This parasitological investigation study made contribution on the parasite fauna of rarely studied fish species that is under the pressure of extinction.
Keywords: vimba, *Vimba vimba*, parasite, prevalence, mean intensity.

SY23.P.18
PARASITE FAUNA OF RUDD, *SCARDINIUS ERYTHROPTHALMUS* L., 1758, COLLECTED FROM LOWER KIZILIRMAK DELTA (SAMSUN) IN TURKEY

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Rudd, *Scardinius erythrophthalmus,* is a common bentho-pelagic freshwater fish species, which is widely distributed throughout streams and lakes in Europe, North Caucasia and in the Black Sea Basin. It is abundant especially in Central and Northern Anatolia where inhabited both streams and lake systems in Turkey. In the present study, the parasite fauna of rudd collected from Lower Kızılırmak Delta in Samsun, Turkey was investigated. This delta has an importance for natural wild life and it is a natural protection area. In this research study, 21 fish specimens were investigated for parasites. Standard parasitological investigation methods were used and standard indices of infection (prevalence (%), mean intensity MI, abundance A) were applied. A total of eleven parasite species including one Protozoa, three monogenea, six Digenea and one Nematoda were identified

and they were *Trichodina* sp., *Gyrodactylus* sp., *Dactylogyrus difformis* Wagener, 1857*, Paradiplozoon homoion* Bychowsky & Nagibina, 1959, *Ascocotyle* sp., *Echinochasmus* sp., *Posthodiplostomum* sp., *Diplostomum spathaceum* (Rudolphi, 1819), *Tetracotyle* sp., *Pseudophylodistomum* sp., *Contraceacum* sp. were identified and their photomicrographs are presented. The overall infection prevalence (%), mean intensity MI and abundance A values were 95.24%; 101.50 ± 79.95 per infected fish; 96.67 ± 76.20 per investigated fish, respectively. These values were also determined for each parasite species. *Ascocotyle* sp. + *Echinochasmus* sp. in the gills of fish had maximum infection prevalence (42.86%) and mean intensity values (209.33 ± 175.40 per infected fish). This research study yielded some valuable information about the parasite fauna and their microhabitat distribution in rudd in a natural protection area which has a biological significance with its wild life diversity.

Keywords: rudd, *Scardinius erythropthalmus*, parasite, Kızılırmak Delta, Turkey.

SY23.P.01

DETECTION OF *TOXOCARA CANIS* DNA WITH DIFFERENT PRIMERS USING PHIRE® ANIMAL TISSUE DIRECT PCR KIT

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The objective of this study was the standardization of a direct PCR for amplification of the second internal transcribed spacer (ITS-2) nuclear ribosomal DNA of *Toxocara canis*. Adult *T. canis* and *A. suum* worms were obtained directly from the small intestine of naturally infected dogs or pigs. The adults of *A. lumbricoides* were obtained from feces of children positive to ascariasis and dewormed with piperazine. Eggs and later larvae were obtained from the adult *T. canis* females. Two protocols were carried out. In the first one (direct protocol) a fragment of the adult worms taken with the Harris Uni-CoreTM or 1 µl of *T. canis* larvae homogenate was added to the reaction mix (10 µl of 2X Phire® Animal PCR Buffer, 0.5 M of each primer, 0.4 µl of Phire® Hot Start II DNA Polymerase and 7.8 µl of water). In the second one (dilution protocol), another fragment of the adults or larvae was diluted in 20 µl of buffer solution preheated to 98°C and 0.5 µl of DNA Release Additive for two minutes; 1 µl of the dilution of each worm was added to the reaction mix. For the detection of ITS-2 fragments, primers (F/R) Tcan1/NC-2, YY1/NC-2 and NC-13/NC-2 were tested. The reaction was carried out under the following conditions: initial denaturing at 98°C for 5 min; 30 cycles of denaturing at 98°C for 5 seconds; for alignment three temperatures were tested 55°C, 57°C and 60°C for 5 seconds and an extension at 72°C for 20 seconds. Final extension was at 72°C for 1 minute. PCR products were separated in 2% agarose gels and stained with ethidium bromide, transilluminated, and photographed for their analysis. Amplifications of *T. canis* ITS-2 were obtained in the dilution protocol with the three tested pairs of primers. Primers YY1/NC-2 amplified an expected fragment of 330 bp in larvae and adult samples of *T. canis*. Primer pairs Tcan1/NC-2 and NC-13/NC-2 amplified expected fragments of 380 and 570 bp respectively from samples from the three parasites. The three pairs of primers amplified at 57°C but not at 60°C. In the direct protocol no amplified products were observable. These results show that direct PCR from samples without prior extraction of DNA is useful for the detection of *T. canis* ribosomal DNA.

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SY23.P.02

PCR-RFLP AND SEQUENCING OF ITS REGION FOR THE GENOTYPING OF *ENTEROCYTOZOON BIENEUSI* ISOLATES FROM TUNISIAN HIV-PATIENTS

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Introduction: *Enterocytoon (E.) bieneusi* is the most common species of Microsporidia in humans. It causes chronic diarrhea and wasting in immunocompromised patients especially those infected by human immunodeficiency virus (HIV). Molecular tools based on analysis of internal transcribed spacer (ITS) of rRNA genes have been developed to delineate the transmission of *E. bieneusi*. The aim of the present study was to compare the efficiency of both restriction fragment-length polymorphism (RFLP-PCR) and ITS region sequencing in determining *E. bieneusi* strains genotypes of Tunisian patients.
Methods: Two methods of genotyping, PCR-RFLP method described by Liguory *et al* and ITS region sequencing adapted by Rinder *et al.*, were applied on DNA extracts from seven stool samples of HIV-infected patients, revealing positive for *E. bieneusi* after light microscopy and PCR screening. Identification of *E. bieneusi* was confirmed by PCR using species specific primers V1/EB450.
Results: PCR-RFLP method detected two types namely: type I (n=2) and IV (n=5). However, ITS region sequencing identified three distinct genotypes which were previously described (B, D and Peru 8). The zoonotic genotype D and the anthroponotic genotype B were characterized in 4 and 2 isolates respectively. The genotype Peru 8, which was only reported in a HIV-patient in Peru, was detected in the last isolate.
Conclusion: *Enterocytoon bieneusi* genotypes were identified for the first time in North Africa. PCR-RFLP and ITS sequencing revealed easy to implement in typing and classification *E. bieneusi* strains. ITS sequencing has higher level discrimination power, compared with PCR-RFLP and permits identification of more genotypes. The obtained data also suggest that both anthroponotic and zoonotic route of transmission co-exist in Tunisia.

SY23.P.03

DIAGNOSIS OF LATENT *PNEUMOCYSTIS JIROVECI* IN SPUTUM SAMPLES OF PATIENTS UNDER CHEMOTHERAPY VIA NESTED PCR

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Diagnosis of latent *Pneumocystis jiroveci* in sputum samples of patients under chemotherapy via Nested PCR Introduction: Using of chemotherapy against patients who suffer from malignancies make them susceptible to opportunistic infections. So, early diagnosis of infection and its causative factor lead to proper treatment of these patients. *Pneumocystis jiroveci* is one of the infections among patients who are under chemotherapy. The aim of this study is application of Nested PCR method for prompt diagnosis of *Pneumocystis jiroveci* presence in the pulmonary samples of these patients. Method: Sputum samples collected from 46 patients of (KOSAR chemotherapy clinic) who suffer from cancer and were under chemotherapy treatment. All samples homogenized with 10mM and their DNA extracted. The presence of special gene of *Pneumocystis jiroveci* (mtLSUrRNA) was evaluated with using of Nested PCR method in two phases, first phase

extracted DNA out of sputum samples, and external primers Paz_102E and Paz_102H and then using first round PCR product in the preliminary phase and internal primers of Paz_102E and Paz_L2. Result Among positive control samples of PCR product in a group from 346 bp and 120 bp appeared, and result of PCR of patient sputum samples showed special genus of *Pneumocystis jiroveci* exist in the 58.69% of patient's sputum who were under chemotherapy. Conclusion Chemotherapy suppresses the humeral and cellular immune system and makes the patients susceptible to many kinds of opportunistic infections. *Pneumocystis jiroveci* has a notable prevalence in pulmonary of patients who have a background of chemotherapy and Nested PCR method was recommended to early diagnosis of infections caused by this fungi.

SY23.P.04

INFECTION RATES OF HELMINTHESIS AT THE CATCHMENT AREA OF PASAK CHOLASID DAM UNDER THE ROYAL PROJECT, LOPBURI PROVINCE AND ITS RELATED AREAS, THAILAND

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The water shed development scheme is an extended plan to achieve the objectives of Pasak Cholasid Dam and its related areas under His Majesty's Initiative Project involving a proper water management for consumption all year round. The dam could have some negative effects on public health. The probable important, helminthic diseases are schistosomiasis, liver fluke, lung fluke, small intestinal fluke infections and intestinal helminthesis. Thus surveillance of these parasites is necessary for public health protection. The objective of this study was investigation for helminthes infections of human, who lived in the catchment area of Pasak Cholasid Dam, Central Thailand. The intestinal helminthes (hook worm, *Teania* spp., *Enterobius vermicularis*, *Strongyloides stercoralis*) and other flukes (*Schistosoma mekongi*, *Opisthorchis viverrini*, intestinal flukes infections) were examined between December 2011 and March 2012. A total of 1,094 stool were designed to obtained from the population by using 30 clusters random sampling under WHO guidelines and calculate minimal sample size at 95% confidence by using Yamane Taro (1967). Formalin Ether Concentration Method (Ritchie, 1948) was applied to the fecal samples to identify the infection rates or prevalence of helminthes infections. Statistical data analysis for prevalence used only the percentage or rate of infections. The infection rates of helminthes were 5.6%; they were 2.3% intestinal helminthesis (Hook worm 1.2%, *Teania* spp.0.4%, *Strongyloides stercoralis* 0.6% *Enterobius vermicularis* 0.1%) and 3.3% trematode infections (*Opisthorchis viverrini* 1.9%, small intestinal flukes 1.3% and *Eurytrema pancreaticum* 0.1%). For the microscopic examination, the highest trematode infections was found at Khoksalung district (5.8%), and Opisthorchiasis was the highest infection (5.2%). However, the molecular study should be designed to confirm accuracy of the helminthes species.

SY23.P.05

MOLECULAR DIAGNOSIS OF A CASE OF GASTRIC ANISAKIASIS ASSOCIATED TO *ANISAKIS PEGREFFII* (NEMATODA: ANISAKIDAE)

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Anisakiasis is a fish- borne zoonosis caused by the accidental ingestion of larval nematodes with raw, undercooked or improperly processed (e.g., marinated) parasitized fish and squid: when raw infected marine fish are consumed accidentally by humans, the *Anisakis* larvae migrate to the

digestive tract and potentially invade the gastric/intestinal walls, causing various symptoms. There is a growing report that these parasites are able to produce a strong allergic reaction: this aspect seems to be more evident when a live larva reaches the gastric submucosa (Gastro-Allergic Anisakiasis) (Daschner et al., 2012). In Italy during recent years, several cases of anisakiasis have been reported on the basis of parasitological findings but, recently, the usefulness of molecular markers as diagnostic tools for the identification of human anisakidosis was demonstrated in those cases where the parasite, endoscopically removed, was available. In the present study, a case of gastric allergic anisakiasis in a Italian man who had consumed raw anchovies (*Engraulis encrasicolus*) and molecular identification of the parasite, endoscopically removed and conserved in formalin, is reported.
A 46 year old man presented epigastric pain and nausea after some hours after ingestion of marinated raw anchovies and he had an allergic reaction. During the esophagogastroduodenoscopy (EGDS) a white color worm was detected and extracted from cardia by means of biopsy forceps. After removing the parasite, all symptoms immediately disappeared. The parasite removed was fixed in formalin and sent for the morphological and molecular identification. The nematode was classified as a L3 larva type I of the genus *Anisakis* by a light microscope. The molecular identification, performed by means of DNA sequencing of mito chondrial (mtDNA *cox2* and *rrnS*) and nuclear (ITS1-ITS2 of the rDNA) genes (S. Mattiucci, 2011), allowed the identification as *A. pegreffii*.
Development of molecular tools for the diagnosis of human anisakiasis has resulted with an increase in the frequency of the reports in many parts of the world and the accurate identification has important diagnostic as well as epidemiological implication. In Italy only three human cases have been identified by molecular methods (PCR-RFLP analysis of the ITS region or sequences analysis of the mtDNA *cox2* gene) as *A. pegreffi*: in these cases the larvae, removed from the stomach by endoscopy, were fresh fragments or preserved in ethyl alcohol.
A. pegreffi is the most common species detected in fish from the Mediterranean Sea and is a zoonotic agent of a human anisakiasis in Italy.

SY23.P.06
LETHAL *PNEUMOCYSTIS JIROVECI* PNEUMONIA 24 YEARS AFTER KIDNEY TRANSPLANTATION: A CASE STUDY

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Pneumocystis jiroveci is an opportunistic infections fungus among immunosuppresses patients particularly AIDS patients. *Pneumocystis jiroveci* is attached to type 1 pneumocytes of the lung. This causes disorder in oxygen exchange resulting in progressive shortness of breath and death if left untreated The use of immunosuppressive drugs especially corticosteroids predisposes the transplanted patients to a variety of infectious diseases including *Pneumocystis*. In many developed countries, the incidence of *Pneumocystis jiroveci* pneumonia (PCP) is dwindling in transplant patients receiving appropriate prophylaxis. In this review, definitive diagnosis of *Pneumocystis* in a patient receiving the kidney transplant is presented.
Case Presentation: The patient was a 45 year old man with a history of kidney transplantation 24 years ago who was admitted to a specialized hospital in Tehran due to fever and respiratory distress. Upon admission, the patient showed symptoms of impaired consciousness and shortness of breath. Paraclinical tests and complementary examinations confirmed the definitive diagnosis of *Pneumocystis* using imaging techniques, microscopic observation and molecular analysis. Specific treatment with trimethoprim/sulfamethoxazole was carried out alongside other therapeutic measures, but unfortunately the patient did not respond to specific treatment and died in the course of a progressive disease.

The above review suggests that despite 24 years of transplant, the risk of opportunistic infections in transplant patients is still there. Therefore, considering the likelihood of opportunistic infections in these patients is critical. The disease progress in these patients can still be fast and deadly. The use of rapid molecular diagnostic techniques in order to start appropriate and timely treatment is essential. Launching these diagnostic methods is recommended in our country.

SY23.P.07

COMPARISON OF MICROSCOPY, CULTURE AND DNA BASED METHODS TO DETECT *BLASTOCYSTIS* SP. IN FECAL SAMPLES

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In this study we compared 3 diagnostic methods for identifying *Blastocystis* sp. in routine stool samples. During one year 150 routine stool samples submitted for parasitological analysis to the reference laboratory of social security organization. All stool samples were examined with direct-light microscopy, culture and conventional PCR. Direct wet mount examination and culture were performed immediately on all fresh fecal samples after arrival at the laboratory. For DNA extraction and further PCR analysis about 200 mg of stool samples was frozen. For culture, approximately 10 mg of each sample was inoculated into house made culture media containing 10% horse serum and 500 µl of penicillin-streptomycin solution and incubated at 37°C for 3–4 days. In microscopy method and culture, diagnosis of *Blastocystis* was made based on morphology of parasite. DNA of all samples was extracted at the same time by using the QIAamp™ DNA Stool Minikit (Qiagen, Hilden, Germany) based on manufacturer's instructions. Polymerase chain reaction for diagnosis *Blastocystis* sp. performed by use of specific primers for the SSU rDNA of *Blastocystis* previously designed for detection of all 10 subtypes of parasite. From 150 samples, 42 (28%) were positive for *Blastocystis* in one or more of the diagnostic techniques. In light microscopy examination, 38 samples (28/3%) were positive for *Blastocystis* and 12 samples showed co-infection with the other parasites such as *Entamoeba histolytica*/dispar, Giardia cyst, *Entamoeba coli* and *H*. *nana* ova. Giardia intestinalis cyst was the most common parasite that found in conjunction with *Blastocystis*. Compared with PCR, the sensitivity of culture and direct wet mount examination was 100% and 90.5% respectively.

SY23.P.08

PREVALENCE AND CLINICAL MANIFESTATION OF NEMATODOSIS IN CHILDREN IN SLOVAKIA

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The aim of this study was to record the occurrence of intestinal nematode infections in hospitalised children in Slovakia and to assess the correlation between the intensity of parasite infections with clinical symptoms, haematological and biochemical abnormalities. A study involved a total of 1800 children (9 months to 16 years old) hospitalised for acute or chronic respiratory and gastrointestinal infections examined by Hein' s method and confirmed the occurrence of *Ascaris lumbricoides* (2.38%), mix infection *Ascaris lumbricoides / Trichuris trichiura* (0.72%) and *Trichuris trichiura* (0.8%) infection only. The mean count of *A. lumbricoides* eggs in positive patients was by McMaster method assessed 1050 eggs per gramme (epg) in range 150-4500 epg. The mean

count of *T. trichiura* eggs was 150 epg (50-250 epg). The highest intensities of *A. lumbricoides* and *T. trichiura* infection and reinfection occurred particularly in children 3-5 years of age living in communities with low hygienic standards.
Clinical signs and symptoms in infected children varied according to the intensity of infections, larval stages and degree of larval migration. Most common clinical conditions in all the patients included anaemia combined with complicated bronchopneumonia, colitis and gastritis. The strongest correlation between the intensity of parasite infection and selected laboratory test data (eosinophil count, haemoglobin, total serum iron) was found in children of 2 years of age (p<0.05) and decreased with age.
In conclusion, our study reported 2.55% rate of infections with a both nematodes in the group of hospitalised children indicating that these infections have not been eradicated in Slovakia.

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SY23.P.09
IDENTIFICATION OF BINDINGS PARTNERS OF CAGA *HELICOBACTER PYLORI* VIRULENCE FACTOR

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Helicobacter pylori is able to colonize more than half of the entire earth polulation. *H. pylori* strains carrying the cagA gene are more virulent than cagA-negative strains and are associated with the development of gastric adenocarcinoma. CagA is injected into the host cell and tyrosine-phosphorylated. In this study we investigate the relationship of cagA with his binding partner in lipid rafts. Rafts are highly structural ordonated lipid domains of host cells membrane able to function as devices or signaling platform and entery sites into the host cells. Elucidation of interaction host – parasites is crucial in order to clarify and develop neu strategy for treatment and prevention of infections. AGS (adenocarcinom gastric cells) were infected with P1 strain of *H. pylori*. The RAFTS were isolated after differential centrifigation in sucrose gradient. The complexes CagA x parteners were isolated using affinity chromatography on Sepharose protein A and G, resolved by SDS-PAGE, in gel digested and supose to MALDI mass spectometric analysis. The following proteins could be identified with higher probability: Host cells proteins: cytoskeletal proteins: annexin, actin, kerratin, filamin FLIP 1 or noncytoskeletal: cAMP dependent protein kinase, HSP 90, mortalin-2, sphingosynkinase 1, metalloproteinase 23B. *Helicobacter pylori* proteins: VirB (that belong to the type four secretion system), NFkB, HspB, methyl transferase, CagA. The founded proteins were in accordance with the expectation that cagA needs for host cells interaction, chaperones like VirB or HspB, that appear necessary for stabilization of cagA structure during translocation. We concluded: (1). cagA is recruted on lipid rafts after infection; (2) cagA interract with proteins implied in his stabilization (chaperone); (3). a lot of host cytoskeletal proteins are implied in cagA binding after infection: annexin, actin, kerratin, filamin FLIP 1; (4). cagA interraction with VirB and HspB prove the important role of these protein in cagA translocation process and structure stabilization.

SY23.P.10

UNUSUAL CRYPTOSPORIDIOSIS CASES IN SWEDEN – *CRYPTOSPORIDIUM VIATORIS* AND *CRYPTOSPORIDIUM* CHIPMUNK GENOTYPE I

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Cryptosporidium spp. are frequent causes of diarrheal disease in both immunocompetent and immunocompromised hosts. Even though most human cases are caused by *C. hominis* or *C. parvum* many other species or genotypes have also been detected in humans.
During a genotyping study we identified two unrelated patients, both infected in Sweden, who carried *Cryptosporidium* chipmunk genotype I. Two other patients who had travelled to Kenya and Guatemala, respectively, were infected with the recently described species – *C. viatoris*. All patients were immunocompetent and suffered from diarrhea. One of the *C. viatoris* patients was also infected with *Giardia intestinalis.*
The *Cryptosporidium* oocysts were identified using acid fast staining and were indistinguishable from other *Cryptosporidium* oocysts usually found in human stool. Initial molecular analysis using PCR-RFLP (SSU rRNA and COWP genes) showed inconsistent results but sequencing of a fragment of the SSU rRNA gene identified *Cryptosporidium* chipmunk genotype I and *C. viatoris*. The isolates were further characterized at the actin and HSP70 loci. While sequences from the two *Cryptosporidium* chipmunk isolates were indistinguishable from each other at those loci, the two *C. viat*ori isolates slightly differed from each other at the repetitive part of the HSP70 gene, suggesting two different subtypes of this species.
Cryptosporidium chipmunk genotype I has been isolated from red squirrel (Italy), chipmunk, eastern grey squirrel and deer mouse (USA) and from a few human cases in Wisconsin and France. *Cryptosporidium viatoris*, which has fairly recently been proposed as a new *Cryptosporidium* species, has previously been reported only from patients who have travelled to India, Nepal or Bangladesh. So far this novel species has not been reported from any animals.
This study shows the importance of molecular analysis, including sequencing, of *Cryptosporidium* isolates both from patients with domestically acquired infections and from returning travelers.

Y23.P.11

MICROSPORIDIA IN IMMUNOCOMPETENT AND IMMUNODEFICIENT PATIENTS IN POLAND

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Microsporidia are opportunistic pathogens responsible for infections in immunocompetent and immunodeficient patients, found frequently in AIDS patients, organ transplant recipient, children and elderly persons. In the present study, investigation on the distribution of two intestinal species *Encepahalitozoon intestinalis* and *Enterocytozoon bieneusi* was performed in the different groups

of hospitalized patients in Central Poland. Fecal samples from 315 patients were tested, including 32 children with Primary Immunodeficiency Diseases [PIDs], 38 children with diarrhea or other intestinal disorders, 146 children - liver transplant recipients, 44 adult patients under medical immunosuppression following transplantation (kidney, liver, or bone marrow transplant recipients) or patients undergoing treatment before the transplantation of bone marrow, and 55 adults with diarrhea and with serious chronic intestinal disorders undergoing steroid therapy (due to colitis ulcerosa, cancers) or diabetics. Microsporidia infections were identified in stool samples by modified trichrom staining procedure and PCR targeting SSU rRNA gene, for the microsporidial species determination. Overall microsporidia were identified in 13 of 315 patients (4.1%). Spores were detected by microscopy in the fecal smears of 10 out of 293 examined persons (3.4%). A species specific PCR detected microsporidia DNA in 2.2% of samples (7/315). Molecular typing confirmed infection of *E. bieneusi* in one patient, liver transplant recipient. Original sequence was deposited in GenBank data base under accession no. JN107808. To our knowledge, this is the first report describing of *E. bieneusi* infection in transplant recipient in Poland. Only four of microsporidia - infected persons displayed gastrointestinal symptoms, such as diarrhea. The prevalence of microsporidia infection was higher in the group of adult transplant recipients in comparison to group of children - liver transplant recipients (18% versus 0.7%, respectively). We have documented that microsporidiosis occurs in immunodeficient persons in Poland and that immunosuppression constitutes risk factor for microsporidian infection. Our findings also show that these infections are rather asymptomatic, not associated with the intestinal disorders.

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SY23.P.12

OCCURRENCE OF INTESTINAL PARASITES AMONG REFUGEE SEEKERS FROM HUMENNÉ REFUGEE CAMP IN SLOVAKIA

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Monitoring of parasitic diseases is very important among immigrants coming from endemic areas.
This study was performed to investigate the presence of causative agents of parasitic diseases among the legal and illegal immigrants in Slovakia in order to protect the infestation of Slovak citizens.
From July to September 2009, in total 213 stool samples of refugees placed in the refugee camp in Humenné (Slovakia) were examined for the presence of intestinal parasites. Each stool was examined with the methods KATO and MIFC.
In total 213 of collected stool samples from two groups of refugees localized in a refugee camp of the Ministry of Interior in Humenné was examined for the presence of intestinal parasites.
The first study group consisted of 109 (104 men and 5 women) asylum seekers coming from 21 different countries of Europe, Asia and Africa. 29 persons (26.6%) were positive for intestinal parasites. Except of the comensals, we diagnosed *Entamoeba histolytica*, *Giardia intestinalis*, *Blastocystis hominis* (14 persons). Eleven persons were posive for Ancylostoma duodenale infestation.
Among 98 Palestinian refugees seekers 24.5% were infested by intestinal parasites.
Except of the protozoan comensals, we diagnosed *Giardia intestinalis* and *Blastocystis hominis* (in 10 persons).
Occurrence of the pathogenic protozoans was quite low in the group of legal immigrants (*G. intestinalis* 1. 0 - 2.9%, *E. histolytica/dispar* 0.7%, *B. hominis* 1.4 - 1.6%).
The prevalence of parasitic protozoans was higher in the illegal immigrant group. *G. intestinalis* occurred in 11.4%, in some groups *E. histolytica/dispar* accounted for 7.7% and *B. hominis* 3.3% respectively.
In our study we focused to investigate the presence of causative agents of intestinal parasitic diseases among the legal and illegal immigrants in Slovakia in order to protect the infestation of parasites to Slovak citizens.

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SY23.P.13

CRYPTOSPORIDIUM SPECIES, GP60 SUBGENOTYPES AND CLINICAL MANIFESTATIONS IN AIDS PATIENTS FROM ROMANIA

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In the present study, we analyzed genetic variation in *Cryptosporidium* species found in patients with AIDS in Romania. To establish whether certain clinical manifestations are associated with *Cryptosporidium* genotypes and subtypes, we analyzed over a period of three years a cohort of 257 patients with human immunodeficiency virus (HIV) and diarrhea syndrome. Fecal samples were analyzed by conventional diagnostic methods (microscopic examination and identification of specific antigens by ELISA). From a total of 21 cases found positive for *Cryptosporidium* infection, 20 were genotyped. Sequence variation was assessed in regions of the small subunit of nuclear rRNA (p-SSU), and the 60-kDa glycoprotein (p-gp60) genes using PCR-RFLP and sequencing analyses. The data obtained by PCR-RFLP showed that 14 patients had an infection with *Cryptosporidium parvum* and 6 with *Cryptosporidium hominis*. Initial correlation between clinical presentation and genotype showed that *Cryptosporidium hominis* is associated with chronic diarrhea, prolonged excretion of oocysts, general malaise, weight loss, and severe dehydration syndrome over 5% (hyponatremia, hypocalcemia and hypokalemia). Instead *Cryptosporidium parvum* was associated with transient diarrhea syndrome limited to a shorter period of time. Molecular analysis of p-gp60 revealed for *Cryptosporidium hominis* subtypes Ia and Ib. The subtype Ia was associated with severe diarrhea (more than 4 unformed stools in 8 h). Ib subtype was also associated with nausea, vomiting and possible risk of biliary extra-intestinal dissemination. Molecular analysis of p-gp60 for *Cryptosporidium parvum* revealed 3 subtypes (IIa, IIb and IId). All subtypes of *Cryptosporidium parvum*, with the exception of IId, were associated with moderate-intensity infections. In conclusion, the clinical manifestations of infection with *Cryptosporidium* in patients with HIV showed a great diversity and range of diversity can be attributed to different *Cryptosporidium* species and subtype families. Due to the need to elucidate the genetic diversity of human isolates *Cryptosporidium,* this approach herein reported may represent a useful tool for correlating different symptoms with subtypes.

SY23.P.14

GEOGRAPHICAL DISTRIBUTION OF *ASCARIS LUMBRICOIDES* AND *TRICHURIS TRICHIURA* IN SERBIA

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Historically, a wide range of intestinal parasites were endemic in Serbia, at high prevalence; today, *Ascaris lumbricoides* and *Trichuris trichiura* are still widespread and therefore a health concern requiring constant surveillance and control of potential risk factors. Geographical Information Systems (GIS) technology is a powerful novel tool to analyze geographical parameters of potential influence for STH distribution. We here re-analyze our earlier research data on the distribution of

Ascaris and *Trichuris* in Serbia using GIS. Cross-sectional copromicroscopic (direct microscopy and the Kato and Lörincz techniques) studies of STH infections in Serbia were carried out over an 11-year period (1985-1995), involving a total of 4,913 asymptomatic school-children (2388 F, 2525 M), 7-11 years of age, representing more than 10% of the total age-matched population (n = 69,232), from 96 settlements within 16 administrative districts throughout central Serbia. Of the 16 districts examined, STH infections were found in all but one (Bor). However, *Ascaris* was found in 11 (68.7%) and *Trichuris* in 13 (81.2%) districts, with an overall prevalence of 3.8% (0.3-12.3%) and 1.8% (0.3-4.7%) respectively, but with a highly significant (p=0.001) geographical heterogeneity. Both parasites were most prevalent in South-Western (Ivanjica, Novi Pazar) and Eastern (Žagubica) Serbia. Of the 96 settlements examined, 40 were STH-free. The individual prevalence rates in the 56 (58.3%) STH-positive settlements varied from a low of 0.5% to a maximum of 37.5%. Moreover, the STH prevalence was above 20% in as many as 10 settlements. The data were mapped using GIS (ARC GIS software version 10.0), and spatial analysis (using the Kriging method) was performed to identify the relative influence of geo-environmental factors (surface temperature, altitude, soil type, and rainfall) on the geographic distribution of STHs. These analyses showed differences in the STH prevalence according to settlement elevation. The elevation of settlements ranged from 44 to 1,440 m above sea level, divided into three strata, namely below 500, from 500 to 1000 and above 1000 m. The prevalence in the respective strata differed significantly; for *Ascaris*, 1.6%, 5.4% and 2.1% (p=0.000); and for *Trichuris*, 1.3%, 2.6% and 0.6% (p=0.050), but interestingly, the prevalence of both parasites was the highest at the middle altitudes. Given the public health significance of STH infections, further GIS-based analysis of other environmental factors may be significant for effective control strategies and monitoring of their implementation.

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