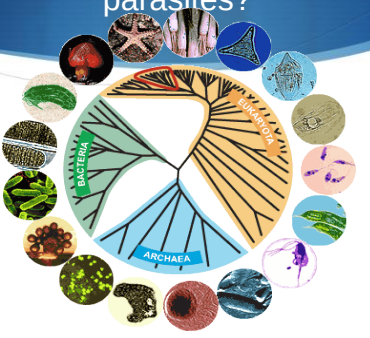
Parasitic infections I.

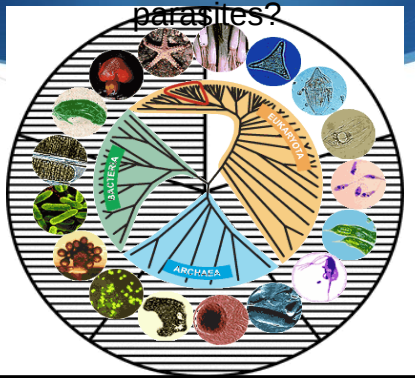
RNDr. et M.Res Lenka Richterová Ph.D.
NRL for diagnosis of tropical parasitological infections

Nemocnice Na Bulovce

What proportion of species are parasites?



What proportion of species are parasites?



World Health Organization

Neglected tropical diseases 2017

12/20

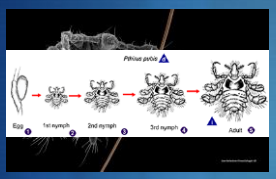
- Buruli ulcer
- Chagas disease
- Dengue and Chikungunya
- Dracunculiasis (guinea-worm disease)
- Echinococcosis
- Foodborne trematodiases
- Human African trypanosomiasis (sleeping sickness)
- Leishmaniasis
- Leprosy (Hansen's disease)
- Lymphatic filariasis
- Mycetoma, chromoblastomycosis and other deep mycoses
- Onchocerciasis (river blindness)
- Rabies
- Scabies and other ectoparasites
- Schistosomiasis
- Soil-transmitted helminthiases
- Snakebite envenoming
- Taeniasis/Cysticercosis
- Trachoma
- Yaws (Endemic treponematoses)

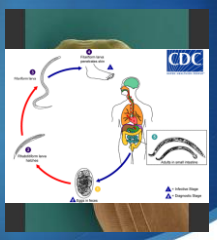
DNDi
Drugs for Neglected Diseases *initiative*

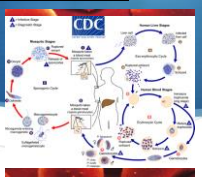
Parasites as seen by medicine

Parasites as seen by medicine

Parasites as seen by medicine



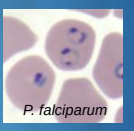




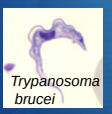
Tropical tissue parasites protozoans


Leishmania sp.


Plasmodium falciparum

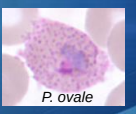

P. falciparum


T. cruzi


Trypanosoma brucei


Babesis sp.


P. malariae


P. ovale

https://www.cdc.gov/dpdx/

Intestinal parasites

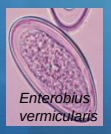

Microsporidia


Cyclospora cayetanensis


Entamoeba


Trichuris spp.


Taenia saginata


Enterobius vermicularis


Cryptosporidium

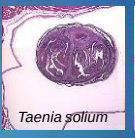

G. intestinalis

https://www.cdc.gov/dpdx/

Tissue helmints


Toxocara sp.

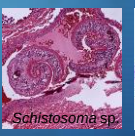

Echinococcus multilocularis


Taenia solium


O. volvulus

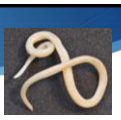

Trichinella sp.

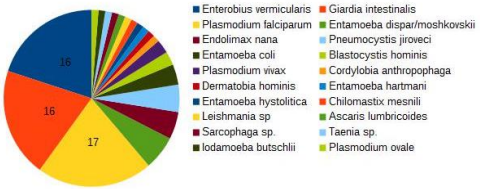

Fasciola hepatica

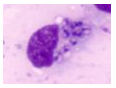

Schistosoma sp.


Paragonimu ssp.

https://www.cdc.gov/dpdx/



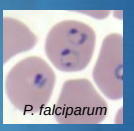




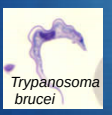
Tropical tissue parasites protozoans


Leishmania sp.


Plasmodium falciparum


P. falciparum


T. cruzi


Trypanosoma brucei


Babesis sp.

P. malariae

P. ovale

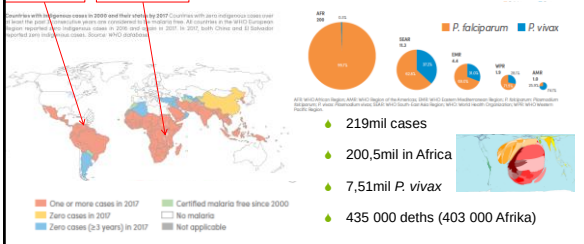
https://www.cdc.gov/dpdx/

World malaria report 2018

2016 for the first time in 20 years number of cases increased!!!

Venezuela 1mil 2018

Burundi 7,2 mil 2019



- 219mil cases
- 200,5mil in Africa
- 7,51mil *P. vivax*
- 435 000 deths (403 000 Afrika)

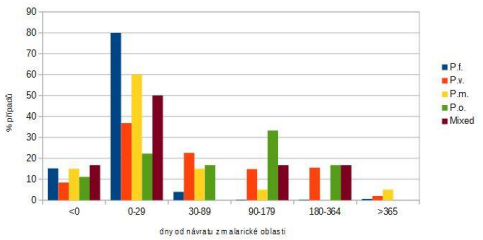
https://www.who.int/malaria/publications/world-malaria-report-2018/en/

Plasmodium falciparum - life cycle

http://magazine.jhsph.edu/2011/malaria/online_extras/galleries/malaria_life_cycle/

Interval mezi návratem z malarické oblasti a prvními příznaky (US 2002)

(Snehal S., et al. MMWR Surveill Summ. 2004 Apr 30;53(1):21-34.)



Malaria Clinical presentation

- symptoms of **uncomplicated** malaria can be rather **non-specific**
- **untreated** malaria can progress to severe forms that may be rapidly **(<24 hours) fatal**
- most frequent symptoms include **fever** and **chills**, which can be accompanied by **headache, myalgias, arthralgias, weakness, vomiting,** and **diarrhoea**
- Other clinical features include **splenomegaly**, anemia, **thrombocytopenia**, hypoglycemia, pulmonary or renal dysfunction, and neurologic changes

Malaria

- **RDT antigen** – *P. falciparum/ P.spp*
- **Microscopy** – species determination, parasitemia
- **PCR** – detection of sub microscopical parasitemia, mixed infections, damaged plasmodia

Malaria RDT

- **HRP – 2**

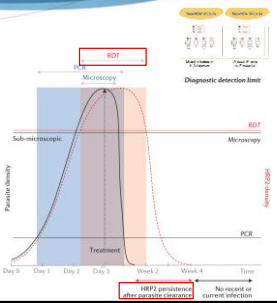
Plasmodium falciparum

Sequence polymorphysm

- **Aldolase**

Plasmodium spp.

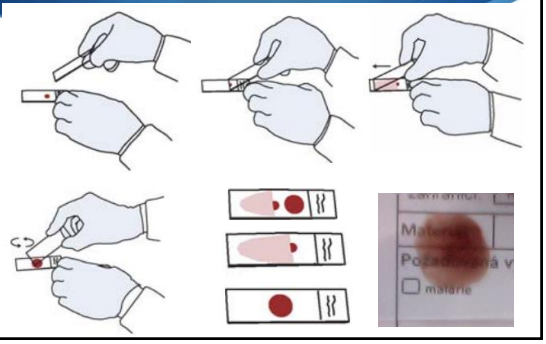
High false negativity *P. ovale* a *P.malariae*



Malaria–RDT – HRP2

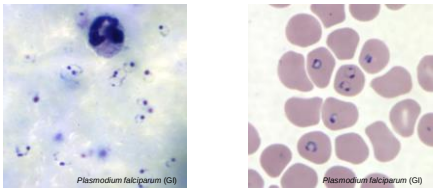
Pfhrp2 mutace 2000 | *Pfhrp2* mutace 2016

Thin and thick smear



Malaria microscopy

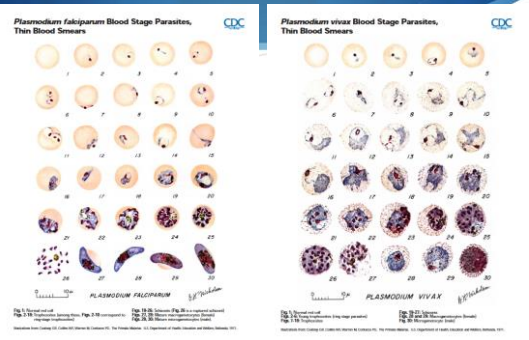
Thick smear | Thin smear



10x senzitivity | Morfology

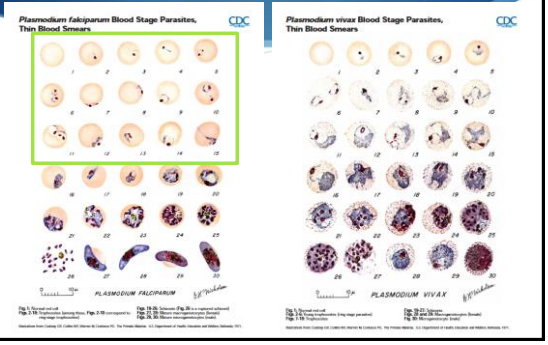
Thin smear

Plasmodium falciparum | ***Plasmodium vivax***



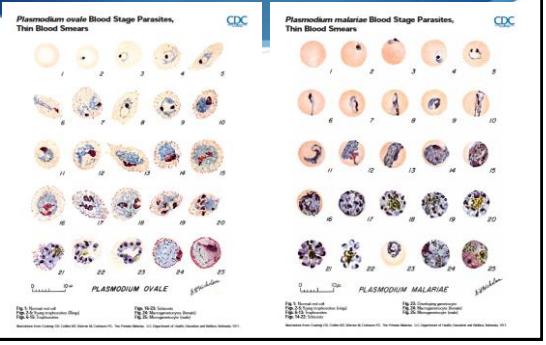
Thin smear

Plasmodium falciparum | ***Plasmodium vivax***



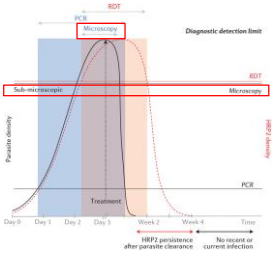
Thin smear

Plasmodium ovale | ***Plasmodium malariae/knowlesi***



Malaria microscopy

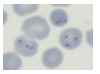
- Esential for parasitaemia
- Detection minimum parasitaemia 0.001%
- Species identification

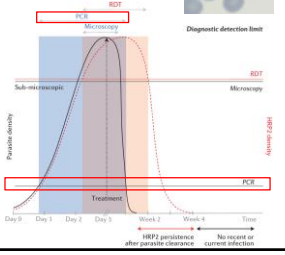


Malaria PCR

- *Plasmodium falciparum*
- *Plasmodium vivax*
- *Plasmodium ovale*
- *Plasmodium malariae*
- *(Plasmodium knowlesi)*

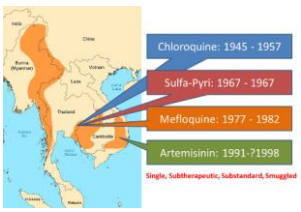
Detection of mixed infections





Malaria treatment

ACT – first line treatment



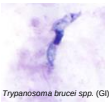
Intermitent preventive treatment in pregnancy (IPTp)

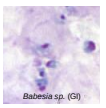
•2010-2015 6% - 31% increase

IPTp (2 doses sulfadoxine-pyrimethamine) female15-49 last pregnancy

Malaria and other parasites in blood microscopy

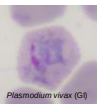
Thick smear

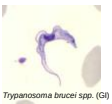


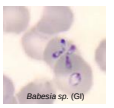


10x sensitivity

Thin smear



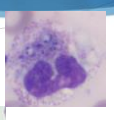


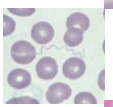


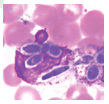
Morfology

What else can be found in blood?

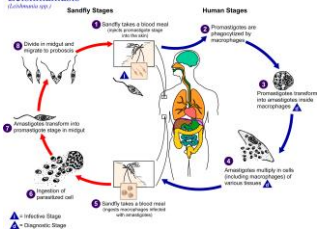
- *Histoplasma*
- *Borelia*
- *Candida*





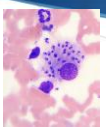


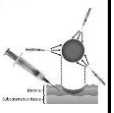
Leishmaniasis



- 12-15 mil cases / year
- 40 000 deaths
- Over 20 species of *Leishmania*
- 30 species of sandfly can transmit

Leishmaniasis

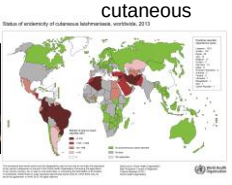


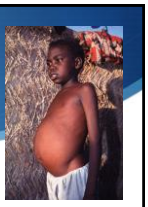
visceral

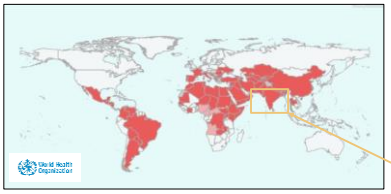
cutaneous



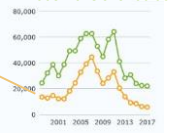


Status of endemicity of visceral leishmaniasis:



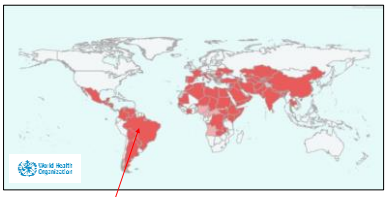
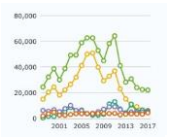
Number of cases
India/ World
2005-2013 over 60 000



http://apps.who.int/neglected_diseases/ntddata/leishmaniasis/leishmaniasis.html

Status of endemicity of visceral leishmaniasis:

Number of cases 2017
World 22 145

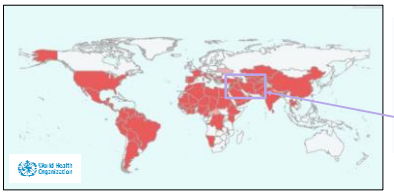
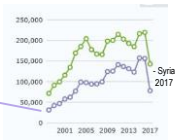
South-East Asia 6 176
Africa 6 050
Eastern Mediterranean 5 127
Americans 4 422

http://apps.who.int/neglected_diseases/ntddata/leishmaniasis/leishmaniasis.html

1990-2016 Brazil increase by 52,9%

Status of endemicity of cutaneous leishmaniasis:

Number of cases 2016
Eastern Mediterranean/ World 219 200

1. Syria 47 377
2. Afghanistan 35 184
3. Pakistan 27 151
4. Iraq 18 258

58%
71%

http://apps.who.int/neglected_diseases/ntddata/leishmaniasis/leishmaniasis.html

Leishmaniasis Clinical presentation

- **Cutaneous leishmaniasis** is characterized by one or more cutaneous lesions on areas where sandflies have fed.
- **sores** can be painless or painful
- Some people have swollen glands near the sores
- **Visceral leishmaniasis** usually have fever, weight loss, and an enlarged spleen and liver
- Some patients have swollen glands.
- **low blood counts,** including a low red blood cell count (anemia), low white blood cell count, and low platelet count.
- Some patients develop post kala-azar dermal leishmaniasis.

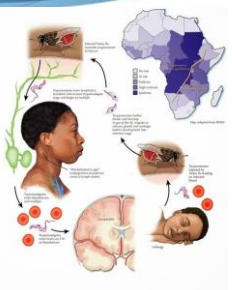
Leishmaniosis - *Leishmania* spp.

- **Microscopy** – scrapings of the ulcer, bone marrow, histological slides
- **Serology** – semiquantitative haemaglutation IgG *Leishmania donovani*
- **Cultivation + Sequencing** – species determination



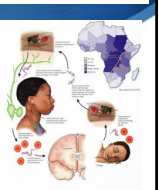
Sleeping sickness- *Trypanosoma gambiense,T. rhodesiense*

- **Microscopy** – ulcer, aspirate from lymph nodes, blood, CSF,
- **PCR** – kinetoplastid DNA
- **Serology** –
- HAT Ag for *T. gambiense*



Sleeping sickness *T. gambiense, T. rhodesiense*

DNDi Drugs for Neglected Diseases initiative



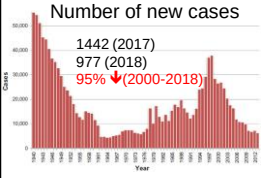
WHO adds first all-oral sleeping sickness drug to Essential Medicines List

Published 10 July 2019

Fexinidazole, the first all-oral treatment for sleeping sickness, approved in Democratic Republic of Congo

Paris/Geneva – 30 January 2019

Number of new cases

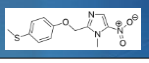


1442 (2017)
977 (2018)
95% ↓(2000-2018)

Melasporol 1/20 killed
↓
Eflornithine difficult admin.
↓
2009 **Nifurtimox-eflornithine**
↓
2018 Fexnidazole

Nitroimidazole derivatives - **Fexinidazole**

DNDi Drugs for Neglected Diseases initiative



Fexinidazole Winthrop, a Sanofi-Aventis product developed with the DNDi, received a positive endorsement from the European Medicines Agency on **November 15th, 2018**, for use in non-European markets

- **(metabolit=sulfoxid a sulfon) antiparazitikum s aktivitou proti**
- *Trypanosoma cruzi*
- *Trichomonas vaginalis*
- *Entamoeba histolytica*
- *Trypanosoma brucei*
- *Leishmania donovani*

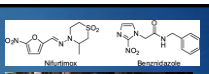
- enzyme-mediated reduction by nitroreductases to generate cytotoxic species that cause DNA, lipid and protein damage

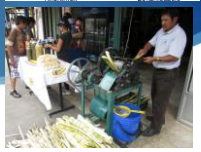
Chagas disease

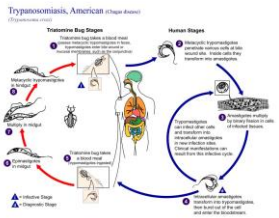
- Estimate 6-7 mil people, mainly in latin america are infected

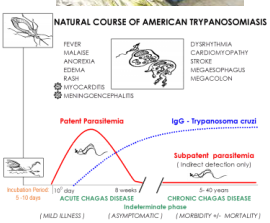
N. Of cases 2013 WHO

Chagas disease







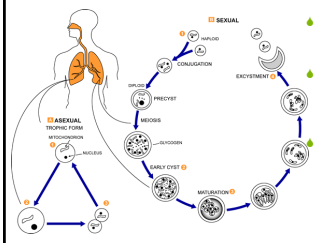


Chagas disease *Trypanosoma cruzi*

- **Microscopy** – acute – blood, CSF

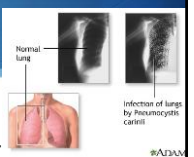
  chronic – histological slides from biopsy
- **Serology** – quantitative IgG *Trypanosoma cruzi*
- **PCR** – kinetoplastid DNA
- Diagnostics – kcongenital Chagas disease

Pneumocystis jiroveci



- These **fungi** are found in the lungs of mammals
- until the host's immune system becomes debilitated - **oftentimes lethal pneumonia**
- *Pneumocystis jirovecii* **cysts** are thick-walled, rounded and approximately 5-8 µm in size

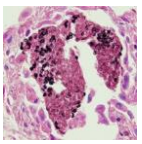
Pneumocystosis – Clinical presentation



- **dyspnea**, **nonproductive cough**, and **fever**
- Chest radiography demonstrates bilateral infiltrates
- Extrapulmonary lesions occur in a minority (<3%) of patients, involving most frequently the lymph nodes, spleen, liver, and bone marrow
- Typically, in untreated PCP increasing pulmonary involvement leads to death.

Pneumocystosis – diagnostics and treatment

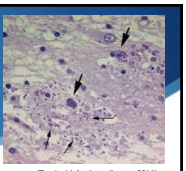
- Up to 80% of population have Ab
- Diagnosis from **BAL**, **sputum** or oral wash,
- **microscopy or PCR**



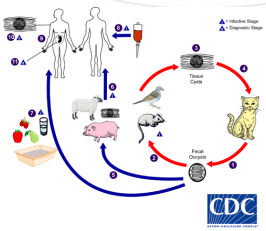
- **Treatment:** Trimetoprim-sulfamethoxazol (TMP-SMX, Co-trimoxasol), Pentamidin
- HIV+ 21 days, non-HIV 14-21 days

Toxoplasma gondii

tissue cysts: skeletal muscle, myocardium, brain, and eyes



(Tropical infectious disease 2011)



- serology, stained biopsy, amniotic fluid PCR

- 1) oocysts
- 2) tachyzoits
- 3) bradyzoits (tissue cyst)
- Eating **undercooked meat** with bradyzoites
- **Food or water contaminated** with cat feces with oocysts
- **Blood transfusion or organ transplantation**
- **Transplacentally** from mother to fetus

Toxoplasmosis clinical presentation

MR Lesion and edema

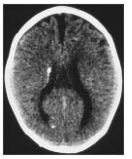
- in **immunocompetent** persons is generally an **asymptomatic**
- 10% to 20% with acute infection cervical **lymphadenopathy** and/or a **flu-like illness**
- **Immunodeficient** patients - **CNS disease** but may have retinochoroiditis, pneumonitis, or other systemic disease
- **AIDS, toxoplasmic encephalitis** is the most common cause of intracerebral mass lesions (reactivation of chronic infection)

(Tropical infectious disease 2011)

Congenital toxoplasmosis

- from an acute **primary infection** acquired by the mother **during pregnancy**
- **incidence and severity** vary with the **trimester** during which infection was acquired
- **treatment of the mother** reduce sequelae in the infant (**pyrimethamin + sulfadiazin + acidum folinicum**)
- Ocular *Toxoplasma* infection – retinochoroiditis, hydrocephalus, psychomotor retardation

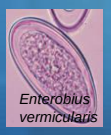
retionochoroiditis



Calcification and mild hydrocephalus

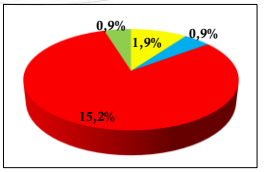
(Tropical infectious disease 2011)

Intestinal parasites



https://www.cdc.gov/dpdx/

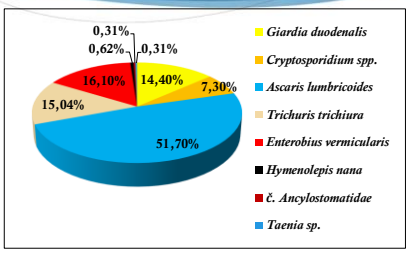
Main population in Košice



Giardia duodenalis *Ascaris lumbricoides* *Enterobius vermicularis* *Taenia* spp. *Hymenolepis nana*

G. Štrkolcová[1], M. Goldová[1], M. Maďar[2]
[1] Ústav parazitológie, UVLF, [2]Katedra mikrobiológie a imunológie, UVLF v Košiciach

Getho near Košice



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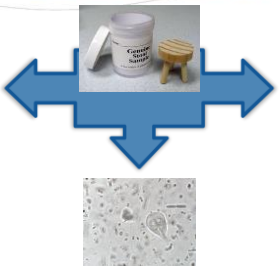
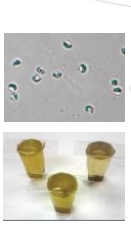
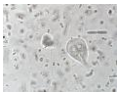
Parasitological examination of stool

Parasitological examination of stool

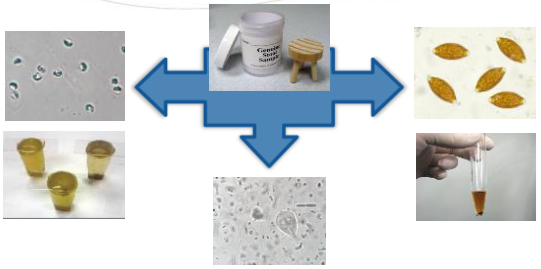


Parasitological examination of stool



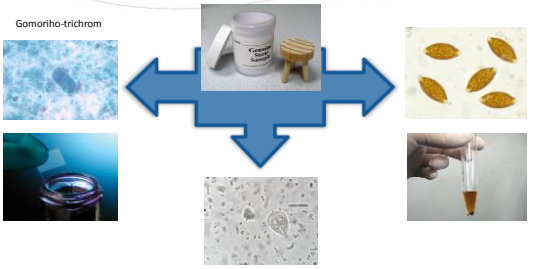


Parasitological examination of stool



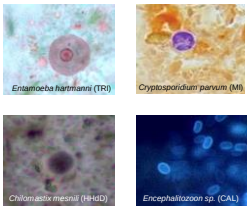
Parasitological examination of stool

Gomoriho-trichrom



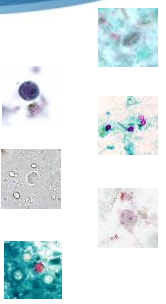
Parasitological examination of stool

- Stain (Trichrom, Miláček, Heidenheim, Calcofluor white)



PCR multiplex – stool parasites

- - *Giardia lamblia*
- - *Entamoeba histolytica*
- - *Cryptosporidium* spp.
- - *Blastocystis hominis*
- - *Dientamoeba fragilis*
- - *Cyclospora cayetanensis*

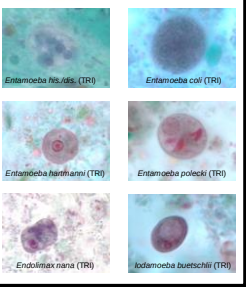


Diagnostical-RDT

Amoebiasis - *Entamoeba histolytica*

- Microscopy

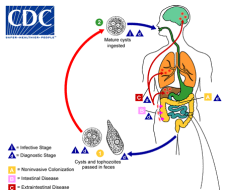
Differencitation *Entamoeba histolytica* from non pathogenic

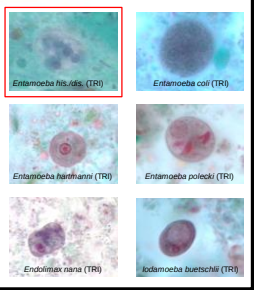


Amoebiasis - *Entamoeba histolytica*

- Microscopy

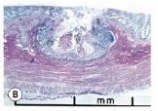
Differentiation *Entamoeba histolytica* from nonpathogenic amoebs





Amoebiasis – Clinical presentation

- from **asymptomatic infection** ("luminal amebiasis")
- to **invasive intestinal amebiasis** (dysentery, colitis, appendicitis, toxic megacolon, amebomas)
- to **invasive extraintestinal amebiasis** (liver abscess, peritonitis, pleuropulmonary abscess, cutaneous and genital amebic lesions)

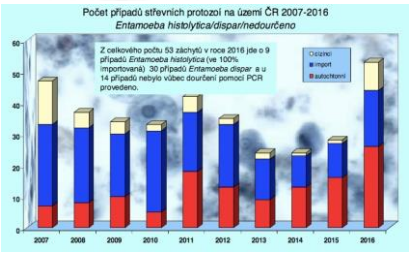


Mucosal ulcer colon

Liver abscess (Tropical infectious disease 2011)

Amoebiasis - *Entamoeba histolytica*

100 000 deaths worldwide annually



9 *E.h.*

Amoebiasis - *Entamoeba histolytica*

- **Microscopy** – stool, cyst aspirate, histological slides from biopsy
- **Serology** – Quantitative detection of IgG *Entamoeba histolytica*
- **PCR** – stool, cyst aspirate, histological slides from biopsy

Amoebiasis - *Entamoeba histolytica*

- **Microscopy**

Differentiation *Entamoeba histolytica* from nonpathogenic amoebs

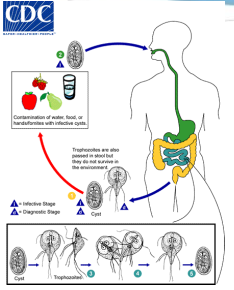
- **PCR**

Differentiation *Entamoeba histolytica from E. dispar* and *E. moshkowskii*

(stool, aspitrate, biopsia)

- **Serology**
- 95% extraintestinal am.
  70% activ intestinal inf.
  10% asymptomatic inf.

Giardia intestinalis (syn. G.lamblia, G.duodenalis)



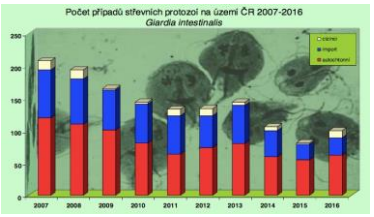
- The cysts are hardy and **can survive several months** in cold water
- **lumen of the proximal small bowel** where they can be free or attached to the mucosa by a ventral sucking disk
- cysts are infectious when passed in the stool or shortly afterward

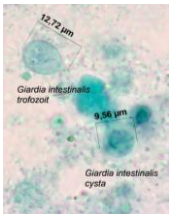
Giardia intestinalis Clinical Presentation

- from **asymptomatic** carriage to severe **diarrhoea** and **malabsorption**
- Acute giardiasis develops after an **incubation period of 1 to 14 days**
- usually lasts 1 to 3 weeks
- Symptoms include **diarrhoea, abdominal pain, bloating, nausea, and vomiting**.
- In chronic giardiasis the symptoms are recurrent

Giardiosis

- Most prevalent GI pathogenic protozoa estimate 200 mil./year





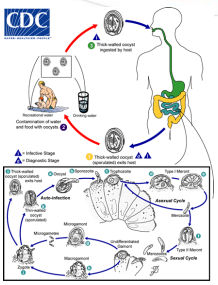
Giardia intestinalis/duodenalis/lamblia

- ***Giardia lamblia* A** (**humans**, primates, dogs, cats, cows, sheep, deer, rodents)
- ***Giardia lamblia* B** (**humans**, primates, dogs, cats, horses, **beavers**)
- *Giardia lamblia* C,D (digs)
- *Giardia lamblia* E (cows, sheep, goats, pigs)
- *Giardia lamblia* F (cats)
- *Giardia lamblia* G (pigs)
- *G.agilis, G. ardae, G.psittaci, G.microti, G. muris*

Diagnostics

- **Microscopy**
- Special stain Trichrom (50-70% 1stool., 90% 3stool.)
- **PCR** detekction (for therapy monitoring)
- Cysts 6-10um x 8-12um
- Trofozoites 5-15um x 9-21um (duoden st.)

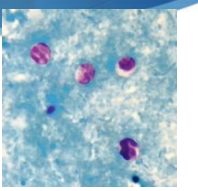
Cryptosporidium sp.



- Sporulated **oocysts**, containing 4 sporozoites, are excreted by the infected host through feces (**contaminated water**)
- sporozoites are released and **parasitize epithelial cells**
- Two different types of **oocysts** are produced, the **thick-walled**, which is commonly **excreted** from the host
- and the **thin-walled oocyst** which is primarily involved in **autoinfection**

Cryptosporidiosis - Clinical presentation

- from asymptomatic infections to severe, life-threatening illness
- **incubation period** is an average of **7 days**
- **Watery diarrhoea** is the most frequent symptom
- dehydration, weight loss, abdominal pain, fever, nausea and vomiting
- **Immunocompetent 1 to 2 weeks**



- chronic and more **severe in immunocompromised patients**
- CD4 counts < 200/µl

(Tropical infectious disease 2011)


http://www.cdc.gov/dpdx/
http://mikrobiologie.lf3.cuni.cz/
Tropical infectious disease 2011
Nemocnice
Na Bulovce