FOLIA VETERINARIA

The scientific journal of the
UNIVERSITY OF VETERINARY MEDICINE AND
PHARMACY IN KOŠICE — The Slovak Republic

ISSN 0015-5748

2

LVII • 2013

FOLIA VETERINARIA is issued by the *University of Veterinary Medicine and Pharmacy in Košice (UVMP);* address: Komenského 73, 041 81 Košice, The Slovak Republic (tel.: +421 55 632 52 93, fax: +421 55 632 52 93, E-mail: Milada.Vargova@uvlf.sk).

The journal is published quarterly in English (numbers 1—4) and distributed worldwide.

Subscription rate for 1 year is 120 €. Orders are accepted by *The Department of The Scientific Information — The Library of The University of Veterinary Medicine and Pharmacy in Košice* (UVIK), E-mail: Natalia.Palencarova@uvlf.sk; the subscription is accepted by the State treasure.

Bank contact: State treasure, Radlinského 32, Bratislava 15, The Slovak Republic; **account number:** 7000072225/8180.

FOLIA VETERINARIA, vydáva *Univerzita veterinárskeho lekárstva a farmácie v Košiciach (UVLF),* Komenského 73, 041 81 Košice, Slovenská republika (tel.: 055/632 52 93, fax: 055/632 52 93, E-mail: Milada.Vargova@uvlf.sk).

Časopis vychádza kvartálne (č. 1—4) a je distribuovaný celosvetove.

Ročné predplatné 120 €. Objednávky prijíma *Ústav vedeckých informácií a knižnice Univerzity veterinárskeho lekárstva a farmácie v Košiciach* (UVIK), E-mail: Natalia.Palencarova@uvlf.sk; predplatné Štátna pokladnica (na nižšie uvedené číslo účtu).

Bankové spojenie: Štátna pokladnica, Radlinského 32, Bratislava 15; **číslo účtu:** 7000072225/8180.

Tlač: **Univerzita veterinárskeho lekárstva a farmácie** Komenského 73, 041 81 Košice

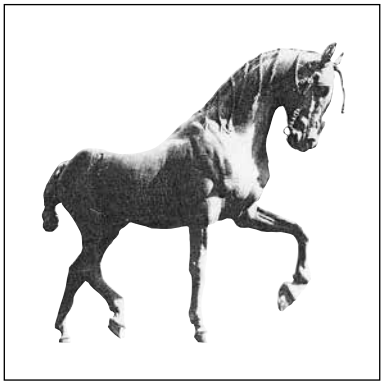
Sadzba: **Sapfo publishers**, Szakkayho 1, 040 01 Košice

EV 3485/09

For basic information about the journal see Internet home pages: www.uvm.sk; www.uvlf.sk

Indexed and abstracted in AGRIS, CAB, EBSCO

FOLIA VETERINARIA, 57, 2, 2013



56th STUDENT SCIENTIFIC CONFERENCE

April 17th, 2013

The aim of the 56th Student scientific conference (ŠVOČ) organised in the academic year 2012/2013 was to present results of scientific investigations carried out by undergraduate and PhD. students. Papers included in this issue were selected by the Organisation committee from among 63 papers presented in the following six sections:

I. Pre-clinical section; II. Clinical section; III. Hygiene of food and the environment; IV. Pharmacy; V. Bachelor's study; VI. Foreign students

UNIVERSITY OF VETERINARY MEDICINE AND PHARMACY IN KOŠICE

FOLIA VETERINARIA, 57, 2, 2013

CONTENTS

FOLIA VETERINARIA, 57, 2: 61—64, 2013

MONITORING OF SEASONAL CHANGES IN BLOOD PARAMETERS OF SNAKES *LAMPROPELTIS TRIANGULUM SINALOE*

Žilinčík, M., Halán, M.

Institute of parasitology, University of Veterinary Medicine and Pharmacy, Košice
The Slovak Republic

halan@uvlf.sk

ABSTRACT

This study was aimed at assessing the extent of the changes in blood parameters over a one year period in milk snakes (*Lampropeltis triangulum sinaloe*). The study included 6 individuals of this specie. Blood samples were taken at monthly intervals. The main objective of this study was to evaluate the blood films and leucograms by means of light microscopy. After the evaluation and recording of a finite number of different types of leukocytes (Leu) throughout the year, we concluded that the seasonal changes in the blood parameters were most pronounced during hibernation (January and February). Our results showed a decrease in the lymphocyte (Ly) count on average by 21.58 %, compared to our calculated reference value for lymphocytes. Conversely, when evaluating the granulocytic and monocytic line of leukocytes, our results showed a progressive increase in numbers. In azurophils (Az) we recorded a mean increase of 68.09 % as compared to our reference calculations. The increase in the number of heterophils (Het) averaged up to 124.16 % compared to our reference value. The numbers of basophils (Ba) were most pronounced during hibernation by up to 187.35 % over our reference value for basophils. After hibernation, the numbers of different types of leukocytes gradually approached the physiological calculated levels. Our results suggest that in clinical practice it is necessary to relate changes in haematological parameters to changes in seasonal activity, not only in snakes of the genus *Lampropeltis*, but in all kinds of snakes.

Key words: blood cells; blood count; snake

INTRODUCTION

The monitoring and evaluation of biochemical and hematological parameters of blood is an essential part of any comprehensive examination to assess a patient's condition whether in humans or any other animal. Increasingly, in clinical practice, veterinarians encounter exotic species of animals for which common methods of clinical examination meet with technical difficulties or established published health parameters are lacking. In such cases, hematology comes to the foreground as one of the main components necessary to obtain a correct diagnosis.

Changes in the percentage of leukocytes in the peripheral blood in response to pathological processes and various diseases, have been used only marginally in clinical practice involving reptiles [3]. The evaluation of haemograms and blood films provides a quick, valuable method for assessing the current health status and identifying ongoing disease processes [5]. The blood of reptiles performs essentially the same functions as in mammals. Their constituent blood levels correspond to the haemopoiesis of a three-month human embryo. Unlike mammals, in the blood of reptiles, we find (as in fish and amphibians) more immature blood elements and their arrested developmental stages [3]. The cellular components of the reptile's peripheral blood consists of erythrocytes, lymphocytes, leucocytes (Leu), heterophils (Het), eosinophils, basophils (Ba), azurophils (Az), monocytes and platelets. However, in individual reptile orders, we find variations in the prevalence of certain blood cells. The presence or absence of eosinophils is disputed in snakes. The literature sources demonstrate that eosinophils are present in several species of snakes. They are described by some authors [2],

[6,] [7], [8], but others consider them as a secondary type of heterophils [1]. Azurophils are found in lizards only in small numbers and their increase is interpreted as a condition of monocytosis. Therefore, it was proposed to declare them as monocytes or monocytic azurophiles. Individual blood counts may vary with respect to environmental and physiological factors [10], [11].

The main objective of this study was to evaluate the blood films and leucograms of snakes by means of light microscopy.

MATERIALS AND METHODS

The subject of our investigation was the milk snake (*Lampropeltis triangulum sinaloe*). We investigated 6 milk snakes, 3 to 9 years old (2 males and 4 females). They were reared in an indoor terrarium in the facilities of the AQUA-TERA club of the University of Veterinary Medicine and Pharmacy (UVMP) in Kosice. The snakes were fed large laboratory rodents at regular intervals every 14 days. Drinking water was provided *ad libitum*. In the terrarium we kept a constant temperature of 24 °C and used local heating to reach temperature of 32 °C in the xarea of the snakes. In the second half of December, after the period of preparation for the hibernation and emptying of the digestive tracts, the milk snakes were transferred to linen bags which were stored in a separate room for hibernation at a temperature of 10—14 °C. Hibernation lasted 70 days and during this period, the snakes were fed every 2 weeks. After hibernation, we gradually increased the temperature to the above mentioned values.

The blood sampling took place regularly once a month from September 2011 to August 2012. From each snake we collected a small amount of blood (approximately 0.2 ml). As the most appropriate technique for blood collection, we selected the ventral tail vein (*v. coccygea ventralis*). It is a very suitable technique for serial blood sampling in snakes [4]. For collection, we used a short needle, size 25 G (0.5 mm). Immediately after collection, we prepared two blood films from each sample. The blood films were stained with a dye kit (Hemacolor) for microscopic examination. After staining, the films were allowed to dry and were thoroughly suffused with the fixation medium (Entellan). For the evaluation of the blood films we used a light microscope (Motic B3, Professional Series) with the immersion lens at 1000 × magnification. Individual films were examined by a meandering motion, in order to obtain the best possible objectivity of the results.

RESULTS

A literature review did not reveal any reference values for leukocyte types in milk snakes (*Lampropeltis triangulum sinaloe*). Therefore, the means of the values of leukocytes measured during the periods of least variability from our experimental data were used as the calculated reference values for the different types of leukocytes. The mean number of leukocytes in % during the period of observation is summarized in Table 1.

The lowest mean lymphocyte counts were measured in the month of January. During this month, the mean number of lymphocytes was 64.5 Ly.100 $Leu^{-1,}$ with the highest number of 72 Ly.100 Leu^{-1} and the lowest number of 58 Ly.100 Leu^{-1}. On average, there was a decrease of up to 21.58 %. At the

Table 1. The mean number of leukocytes in % during the period of observation

Date of sampling	Lymphocytes	Heterophils	Basophils	Azurophils
Sept. 28, 2011	83.5	8.3	0.67	7.83
Oct. 25, 2011	81	8.5	1.17	9.3
Nov. 20, 2011	84.67	6.5	0.83	8.17
Dec. 30, 2011	79.17	9.17	1.17	9.83
Jan. 28, 2012	64.5	18.83	2.5	14.17
Feb. 26, 2012	65.17	17.83	2.5	12.83
March 22, 2012	75.67	10.83	1.83	11.67
April 29, 2012	79.17	9.3	1.33	9.3
May 19, 2012	79.3	7.83	1.5	11.3
June 21, 2012	81.67	8	0.83	9.5
July 24, 2012	82	9	0.83	8.17
Aug. 27, 2012	81.67	8.5	1	8.83

highest number of lymphocytes in this period, there was a decrease of 12.46 % and in the lowest number, a decrease up to 29.48 %. The highest number of cells was recorded during the month of November, when the average number of cells reached 84.67 Ly.100 Leu^{-1}. The highest number in this period was 90 Ly.100 Leu^{-1} and the lowest 81 Ly.100 Leu^{-1}. It was an average increase up to 2.94 %. At the highest number this was an increase of up to 9.42 %.

The mean highest number of azurophils was observed in January, when it reached 14.17 Az.100 Leu^{-1}. The highest number in this period was 17 Az.100 Leu^{-1} and the lowest 12 Az.100 Leu^{-1}. It was a mean increase by up to 68.09 %. The highest number means an increase by up to 101.66 %. With respect to the minimum number of azurophils in January, this was an increase of up to 42.34 %. The lowest number of azurophils was recorded during the month of September. During this period, the mean number was 7.83 Az.100 Leu^{-1} with the highest number of 12 Az.100 Leu^{-1} and the lowest number 4 Az.100 Leu^{-1}. The mean decrease was about 7.11 % compared to the calculated reference value. In the case of the highest number of azurophils in that period, this was an increase by up to 42.34 %, compared to the reference value and in the case of the lowest number, a decrease by 52.55 %.

The highest mean number of heterophils was observed in January when it reached 18.83 Het.100 Leu^{-1}. The highest number in this period was 24 Het.100 Leu^{-1} and the lowest, 12 Het.100 Leu^{-1}. It was a mean increase by 124.16 %. In the case of the highest number, this was an increase by up to 185.71 %. At the minimum number of heterophiles in January, there was an increase by up to 42.86 %. The lowest number was observed in November. During this period, the mean number of heterophils was 6.5 Het.100 Leu^{-1}, the highest number 9 Het.100 Leu^{-1} and the lowest number, 4 Het.100 Leu^{-1}. The mean count constituted a decrease by up to 22.62 % compared to the reference value. The highest number of heterophils in this period corresponded to an increase by up to 7.14 % and the lowest, a decline by about 52.38 %.

The highest number of basophils was recorded in January and February, when the mean reached 2.5 Ba.100 Leu^{-1}. The highest number in that period was 4 Ba.100 Leu^{-1} and the lowest 1 Ba.100 Leu^{-1}. It was a mean increase by 187.35 %. The highest number corresponded to an increase by 359.77 %. The minimum level of basophils in this period, corresponded to an increase by 14.94 %. The lowest number of basophils was recorded in September. During this month, the mean number of basophils was 0.67 Ba.100 Leu^{-1}, the highest 1 Ba.100 Leu^{-1} and the lowest 0 Ba.100 Leu^{-1}. The mean level was a decrease by 22.98 %, compared to the reference value. At the highest number of basophils in this period, there was an increase by 14.94 % compared to the reference value.

DISCUSSION

Reference values for the numbers of leukocytes types of milk snakes (*Lampropeltis triangulum sinaloe*) were not mentioned in any literature sources surveyed, which was the main reason for developing our own benchmarks. Therefore, in our study we used the mean numbers of leukocytes over the period of least variability. Our results showed that seasonal changes in hematological parameters are most evident during hibernation. This finding is confirmed by several authors [7], [10], [11]. Lymphocyte counts recorded by us indicate that the period of hibernation strongly influences the number of lymphocytes in the peripheral blood. The numbers were reduced on average by 21.58 %, compared to the calculated benchmarks set by us. Such a reduction was reported by Wallach and Boever [9], Sypek and Borysenko [7], as well as other authors. In our opinion, a decrease in the number of lymphocytes may result from low temperatures which decreased during hibernation down to 10 °C. Other reasons for the low number of lymphocytes during hibernation could be food shortages and thus lack of protein availability needed for cell formation or a short lifetime of lymphocytes. Wright and Cooper [11] are inclined to believe that all this is a relative inability to generate a primary immune response during low temperatures. On the other hand, an increase in the number of lymphocytes in the run-up to the hibernation (on average by 2.94 %) may be due to the temperature decrease, limited food intake and an increase in moisture, which may lead to increased stress on the individuals studied, and therefore increase the peripheral blood lymphocyte count. In our study, the azurophils increased notably during hibernation by about 68.09 % on average. Heterophils also increased during hibernation (124.16 % on average). The increase in the number of basophiles during hibernation reached up to 187.35 % compared to our reference calculations. Our findings suggest that the increased numbers of cells with non-specific immune response (heterophils, basophils and azurophils) may result from the reduction in the number of lymphocytes and the reduced primary immune response at low temperatures.

CONCLUSION

After evaluation and recording the final numbers of leukocytes throughout the year of study we can conclude that seasonal changes in blood parameters were most pronounced during hibernation in January and February. After hibernation, the leukocyte counts approached gradually over several months to the levels set as benchmarks. A gradual increase was observed during the preparation for hibernation. The results of our study indicate that seasonal changes in the activity of milk snake (*Lampropeltis triangulum sinaloe*), mostly the period of hibernation, have a major impact on the change in blood parameters. Our results showed that in clinical practice it is necessary to relate changes in haematological parameters to changes in seasonal activity, not only in snakes of the genus *Lampropeltis*, but in all kinds of snakes.

REFERENCES

1. Alleman, A. R., Jacobson, E. R., Raskin R. E., 1999: Morphologic, cytochemical staining and ultrastructural characteristics

of blood from eastern diamondback rattlesnakes (*Crotalus adamanteus*). *Am. J. Vet. Res.*, 60, 507—514.

2. Campbell, T. W., 2006: Clinical pathology. In *Reptile Medicine and Surgery,* 2nd edn., **Mader, D. R.** (Ed.), Saunders Elsevier, St. Louis, 1264 pp.

3. Dubanský, V., Pravda, D., Knotek, Z., 1996: Blood of reptiles II. Cytology of blood elements (In Czech). *Veterinářství*, 46, 467—473.

4. Jacobson, E. R., 1993: Blood collection techniques in reptiles: laboratory investigations. In *Zoo and Wild Animal Medicine*, 3rd edn., WB Saunders Co., Philadelphia, 644 pp.

5. Jacobson, E. R., 2007: *Infectious Diseases and Pathology of Reptiles*, 1st edn., CRC Press, Boca Raton, 716 pp.

6. Salakij, C., Salakij, J., Apibal, S. et al., 2002: Hematology, morphology, cytochemical staining, and ultrastructural characteristics of blood cells in king cobras (*Ophiophagus Hannah). Vet. Clin. Pathol*, 31, 116—126.

7. Sypek, J., Borisenko, M., 1988: Reptiles. In **Rowley, A. F., Ratcliffe, N. A.:** *Vertebrate Blood Cells.* University Press, Cambridge, 245 pp.

8. Troiano, J. C., Vidal, J. C., Gould, J., Gould, E., 1997: Haematological reference intervals of the South American rattlesnake (*Crotalus durissus terrificus*) in captivity. *Comparative Haematology International*, 1, 109—112.

9. Wallach, J. D., Boever, W. J., 1983: *Diseases of Exotic Animals, Medical and Surgical Management*. WB Saunders Co., Philadelphia, 1159 pp.

10. Work, T. M., Raskin, R. E., Balazs, G. H., Whittaker, S. D., 1998: Morphologic and cytochemical characteristics of blood cells from Hawaiian green turtles. *Am. J. Vet. Res.*, 59, 1252—1257.

11. Wright, R. K., Cooper, E. L., 1981: Temperature effect on ectothermic immune responses. *Development and Comparative Immunology*, 5, 117–122.

*

Selected papers from the 56th STUDENT SCIENTIFIC CONFERENCE, Section I, held at the University of Veterinary Medicine and Pharmacy in Košice on April 17, 2013.

FOLIA VETERINARIA, 57, 2: 65—67, 2013

THE INFLUENCE OF CATIONIC PEPTIDES AND DIETARY NUCLEOTIDES ON PARAMETERS OF NON-SPECIFIC IMMUNITY IN DOGS

Bratičáková, M., Smrčo, P., Bečárová, L.

Institute of Epizootology and Preventive Veterinary Medicine
University of Veterinary Medicine and Pharmacy, Komenského 73, 041 81 Košice
The Slovak Republic

smrco@uvm.sk

ABSTRACT

Our experiment was conducted on 16 dogs of different age, sex and breed showing clinical symptoms of gastroenteritis of varying etiology. They were divided into two groups, A and B, with 8 animals per group. During four months, the dogs from group A received an immunostimulant preparation containing dietary nucleotides and cationic peptides, while group B served as a control. Both groups were examined for the parameters of non-specific immunity. The results of the immunological examination were compared and evaluated statistically.

Key words: cationic peptides; dietary nucleotides; dog; non-specific immunity

INTRODUCTION

Gastroenteritis is among the most common diseases of small animals. The most frequent causes include: dietetic factors, ingestion of toxins including medicines or foreign bodies, parasites, infectious agents (viruses and bacteria), inadequate response to food and some systemic diseases [5].

Dietary nucleotides are low-molecular intracellular components. Although their deficit is not associated with any specific disease, scientists recorded their beneficial effect on the young due to their positive influence on metabolism of lipids, immunity, growth, development and tissue repair [1]. Rapidly proliferating tissues, such as the immune system and intestines, are not capable of satisfying the cellular nucleotide needs purely by *de novo* synthesis and preferably use nucleotides or nitrogen bases from the blood and feed. Exogenous supply of these compounds by food may become necessary for the maintenance of growth and cellular functions in these tissues [1].

Cationic peptides usually have chains with 12—50 amino acids. They are produced by all organisms as the main component of their immediate, effective, non-specific defense to infections [2]. These peptides form two main classes of potential medicines:

a) peptides with direct antimicrobial and/or anti-biofilm activity;
b) peptides with immunomodulation and/or anti-inflammatory activity [4].

The aim of the study was to investigate the effect of the administration of immunostimulant preparations on dogs showing clinical symptoms of gastroenteritis.

MATERIALS AND METHODS

Animals

Group A consisted of 8 dogs of different breed, sex and age. They suffered from long-standing gastroenteritis of varying etiology and were administered Aminex (1drop per 1 kg b. w. daily) *per os* in the form of syrup (UNIREGEN spol. s r. o., CR) for four months. Group B included 8 sick dogs of different breed, sex and age that were not administered Aminex and served as a control.

Table 1. Parameters of non-specific immunity in group A and group B during 4 samplings

	Le	PANe	PINe	PALe	PILe	IMA	SI
				1st sampling			
A	5325 ± 634.4	81.87 ± 4.28	14.1 ± 0.4	49.75 ± 5.56	16.76 ± 0.30	2.14 ± 0.81	1.36 ± 0.35
B	5275 ± 359.4	80.225 ± 7.2	15.4 ± 0.58	52.86 ± 4.96	17.85 ± 0.79	2.49 ± 0.19	1.19 ± 0.07
P	ns	ns	* P < 0.028	ns	ns	ns	ns
				2nd sampling			
A	8200 ± 1476.48	80.39 ± 9.14	11.89 ± 2.79	48.25 ± 9.32	13.72 ± 2.99	1.89 ± 0.64	1.42 ± 0.07
B	6200 ± 141.4	79.54 ± 7.55	14.245 ± 0.35	51.26 ± 4.77	16.24 ± 0.63	2.15 ± 0.06	1.39 ± 0.07
P	ns	ns	ns	ns	ns	ns	ns
				3rd sampling			
A	8825 ± 543.9.8	74.9 ± 0.94	10.14 ± 0.98	44.72 ± 5.24	9.87 ± 0.65	2.41 ± 0.205	1.75 ± 0.28
B	7200 ± 163.29	78.39 ± 7.15	12.88 ± 0.52	48.39 ± 4.00	12.51 ± 0.76	1.85 ± 0.100	1.34 ± 0.04
P	*P < 0.028	ns	*P < 0.02	ns	*P < 0.02	*P < 0.028	*P < 0.0286
				4th sampling			
A	10525 ± 6393.7	73.70 ± 0.49	9.23 ± 0.614	41 ± 4.76	6.805 ± 0.92	1.95 ± 0.08	2.02 ± 0.206
B	8125 ± 95.74	75.615 ± 6.35	12.48 ± 0.41	45.00 ± 3.66	11.16 ± 0.48	1.47 ± 0.13	1.53 ± 0.05
P	*P < 0.02	ns	*P < 0.028	ns	*P < 0.02	*P < 0.0294	*P < 0.028

Le — leukocytes; PANe — phagocytic activity of neutrophils; PINe — phagocytic index of neutrophils; PALe — phagocytic activity of leukocytes PILe — phagocytic index of neutrophils; IMA — index of metabolic activity; SI — stimulation index; ns — not significant

Table 2. Statistical comparison of parameters of non-specific immunity between individual samplings in group A

Group A	Le [µl]	PANe [%]	PINe	PALe [%]	FILe	IMA	SI
1st versus 2nd sampling	*	ns	ns	ns	ns	ns	ns
1st versus 3rd sampling	*	*	*	ns	*	ns	ns
1st versus 4th sampling	*	*	*	ns	*	ns	*

Sampling of blood

Blood was collected by the puncture of the *v. jugularis* and *v. cephalica antebrachii*. We carried our four samplings at monthly intervals and used the samples for the determination of the parameters of non-specific immunity.

Immunological analysis

The phagocytic activity of blood leukocytes (PALe) and neutrophils (PANe) was determined by evaluation of the ingestion of 2-hydroxyethylmethacrylate particles (MSHP, diameter 1.2 µm, ARTIM Prague) [6]. The phagocytic activity of leukocytes and neutrophils was expressed as the percentage of leukocytes or neutrophils phagocytising 3 and more MSHP particles. The phagocytic index of leukocytes (PILe) and neutrophils (PINe) was determined as the ratio of phagocytised particles and all potential phagocytes.

The metabolic activity of leukocytes was determined by iodonitrotetrazolium (INT) test adjusted according to Mareček and Procházková [3]. The index of metabolic activity (IMA) of leukocytes was expressed as the ratio of the activity of stimulated cells and the activity of non-stimulated cells.

The blastic transformation of lymphocytes was evaluated by ELISA BrdU (colorimetric) test using phytohaemaglutinine PHA-P (Sigma, USA) of concentration 20 µl.ml^{-1}. The level of blastogenic response of the lymphocytes was expressed as the stimulation index (SI). The total number of leukocytes was determined by means of Tűrk solution in a Bűrker counting chamber.

The results were processed statistically by Mann-Whitney test using software GraphPad Prism 5.0.

RESULTS AND DISCUSSION

Parameters of non-specific immunity were observed and compared in both groups of dogs, A and B (Table 1). The level of leukocytes (Le) was decreased in both groups in comparison with the normal physiological range. Group A showed a significant increase in Le ($P < 0.05$) at the third sampling in comparison with group B. At the first sampling, we recorded in individual dogs from both groups, an increased phagocytic index of neutrophils and leukocytes and phagocytic activity of neutrophils and leukocytes in comparison with physiological level. During the experiment we observed a significant decrease in PILe and PINe in group A, while in group B the PILe did not decrease down to the physiological range throughout the experiment.

The level of stimulation index was also decreased in both groups of dogs. In group A, we observed a significant increase in SI ($P < 0.05$) starting from the third sampling in comparison with group B. SI in group B did not increase to the physiological range throughout the experiment.

The significance of difference at individual samplings for group A is shown in Table 2. The significant differences between 1st and 2nd samplings were observed for PINe ($P < 0.05$), PILe ($P < 0.05$) and PANe ($P < 0.05$) and between 1st and 4th sampling for SI ($P < 0.05$). Results of Le differed significantly starting from the first sampling ($P < 0.001$).

CONCLUSION

One of the potential ways of correction of the altered immune system is based on the use of immunostimulant substances which can stimulate and restore the immune system. Their use is prospective in the treatment of various diseases in human and veterinary medicine. The dogs which were administered Aminex exhibited correction of immunological parameters after 60 days of administration. From among the parameters of non-specific immunity, the most significant influence was observed at the level of phagocytic index of leukocytes and neutrophils, stimulation index and the level of leukocytes.

ACKNOWLEDGEMENT

The study was supported by the project VEGA 1/0835/12 and Pharmacopola s.r.o.

REFERENCES

1. Gil, A., 2001: New additions to infant formulas. In *Pediatric Gastroenterology and Nutrition in Clinical Practice*, **Liftschitz, C.** (Ed.), Marcel Dekker, New York, 113—135.

2. Hancock, R. E. W., 2001: Cationic peptides: effectors in innate immunity and novel antimicrobials. *Lancet Infect. Dis.,* 1, 156—164.

3. Mareček, D., Procházková, J., 1986: *Selected Diagnostic Methods of medical Immunology* (In Czech). Avicenum, Prague, 219 pp.

4. Mookherjee, N., Chow, L. N. Y., Hancock, R. E. W., 2012: Immunomodulatory cationic peptide therapeutics: A new paradigm in infection and immunity. In **Rajasekaran, K. et al.:** *Small Wonders: Peptides for Disease Control,* ACS Symposium Series, American Chemical Society, Washington DC.

5. Svoboda, M. et al., 2000: *Diseases of Dogs and Cats* (In Czech). Part I, ČAVLMZ — Noviko, Brno, 1014 pp.

6. Větvička, V., Fornusek, I., Kopeček, J., Kamínková, J., Kašpárek, I., Vránová, M., 1982: Phagocytosis of human leucocytes. *Immunol. Lett.* 5, 97—100.

*

Selected papers from the 56th STUDENT SCIENTIFIC CONFERENCE, Section I, held at the University of Veterinary Medicine and Pharmacy in Košice on April 17, 2013.

FOLIA VETERINARIA, 57, 2: 68—70, 2013

FACTORS AFFECTING THE EFFECTIVENESS OF VACCINATION OF DOGS AND CATS

Bučková J., Bečárová L., Vojtek, B.

Institute of Epizootology and Preventive Veterinary Medicine
University of Veterinary Medicine and Pharmacy, Komenského 73, 041 81 Košice
The Slovak Republic

jana.bučkova88@gmail.com

ABSTRACT

Vaccination is a common method of preventing infectious diseases in humans and other animals. One of the ways of increasing the effectiveness of a vaccination is based on the use of immunostimulants. The aim of this study was to observe the influence of β-(1,3)-D polymers of glucose on the specific immune response after vaccination against canine parvovirus disease; and also to observe the influence of cationic peptides and dietary nucleotides on the specific immune response after vaccination against feline panleucopenia. Specific antibodies were detected by means of the ELISA test and the haemagglutination-inhibition test (HIT). The observed animals responded positively to the selected immunostimulants, in terms of more rapid antibody response as reflected in the parameters of specific immunity.

Key words: Aminex; glucan; immunostimulants; vaccination

INTRODUCTION

According to their origin, immunostimulants are divided to substances of biological origin (vitamins, extracts of mushrooms or isolates from bacteria), products of the immune system (interferons, lymphokines, growth factors) and synthetic products (levamisole, isoprinosine).

Glucans are substances of biological origin that are frequently used for this purpose. They are β-(1,3)-D polymers of glucose that occur in nature as the basic components of cell walls of mushrooms and yeasts. Generally they induce non-specific stimulation of the immune system [5], increase proliferative and functional activity and affect specific immunity by increasing the activity of T lymphocytes and production of cytokines [3].

Nucleotides have a positive influence on lipid metabolism, immune system, growth, development and reparation of tissues [1]. Rapidly proliferating tissues, such as the immune system or intestinal tissue, use nucleotides or nitrogen bases from food and thus their intake with food helps to maintain growth and cellular function in these tissues [1]. Cationic peptides exhibit antimicrobial and immunological activity.

The aim of this study was to observe the influence on the specific immune response of glucans administered to dogs after vaccination against canine parvovirus disease; and of cationic peptides and dietary nucleotides administered to cats after vaccination against feline panleucopenia.

MATERIALS AND METHODS

Animals

- **group G:** 8 healthy dogs (of various breeds), males and females, 5—6 weeks old, were administered glucan in the form of a syrup (Plerasan, PLEURAN, Bratislava) *per os* at a dose of 2 ml.5 kg^{-1} daily for 3 months,
- **group GC:** 8 healthy dogs (of various breeds), males and females, 5—6 weeks old, received no administration of glucan,

Table 1. Levels of antirabies antibody titres (EU.ml^{-1}) in dogs and comparison of groups G and GC

	Day 42	Day 49	Day 56	Day 63	Day 70	Day 77	Day 85	Day 92
Group G	0.367 ± 0.07	0.391 ± 0.05	0.52 ± 0.01	0.796 ± 0.04	1.36 ± 0.11	1.51 ± 0.09	1.905 ± 0.06	2.17 ± 0.2
Group GC	0.328 ± 0.02	0.354 ± 0.03	0.484 ± 0.02	0.623 ± 0.02	0.741 ± 0.03	0.829 ± 0.04	1.12 ± 0.15	1.272 ± 0.12
P	0.1406	0.1403	0.0009	0.0009	0.0002	0.0002	0.0009	0.0009
	ns	ns	***	***	***	***	***	***

ns — not significant; *** — significant at P < 0.001

Table 2. Titres of antibodies to canine parvovirus on individual days of sampling and comparison of groups G and GC

Day	0	7	14	21	28	35	42	49	56	63	70	77
G	52 ± 16.5	26 ± 8.28	22 ± 8.28	15 ± 7.92	38 ± 16.9	84 ± 38.00	128 ± 59.25	192 ± 68.41	384 ± 136.83	640 ± 237.00	832 ± 264.98	1536 ± 547.35
GK	44 ± 22.2	22 ± 11.1	19 ± 8.48	12 ± 4.27	24 ± 8.55	44 ± 16.56	64 ± 29.62	120 ± 63.42	136 ± 53.4	272 ± 106.81	448 ± 118.50	832 ± 264.98
P	0.4437	0.4437	0.4602	0.5169	0.0675	0.0235	0.013	0.0458	0.0015	0.0018	0.008	0.0121
	ns	ns	ns	ns	ns	*	*	*	**	**	**	*

ns — not significant; * — significant at P < 0.05
** — significant at P < 0.01

Table 3. Titres of antibodies to feline panleucopenia and comparison of groups A and AC

	Day 0	Day 21	Day 42	Day 72
Group A	0.08 ± 0.01	0.1235 ± 0.01	0.185 ± 0.01	0.255 ± 0.05
Group AC	0.08 ± 0.01	0.1 ± 0.003	0.15 ± 0.009	0.201 ± 0.005
P	0.93	0.03	0.005	0.01
	ns	*	**	*

ns — not significant; * — significant at P < 0.05
** — significant at P < 0.01

- **group A:** 8 healthy cats (of various breeds), males and females, 7—8 weeks old, were administered a preparation based on cationic peptides and dietary nucleotides (Aminex, AUNIREGEN spol. s r. o., CR) *per os* at a dose of 1 drop.kg^{-1} daily for 3 months,
- **group AC:** 8 healthy cats (of various breeds), males and females, 7—8 weeks old, received no administration of Aminex.

All animals were vaccinated according to a standard vaccination scheme.

Dogs: primo-vaccination (vaccine DHPPi) in week 6 of age (0-sampling), re-vaccination (DHPPi-L) in week 9 (day 21), second re-vaccination (DHPPi-LR) in week 12 of age (day 42). Altogether we carried out 12 samplings at weekly intervals.

Cats: primo-vaccination (Fellocel CVR) in week 9 of age (0-sampling), re-vaccination (Felocell CVR) in week 12 (day 21), second re-vaccination (Rabisin, Merial) in week 15 of age (day 42). Altogether we carried out 4 samplings at 3-week intervals.

Blood was sampled by puncture of *v. jugularis* or *v. cephalica antebrachii*.

METHODS

In groups G and GC, we observed the effectiveness of antirabies vaccination by means of: an enzyme linked immunosorbent assay (ELISA) for the determination of antirabies antibodies; and of vaccination against canine parvovirus disease by means of a haemagglutination-inhibition test (HIT). In groups A and AC we observed the effectiveness of vaccination against feline panleucopenia by means of Feline Panleucopenia Virus Antibody ELISA (B.V. EUROPEAN VETERINARY LABORATORY EVL).

Our results were processed statistically using the Mann-Whitney test.

RESULTS AND DISCUSSION

The level of titres of antirabies antibodies and their statistical comparison is presented in Table 1. Significant differences in production of antirabies antibodies ($P < 0.001$) were recorded from day 56, i. e. two weeks after vaccination. Protective level of antirabies antibodies 1 EU.ml^{-1} (EU — equivalent units) in group G was reached on day 70, i.e. day 28 after vaccination, while in group GC this level was reached on day 85. Haladová *et al.* [2] reported that puppies reached the protective titre of antirabies antibodies on day 28 following the vaccination.

Titres of antibodies to canine parvovirus disease and the statistical comparison of groups G and GC is presented in Table 2. Significant differences in production of antibodies to canine parvovirus ($P < 0.05$) were recorded from day 35 of sampling, i. e. two weeks after re-vaccination. In group G the antibody titre reached the protective level on day 35 of sampling while in group GK on day 49. Navaro-Garcia *et al.* [4] recorded an increased level of antigen-specific antibodies in rats after *per os* administration of glucan.

Titres of antibodies to panleucopenia in cats are presented in Table 3. In group A, significantly increased levels of antibodies ($P < 0.05$) were recorded already on day 21 of sampling. The protective level in this group was reached on day 72 in comparison with group AC where the protective level was not reached.

CONCLUSION

Our results showed that the administration of immunostimulants (preparations of Plerasan and Aminex) increased the effectiveness of vaccination and the immune response of specific antibodies to canine parvovirus and rabies as well as to feline panleucopenia. The administration of glucan and Aminex significantly increased the relevant antibody titres.

ACKNOWLEDGEMENT

The study was supported by the project VEGA 1/0835/12 and Pharmacopola s. r. o.

REFERENCES

1. Gil, A, 2001: New additions to infant formulas. *Pediatric Gastroenterology and Nutrition in Clinical Practice*, Liftschitz, C., (ed.), Marcel Dekker, New York, 113—135.

2. Haladová, E., Mojžišová, J., Smrčo, P., Ondrejková, A., Vojtek, B., Prokeš, M., Petrovová, E., 2011: Immunomodulatory effect of glucan on specific and nonspecific immunity after vaccination in puppies. *Acta Vet. Hung*., 59, 77—86.

3. Kidd, P. M., 2000: The use of mushroom glucans and proteoglycans in cancer treatment. *Alternative Medicine Reviews,* 5, 4—27.

4. Navarro-Garcia, F., Pedroso, M., Lopez-Revilla, R., 2000: Immunomodulation of rat serum and mucosal antibody responses to *Entamoeba histolytica* trophozoites by beta-1,3-glucan and cholera toxin. *Clin. Immunol.,* 97, 182—188.

5. Paulík, Š., Mojžišová, J., Durove, A., Beníšek, Z., Húska, M., 1996: The immunomodulatory effect of the soluble glucan (*Plerotus ostreatus*) on delayed hypersensitivity and phagocytic ability of blood leucocytes in mice. *J. Vet. Med.B*., 43, 129—135.

*

Selected papers from the 56th STUDENT SCIENTIFIC CONFERENCE, Section I, held at the University of Veterinary Medicine and Pharmacy in Košice on April 17, 2013.

FOLIA VETERINARIA, 57, 2: 71—73, 2013

STUDY OF EPIZOOTIOLOGICAL ASPECTS OF FASCIOLOIDOSIS IN SOUTH-WEST SLOVAKIA

Vágó, T., Kočišová, A.

Institute of Parasitology, University of Veterinary Medicine and Pharmacy
Komenského 73, 041 81 Košice
The Slovak Republic

kocisova@uvlf.sk

ABSTRACT

We examined 56 samples of red deer droppings collected in the hunting area of Bodíky and observed 67.9 % prevalence of eggs of *Fascioloides magna* and 64.3 % prevalence of *Paramphistomum cervi*. In one gram of droppings there were on average 68 (15—1976) eggs of *F. magna* and 46 (9—1500) eggs of *P. cervi*. *Post mortem* examination of the infected livers showed a pathological enlargement of this organ caused by pseudocysts of 5—10 cm diameter, which contained mostly two flukes. The surface of the liver was noticeably uneven and arched. The pseudocysts were filled with a dark liquid with a large number of eggs. We isolated from the liver 12 adult *F. magna* in 6 capsules of mean length 69.9 ± 6.09 mm (56—82 mm) and width 31.08 ± 3.42 mm (26—37 mm). During the spring and summer seasons of 2012, we examined 62 snails from families: Lymnaeidae, Viviparidae, Hydrobiidae, Planorbidae, Clausiliidae and Cochlicopidae. Eight (12.9 %) *Lymnaea stagnalis* snails were positive and allowed us to isolate both sporocysts and cercaria.

Key words: *Fascioloides magna*; pathological-anatomical changes; prevalence; snails

INTRODUCTION

The great liver fluke (*Fascioloides magna*) is a very aggressive fluke worm and under European conditions belongs among the most pathogenic parasites of the liver parenchyma of cloven-hoofed game and domestic ruminants.

The aim of the study was to observe the prevalence and intensity of *Fascioloides magna* infection in red deer, evaluate pathological-anatomical changes in the liver infested with flukes and the influence of individual factors (climate, geography, animal, intermediate hosts) that affect the spreading of fascioloidosis in south-West Slovakia.

MATERIALS AND METHODS

Samples of droppings were collected in the area of a flood-plain forest, approximately 10 km north of the Gabčíkovo dam, close to the village of Bodíky, in the district of Dunajská Streda. Using a sedimentation method [2], we examined 56 samples of red deer droppings. In addition, we examined 62 snails from the same area. The collected snails were exposed to light and warmth for 6 hours and their secretions and smears of the content of body cavity were examined microscopically for the presence of cercaria and sporocysts [1]. The liver of a 5 year old hind was subjected to partial parasitological *post mortem* examination using a standard procedure [2].

RESULTS AND DISCUSSION

Of the total number of 56 samples of red deer droppings, 38 (67.86 %) were positive for eggs of *F. magna*. In addition,

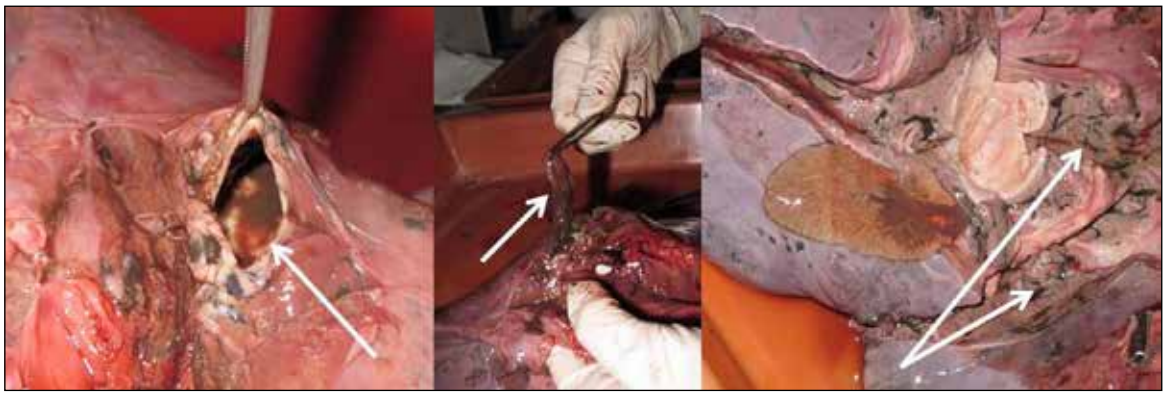


Fig. 1. Pseudocyst in the liver with a dark brown content (left);
extraction of *F. magna* from the liver (centre); liver parenchyma damaged by parasites (right)

Fig. 2. *Fascioloides magna* isolated from the liver; total view (right)
and detail of oral and abdominal suckers (left)

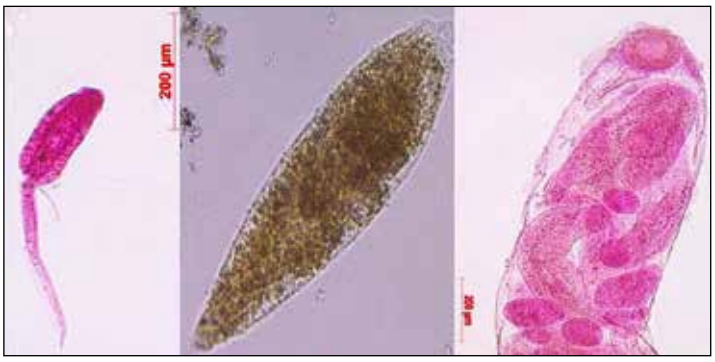


Fig. 3. Cercaria (left); native sporocyst in the smear (centre);
stained sporocyst isolated from snail (right)

eggs of *P. cervi* were found in 36 (64.29 %) of these samples. The intensity of occurrence of *F. magna* eggs ranged from 3 to 395 in 1g of droppings and the mean number of eggs per gram (EPG) was 68.1. Visual examination of the liver showed pathological enlargement of this organ, its anaemic colouration with dark blue to black spots and streaks of varying sizes that signified traces of juvenile stages of *F. magna* migrating through the liver parenchyma (Fig. 1). Helminthological *post mortem* examination allowed us to isolate the parasites from the typical pseudocysts. The pseudocysts contained characteristic dark brown viscous liquid with large number of eggs. Altogether we found 12 adult *F. magna* (Fig. 2) in 6 capsules of mean length 69.9 ± 6.09 mm (56—82 mm) and width 31.08 ± 3.42 mm (26—37 mm). The necessary conditions for spreading of *F. magna* to new places and forming new enzootic areas include mutual occurrence of intermediate hosts, the snails, and definitive hosts, the cloven-hoofed game, their sufficient number and density and a humid environment. If one of these conditions is not met, the parasite cannot develop and spread.

Of the 62 collected and examined snails, positive confirmation was found in 8 individuals (12.9 %). All positive snails belonged to the family *Lymnaeidae*, great pond snail (*Lymnaea stagnalis*). Their examination showed the presence of both sporocysts and cercaria (Fig. 3).

Generally one can assume that the introduction of a foreign parasite into the environment with conditions optimal for its entire developmental cycle and the lack of defence mechanisms in its new hosts, will result in a development of massive enzootic focus in a relatively short time. However, in the long term, a selective adaptation of host animals towards resistance to the parasite most likely develops resulting in equilibrium in the particular environment [6]. Extensive survey of helminth-fauna of cloven-hoofed game in the territory of Slovakia before 1988 [3] failed to diagnose *F. magna,* not even in the area of Žitný ostrov [4], [5]. Only in 1988, the giant liver fluke was found for the first time in the liver of a hind which was found dead on the construction site of the inlet canal of Gabčíkovo dam [6]. The occurrence of fasciloidosis in red deer and roe deer in the area of Gabčíkovo dam has been systematically monitored since 1991. The prevalence and intensity of infection gradually increased reaching a maximum level in 1995 (91.3 %, with 2—107 parasites in red deer and 60 % with 1—6 parasites in roe deer). It is obvious that re-infection of cloven-hoofed game in enzootic areas occurs as a rule every year.

CONCLUSION

The results presented allowed us to state that the prevalence of *F. magna* in the area of a flood-plain forest in the district of Dunajská Streda, north of the Gabčíkovo dam, Slovakia, increased considerably. It is a suitable biotope for the development and long-term maintenance of this parasite.

REFERENCES

1. Faltýnková, A., Horáčková, E., Hirtová, L., Novobilský, A., Modrý, D., Scholz, T., 2006: Is Radix peregra a new intermediate host of *Fascioloides magna* (Trematoda) in Europe? Field and experimental evidence. *Acta Parasitologica*, 51, 87—90.

2. Letková, V., Čisláková, L. et al., 2010: *Laboratory Diagnostic Methods in Veterinary Parasitology* (In Slovak). UVLF in Košice, Editorial centre, 110 pp.

3. Mituch, J., 1992: Helminths of roe deer and red deer in the High Tatras. *Folia Veterinaria*, 22, 47—52.

4. Rajský, D., Beladičová, V., 1988: Study on helminth-fauna in roe deer and red deer in Žitný ostrov (In Slovak). *Veterinářství*, 37, 120—121.

5. Rajský, D., Nagy, T., 1988: Copro-helminthological investigation of field roe deer ecofarmin Žitný ostrov (In Slovak). *Folia Venatoria*, 18, 347—351.

6. Rajský D., Patus, A., Bukovjan, K., 1994: The first finding of *Fascioloides magna* (Bassi, 1875) in Slovakia (In Slovak). *Slovenský Veterinársky Časopis* (Slovak Veterinary Journal), 19, 29—30.

*

Selected papers from the 56th STUDENT SCIENTIFIC CONFERENCE, Section I, held at the University of Veterinary Medicine and Pharmacy in Košice on April 17, 2013.

FOLIA VETERINARIA, 57, 2: 74—76, 2013

THE EFFECTIVENESS OF DIFFERENT TYPES AND FORMS OF ECTOPARASITE CONTROL PREPARATIONS IN DOGS

Pružinská, K., Kočišová, A.

Institute of Parasitology, University of Veterinary Medicine and Pharmacy
Komenského 73, 041 81 Košice
The Slovak Republic

kocisova@uvlf.sk

ABSTRACT

The aim of our study was to observe and compare the effectiveness of selected types and forms of ectoparasitic preparations applied to dogs in 12 different geographical locations in the Prešov district. The dogs were treated with preparations in four different application forms: i. e., collar, spot-on, powder and spray. Before application, we collected 103 ticks from these dogs. Of this, there were 102 *Ixodes ricinus* (99 %) and one *Dermacentor reticulatus*. We also collected 40 fleas. The most prevalent were fleas of the genus *Ctenocephalides canis* (92.5 %) and we also found one (2.5 %) each of; *Ctenocephalides felis, Nosopsyllus fasciatus* and *Archaeopsylla erinacei*. Insecticide-impregnated collars were given to 13 dogs after removal of all ticks. During 6 weeks we found neither ticks nor fleas on these dogs. Fourteen dogs were treated with a spot-on preparation. Preparation Frontline Combo showed 84.4 % effectiveness while with ExSpot, the effectiveness was 100 %. The effectiveness of the powder form varied between 42 % and 86 %. Eleven dogs were treated by spraying and this application was quite demanding because the animals showed signs of fear due to the sound produced by spraying. When evaluating the effect of spraying, we can conclude that the ticks and fleas present on body surfaces were killed during the treatment, however, this application form failed to ensure long-term protection of the animals.

Key words: dog collar; ectoparasite control preparation; flies; powder; spot-on; spray

INTRODUCTION

In the autumn of 2011, at the German Shepherd championship in Kiev, 16 dogs got babesiosis and 4 of them died. Similar problems occurred in 2012 at the Federation Cynologique Internationale (FCI) world championship in Hungary. The disease is stressing and financially demanding for the owners of none-competing dogs, but for the owners of sporting dogs it is a serious issue affecting the sport career of their animals. Treatment and convalescence is demanding and time consuming and sometime the dog cannot be any more subjected to a full training load and the week-lasting stress associated with the competition events. The increasing incidence of canine diseases transmitted by parasites and spreading of parasites to new areas motivated pharmaceutical companies to search for new effective ectoparasite preparations.

The aim of our study was to observe and compare the effectiveness of selected types and forms of ectoparasite control preparations under various conditions of dog breeding in Prešov district.

MATERIALS AND METHODS

This research was carried out in July and October 2011 and March and August 2012. The study included 68 dogs of both sexes, various breeds and ages (6 weeks to 10 years). The dogs originated from 12 geographical locations in the Prešov district, Slovakia. Ectoparasites, ticks and fleas, were collected directly from dogs in order to determine the proportion of species and intensity of in-

Table 1. Number of dogs treated with individual application forms of antiparasitic preparations

Application form	Commercial name	Active ingredient(s)	Number of treated dogs
Collar	Kiltix	Propoxur, Flumetrin	8
	Scalibor	Deltametrin	5
Spot-on	Frontline Combo	Fipronil	8
	Frontline Certifect	Fipronil, (S)-metopren, Amitraz	2
	Ex-spot	Permetrin	4
Powder	Bolfo	Propoxur	10
Spray	Bolfo	Propoxur	10
	Frontline	Fipronil	1

fection before application of the insecticides. We treated 48 dogs which were divided to 4 groups according to the form of antiparasitic preparations administered. The following 4 application forms were tested: dog collar, spot-on, powder, and spray (Table 1). The fifth group (n = 20) served as the control. They were observed for 4—6 weeks after treatment depending on the form of preparation and the information on the guaranteed time of effectiveness provided by the producer.

The percentage effectiveness of preparations was calculated on the basis of the number of parasites on treated dogs compared to the control (untreated) animals.

RESULTS AND DISCUSSION

We collected 103 ticks and 40 fleas from the investigated dogs. Of the collected ticks 102 (99 %) were Ixodes ricinus and one (1 %) belonged to the species Dermacentor reticulatus. Of the fleas, the most frequent was the dog flea Ctenocephalides canis (37; 92,5 %). We also collected one cat flea Ctenocephalides felis (2.5 %) and the fleas; Nosopsyllus fasciatus (1; 2.5 %), and Archaeopsylla erinacei (1; 2.5 %).

When comparing the species proportion of collected ticks and fleas with the results of Polish authors Zygner *et al.* [4], we can see that they collected more ticks Dermacentor reticulatus (64.6 %) and a lower proportion of Ixodes ricinus (35.4 %). In 2009, similar research was carried out in Great Britain, where the proportion of Ixodes ricinus was 72.1 % and of Dermacentor reticulatus only 0.68 % [3]. According to Krämer and Mencke [2] the fleas parasitizing dogs were *C. felis* [2]. Our results agreed with those reported in Hungary according to which *C. canis* was the more frequent parasite on dogs [1].

Dog collars were put on 13 dogs (group 1), which were previously infested with ticks (100 %) and two of them also had fleas (5.4 %). Application of the collar was very simple and the animals showed no signs of unrest, aggressiveness, or fear. Ticks were found on three dogs (23 %) but they were not attached to the surface of hair up to two days after putting collars on dogs. During the 6-week observation period, we found no other ticks or fleas on the dogs.

Spot-on preparations were used to treat 14 dogs (group 2) of which 12 were infested with ticks (85.7 %). Preparation Frontline Certifect was tested on 8 dogs. The first ticks were found at 5 weeks after application. According to the producer, the guarantee period (absence of ticks) of preparation Frontline Combo is 4 weeks. The effectiveness of this preparation after 3 weeks was 87 %, after 4 weeks 83.3 % and after 5 weeks 79.6 %. Four animals in this group were treated with preparation Ex-Spot. The protocol about repeated applications was observed in two dogs and these dogs were free of ectoparasites throughout the observation period. The effectiveness of this preparation after 4 weeks was 87.5 % and after 5 weeks 75 %.

The powdered preparation Bolfo was used to treat 10 dogs (group 3), all of them previously infected with ticks (100 %). According to the producers, the preparation is effective for 4—7 days and at high infestation, the treatment should be repeated 1—2 times per week. The application is time demanding and requires protective devices. There was a problem with the application to young temperamental dogs which tended to touch and lick the powder. Especially problematic was the application to the area of head, abdomen and tail. The effectiveness of the powder was as follows: after 3 days 62.5 %; after four 61.1 %; after five 57.1 %; and after seven days 48.1 %.

Eleven dogs were sprayed with the respective preparations (group 4). This way of application is even more demanding than that of the powder form. The animals showed signs of fear due to the sound produced during spraying.

Particularly problematic was spraying of head, neck, breast and abdomen as the animals resisted the treatment. The effectiveness of this application form reached 80 % on the day of treatment and within two days of treatment it decreased to 56 %, within 4 days to 52 %, within 7 days to 40 %, and within 9 days to 24 %. When evaluating the effect of this form we can state that although it kills ticks and fleas on the surface of the animal's body, it is unsuitable for long-term protection of these animals.

CONCLUSION

The results obtained indicate that the Slovak dog owners have at their disposal antiparasitic preparations with sufficient insecticidal effectiveness from which they can choose according to the costs and length of effectiveness. However, to ensure optimum protection, it is necessary to respect recommendations of producers regarding the way of their application.

REFERENCES

1. Farkas, R., Gyurkovszky, M., Solymosi, N., Beugnet, F,. 2009: Prevalence of flea infestation in dogs and cats in Hungary combined with a survey of owner awareness. *Med. Vet. Entomol.*, 23,187—194.

2. Krämer, F., Mencke, N., 2001: *Flea Biology and Control.* Springer-Verlag Berlin, 192 pp.

3. Smith, F. D., Ballantyne, R., Morgan, E. R., Wall, R., 2011: Prevalence, distribution and risk associated with tick infestation of dogs in Great Britain. *Medical and Veterinary Entomology*, doi: 10.1111/j. 1365-2915.2011.00954.x

4. Zygner, W., Górski, P., Wędrychowicz, H., 2009: New localities of Dermacentor reticulatus tick (vector of Babesia canis canis) in central and eastern Poland. *Polish Journal of Veterinary Science,* 12, 549—555.

*

Selected papers from the 56th STUDENT SCIENTIFIC CONFERENCE, Section I, held at the University of Veterinary Medicine and Pharmacy in Košice on April 17, 2013.

FOLIA VETERINARIA, 57, 2: 77—79, 2013

COMPARISON OF SELECTED METABOLIC PARAMETERS IN OBESE AND NON-OBESE HORSES

Pošivák, J.[1], Izsáková, E.[1], Novotný, F.[1]
Valocký, I.[1], Boldižár, M.[1], Pošiváková, T.[2]

[1]Clinic for horses, University of Veterinary Medicine and Pharmacy
Komenského 73, 041 81 Košice
[2]Department of Ecology, Faculty of Humanities and Natural Sciences
Prešov University in Prešov, 17 Novembra 1, 081 16, Prešov
The Slovak Republic

posivak@uvlf.sk

ABSTRACT

Insulin resistance has acquired great importance in recent years due to its involvement in several equine disorders. It is therefore of interest to determine which factors play a role in the development of this resistance in order to prevent this condition and thus also the consequential disorders. The results of our studies showed that obesity and high insulin levels have a negative effect on the oestrus cycle in mares, as both the oestrus intervals (35 days *vs.* 25 days) and the luteal phases of oestrus were prolonged in affected mares (31 days *vs.* 16 days) ($P < 0.05$). In the obese horses we found significantly higher levels of insulin (14.34 ± 0.84 mmol.l^{-1} *vs.* 6.92 ± 0.62 mmol.l^{-1}) ($P < 0.05$) in blood plasma.

Key words: horse; insulin; insulin resistance; obesity

INTRODUCTION

Obesity has recently become a very popular topic in the field of animal research. Very often we come across this issue in horses. One of the paramount issues, is the impact of various factors on obesity and consequently on the relevant therapy and especially on prevention. Obesity is a pathological condition affected by many factors, especially the diet and exercise. Inappropriate feeding regimen, as well as the composition of the diet, lack of exercise and workload, lead to obesity. The natural tendency to overweight and obesity is observed particularly in primitive breeds of horses and the majority of ponies, but often it also occurs in the noble breeds.

MATERIALS AND METHODS

Five mares were included in this study. Three of them were obese and were fed 3 times a day a total of 2 kg of concentrates. Two mares were feed-restricted during two months, and were fed 3 times per day a total of 0.75 kg concentrates. Hay was fed normally to all horses, 3 times per day. The body weight was estimated in the morning by the use of a standard weight tape and body condition scoring (BCS), using a scale of 0 to 9 [1].

Blood samples were taken in the morning at 9.00 a. m. The blood was collected in a vacutainer, heparin and a serum tube. Thereafter, it was centrifuged at 1800—2000 r. p. m. for 15 minutes to obtain the blood plasma and serum [2]. Insulin was measured in the blood plasma using the ELISA method (set EIA 2340, Roner), while the glucose and triglycerides were measured in the blood serum by biochemical methods (ALIZE, Lisabio, Merieux and Randox). The significance of differences were evaluated by the *t*-test.

RESULTS AND DISCUSSION

The results of insulin, triglycerides and glucose levels in the blood plasma and blood serum of obese and non-obese horses are shown in Table 1 and Fig.1.

Table 1. Insulin, triglycerides, glucose levels in blood plasma and blood serum of obese and non-obese horses

Horse	Insulin		Triglycerides		Glucose	
	Obese [mmol.l^{-1}]	Non-obese [mmol.l^{-1}]	Obese [mg.dl^{-1}]	Non-obese [mg.dl^{-1}]	Obese [mmol.l^{-1}]	Non-obese [mmol.l^{-1}]
1	13.5	7.3	32.8	27.1	84.7	92.8
2	14.5	7.2	33.5	26.6	83.9	94.5
3	14.3	6.8	34.1	25.5	84.4	93.4
4	14.8	6.3	33.6	27.2	84.3	94.2
5	14.6	7.0	32.9	26.7	84.2	92.6
Mean	**14.36**	**6.92**	**33.38**	**26.62**	**84.3**	**93.5**

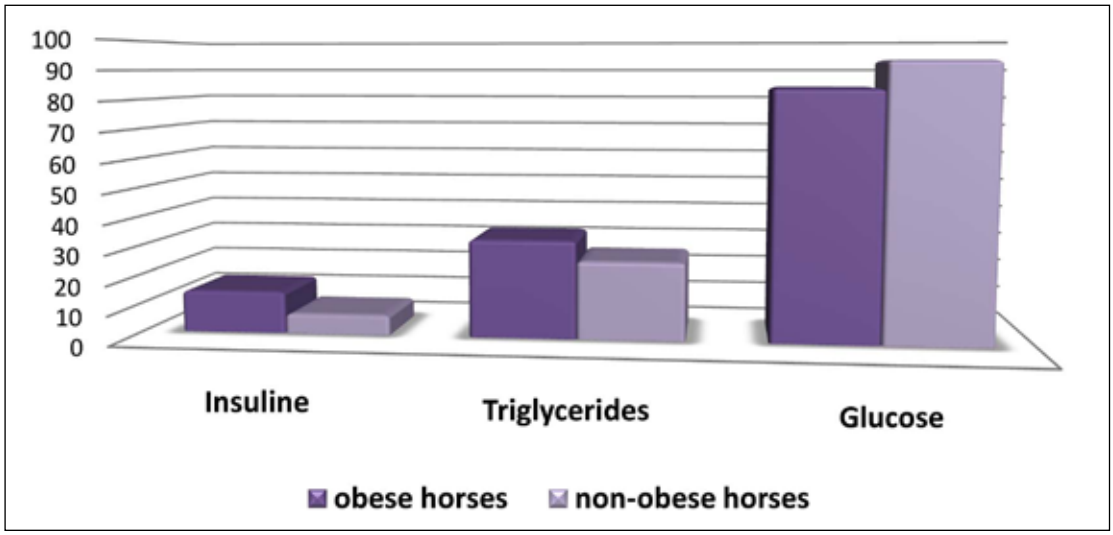


Fig. 1. Insulin, triglycerides, glucose levels in blood plasma and blood serum of obese and non-obese horses

We can conclude that the obesity and hyperinsulinaemia are related as obese horses generally had: higher insulin concentrations in the blood plasma (14.34 ± 0.84 mmol.l^{-1} *vs.* 6.92 ± 0.62 mmol.l^{-1}) ($P < 0.05$); higher concentrations of triglycerides (33.43 ± 0.93 mg.dl^{-1} *vs.* 26.5 ± 1.11 mg.dl^{-1}) ($P < 0.05$) in the blood serum; and lower concentrations of glucose (84.7 ± 0.72 mmol l^{-1} *vs.* 93.07 ± 1.65 mmol l^{-1}) ($P < 0.05$) in the blood serum than non-obese horses. If high levels of circulating insulin are present for longer time, it may eventually lead to the development of insulin resistance [3], [4]. Furthermore, obesity and insulin resistance have a negative effect on the oestrus cycle in mares, as both the oestrus interval (35 days *vs.* 25 days) and the luteal phases of oestrus (31 days *vs.* 16 days) were prolonged in the affected mares ($P < 0.05$). In the examined obese mares we observed an increase in insulin concentrations, lower insulin sensitivity and overall prolonged oestrus interval, meaning that ovulation was delayed in these mares while in the feed-restricted mares after reduction of body weight and body fat, the oestrus interval was not affected and the mares came into season as expected [5]. Moreover, the obese mares had almost twice as long luteal phases than the feed-restricted mares.

ACKNOWLEDGEMENTS

This research was supported by VEGA 1/0498/12.

REFERENCES

1. Carter, R. A., McCutcheon, L. J., Valle, E., Meilahn, E. N., Geor, R. J., 2010: Effects of exercise training on adiposity, insulin sensitivity, and plasma hormone and lipid concentrations in overweight or obese, insulin-resistant horses. *Am. J. Vet. Res.,* 71, 314—321.

2. Carter, R. A., McCutcheon, L. J. George, L. A. Smith, T. L. Frank, N., Geor, R. J., 2009: Effects of diet- induced weight gain on insulin sensitivity and plasma hormone and lipid concentrations in horses. *Am. J. Vet. Res..*, 70, 1250—1258.

3. Firshman, A. M., Valberg, S. J., 2007: Factors affecting clinical assessment of insulin sensitivity in horses. *Equine Vet. J.*, 39, 567—575.

4. Geor, R. J., Pratt, S. E., McCutcheon, L. J., 2008: Repeatability of two methods for assessment of insulin sensitivity and glucose dynamics in horses. *J. Vet. Intern. Med.,* 19, 883—888.

5. Tinworth, K. D., Harris, P. A., Sillence, M. N., Noble, G. K., 2009: Potential treatments for insulin resistance in the horse: A comparative multi-species review. *Vet. J.*, 10, 1016.

*

Selected papers from the 56th STUDENT SCIENTIFIC CONFERENCE, Section I, held at the University of Veterinary Medicine and Pharmacy in Košice on April 17, 2013.

FOLIA VETERINARIA, 57, 2: 80—82, 2013

BACTERIAL DISEASES OF DOGS AND CATS

Juríčková, Z., Fialkovičová, M.

Section of internal diseases, Small Animal Clinic
University of Veterinary Medicine and Pharmacy, Komenského 73, 041 81 Košice
The Slovak Republic

zuzana.jurickova@post.sk

ABSTRACT

This study investigated the intensity of occurrence of individual bacterial diseases of the skin in dogs and cats during a period of 1.5 years. In this period we diagnosed individual bacterial skin disorders in 20 dogs and three cats of ages ranging from 8 months to 12 years. In the patients, we also observed haematological parameters, leucograms and the frequency of occurrence of bacterial agents. The most frequent diagnoses in dogs were pyotraumatic dermatitis (20 %), impetigo (20%) and intertrigo (15 %). Less frequent diseases were; surface folliculitis (10 %), deep pyoderma (10 %) and pododermatitis (10 %). The least frequent disorders included; mucocutaneous pyoderma (5 %), deep folliculitis (5 %) and furunculosis (5 %). In each cat we recorded; surface folliculitis, subcutaneous abscesses and feline acne. The most frequent bacterial agents in dogs were *Staphylococcus intermedius* (33 %) and *Staphylococcus aureus* (29 %).

Key words: cat; dog; pyoderma; skin; *Staphylococcus intermedius*

INTRODUCTION

Bacterial diseases of skin (pyodermies) are pyogenic infections that belong among the most common dermatoses in dogs. These infections occur most frequently due to impaired integrity of the skin surface and the subsequent penetration of bacterial microflora. The high incidence of bacterial diseases of the skin in dogs results from the combination of microbiological, histological, hygiene, epidemiological and iatrogenic factors [2]. Frequently, they occur as secondary infections of; autoimmune, endocrine or allergic diseases. Pyodermies are relatively rare in cats due to the lower numbers of bacteria on healthy skin and hair, and the increased care about their hair which they exhibit. They may occur particularly in association with previous traumas [5].

The aim of this study was to investigate the intensity of occurrence of individual bacterial diseases on the skin in dogs and cats. Additionally, the relationship between age and sex and the development of bacterial skin diseases in dogs and cats and the intensity of occurrence of bacterial agents were also investigated.

MATERIALS AND METHODS

This study was carried out on 20 dogs and 3 cats at the Section of Internal Diseases of the Small Animal Clinic of the University of Veterinary Medicine and Pharmacy in Košice. In addition to obtaining the anamnesis, the patients were subjected to a total clinical examination, which included a dermatological examination. The bacteriological examination of the patients included taking a swab of the skin lesions. The samples were sent for bacteriological cultivation and susceptibility to antibiotics and chemotherapeutics. The dermatological laboratory examination included taking skin scrapings for the determination of the presence of parasites and material was collected for mycological examinations. We determined also

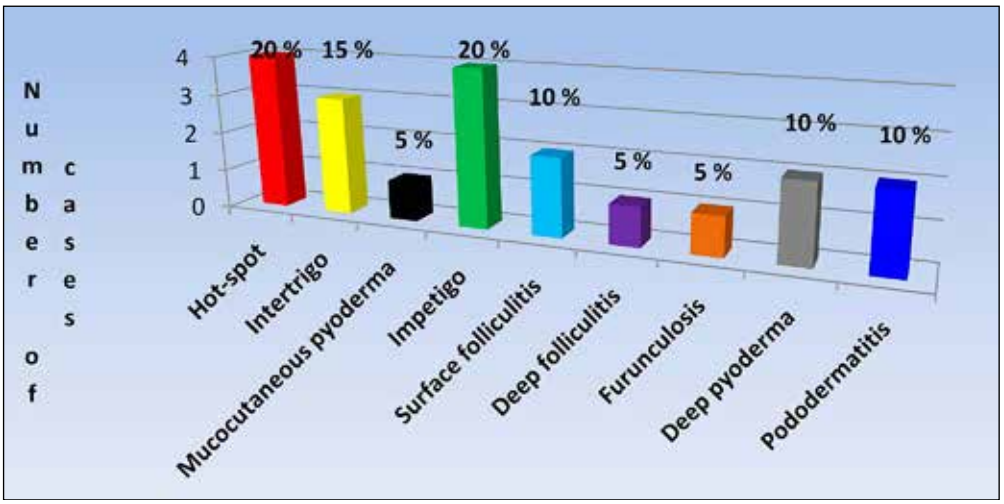


Fig. 1. Percentage of dogs suffering from individual bacterial diseases of the skin ($n_1 = 20$)

the haematological parameters and for the sake of differential diagnostics, particularly to exclude autoimmune and endocrine dermatoses, selected number of dogs ($n_x = 5$) were subjected to biochemical examinations.

RESULTS AND DISCUSSION

Individual bacterial diseases of the skin diagnosed in the observed dogs, were divided into three groups on the basis of the depth of skin affected. The predominant group of diseases were infections of the skin surface (40 %) that included; pyotraumatic dermatitis, intertrigo and mucocutaneous pyoderma. Less frequent (30 %) were surface bacterial infections such as impetigo and surface folliculitis. Deep bacterial infections amounted to 30 %. This group included the following diagnoses: deep folliculitis, furunculosis, deep pyodermy and pododermatitisia a pododermatitída. The most frequent diagnoses were pyotraumatic dermatitis and impetigo. The percentage of individual bacterial diseases of the skin of dogs is shown in Fig. 1. In cats, we diagnosed; feline acne, surface folliculitis and subcutaneous abscess. Of the total number of dogs ($n_1 = 20$), 25 % were females and 75 % males and all of the cats ($n_2 = 3$) were males (100 %). The highest occurrence of the skin diseases were recorded in young dogs between the first and third year of life, and in adult individuals, four to eight years old. The affected cats were 5 to 10 years old.

The cultivation of the samples collected from dogs, showed that the most predominant agents were *Staphylococcus intermedius* (33 %) and *Staphylococcus aureus* (29 %). Other identified bacterial agents included; *Staphylococcus epidermidis*, *Staphylococcus saprophyticus*, *Proteus* spp., *Streptococcus ß-haemolyticus*, *Corynebacterium* spp. and *Enterococcus fecalis.* The cultivation of samples from cats, confirmed the presence of; *Pasteurella multocida,* ß-haemolytic streptococci and *Staphylococcus epidermidis*. The haematological examinations revealed an increased level of leukocytes in 10 dogs (53 %) out of the 19 examined and also in the tomcat with an abscess. The level of erythrocytes was decreased in three dogs. None of the animals showed increased level of erythrocytes.

Pyotraumatic dermatitis is a very frequent dermatosis. The presence of fleas or a foreign body in the auditory canal played a dominant role in its etiology as they provoke the animal towards autotraumatisation of the respective site. Folliculitis is one of the most frequent pyodermies in dogs, but is relatively rare in cats [1], [3], [4], [5], [6]. In cats, the most frequent bacterial infection of the skin and subcutis results in subcutaneous abscesses [5].

CONCLUSION

The occurrence of bacterial skin diseases is frequently associated with insufficient hygiene. Veterinarians can play an important role in the prevention of these diseases by stressing the importance of increased hygiene, educating the owners and recommending preventive application of ectoparasitic preparations.

REFERENCES

1. Foster, A. P., Foil, C. S., 2003: *Bsava Manual of Small Animal Dermatology*. 2nd, edn., BSAVA, 160—161.

2. Guaguére, É., Prélaud, P., 2008: *A Practical Guide to Canine Dermatology*. Merial, 65.

3. Hnilica, K. A., 2011: Small *Animal Dermatology: A Colour*

Atlas and Therapeutic Guide. 3th edn., Elsevier Inc., 37, 41.

4. Nuttall, T., Harvey, R. G., McKeever, P. J., 2009: *Skin Diseases of the Dog and Cat*. 2nd edn., Manson Publishing Ltd, 18.

5. Scott, D. W., Miller, W. H., Griffin, C. E., 2001: *Muller & Kirk's Small Animal Dermatology*. 6th edn., Saunders, 274—335.

6. Svoboda, M., et al., 2000: *Diseases of Dog and Cat* (In Czech). Part I, ČAVLMZ, Brno, 392, 398.

*

Selected papers from the 56th STUDENT SCIENTIFIC CONFERENCE, Section II, held at the University of Veterinary Medicine and Pharmacy in Košice on April 17, 2013.

FOLIA VETERINARIA, 57, 2: 83—85, 2013

COMPARISON OF LEVELS OF BIOGENIC AMINES IN SOME TRADITIONAL FOODS FROM VARIOUS COUNTRIES

Čornejová, T., Dičáková, Z., Lojanová I., Bystrický, P.

Department of Food Hygiene and Technology
University of Veterinary Medicine and Pharmacy, Komenského 73, 041 81 Košice
The Slovak Republic

dicakova@uvm.sk

ABSTRACT

Levels of biogenic amines: histamine, tyramine, putrescine, cadaverine, phenylethylamine, tryptamine, spermine and spermidine in foods from various countries were analysed by thin layer chromatography (TLC). Amines were extracted from samples with trichloroacetic acid and derivatised with dansyl chloride.

Keu words: biogenic amines; food; TLC chromatography

INTRODUCTION

Biogenic amines (BA) present in food are low-molecular compounds that are part of the normal metabolism in micro-organisms, plants, humans, and other animals. On the one hand, they are essential for human organisms (participate in the regulation of body temperature and blood pressure, synthesis of proteins, affect the function of the nervous system and digestive tract, and function as hormones and source of body reserves). On the other hand, their increased concentrations may cause health problems in some individuals. Higher levels of biogenic amines in food may result in gastrointestinal disorders, such as nausea, diarrhoea, allergic reactions (flushing) and even blood pressure disorders and migraine. Almost all foods that contain proteins or free amino acids can contain biogenic amines. The total level of various BA in food depends on its character and the presence of micro-organisms [1], [3]. The content of BA in food can indicate its quantity and quality of the raw materials and also hygiene level, during the technological production process and food storage.

The aim of this study was to develop, as simple as possible, the process for the extraction of histamine (HIS), tyramine (TYR), putrescine (PUT), cadaverine (CAD), phenylethylamine (PHE), tryptamine (TRY), spermine (SPM) and spermidine (SPD) and to determine the levels of these biogenic amines in various foods by the thin layer chromatography (TLC) method.

MATERIALS AND METHODS

Standard mixtures of biogenic amines were prepared using individual amines in the form of chlorides and dansyl chloride (Sigma) which was employed for derivatisation. All other chemicals were used from Fy Lachner. We used an analytical balance, homogenisers, centrifuges, water baths, rotary vacuum evaporators, ultrasound baths, UV boxes, Alltech development chambers, TLC plates ALUGRAM SIL G/UV, and cellulose-acetate syringe filters (ALBET) 0.45 µm.

The samples were obtained from foods purchased in food stores during the period from September 2011 to October 2012. Fresh fruits and vegetables were bought from the market in October 2011.

We used 10 g samples for extraction of BA (fish, meat products, cheeses, fruit, and vegetables). The individual sample was transferred to a graduated cylinder and 5 % trichloroacetic acid solu-

Table 1. Comparison of the level of biogenic amines in milk products [mg.kg^{-1}]

BA	Hermelín	Halloumi	Gouda	Camembert	Niva	Olomoucké syrečky	Bryndza Slatina	Tatranská bryndza
PHE	–	–	–	–	5	–	–	–
HIS	8	1	10	–	–	80	2	6
TYR	10	–	260	–	–	120	90	3
SPM	–	–	–	–	–	10	–	–
SPD	–	–	–	–	–	20	–	–
CAD	2	–	40	3	–	–	10	6
TRY	–	–	–	–	–	–	–	–
PUT	19	–	100	10	–	2	50	20

Table 2. Comparison of the level of biogenic amines in foods [mg.kg^{-1}]

BA	Carp	Mackerel	Smoked meat	Prosciutto	Wine white	Wine red	Beer Pilsner	Beer Heineken
PHE	–	–	–	3	–	–	–	–
HIS	1	2	80	–	1	4	6	2
TYR	–	2	100	2	1	3	3	1
SPM	–	–	–	–	–	–	–	–
SPD	–	–	–	–	–	–	–	–
CAD	–	4	2	–	–	–	6	4
TRY	–	–	–	–	–	–	–	–
PUT	–	1	20	–	2	10	18	8

Table 3. Comparison of the level of biogenic amines in vegetable and fruit [mg.kg^{-1}]

BA	Cucumber	Tomato fresh	Tomato stored	Olives pickled	Apple fresh	Apple pickled
PHE	–	2	2	5	–	–
HIS	–	2	2	100	–	–
TYR	3	2	2	5	–	–
SPM	–	2	2	5	–	–
SPD	5	3	2	5	–	–
CAD	–	–	–	–	–	–
TRY	–	–	–	–	–	25
PUT	12	25	20	20	–	–

tion was added up to the 50 ml mark. For the testing of liquids, we used 25 ml samples to which we added 500 mg of crystalline trichloroacetic acid. The sample solutions were then homogenized and centrifuged (3600 r. p. m.; 4 °C; 10 min) and filtered by means of a syringe membrane filter.

Derivatisation: to 1 ml of the filtrate we added 0.5 ml of saturated hydrogen sodium carbonate and 0.5 ml of a dansyl chloride solution in acetone and incubated the mixture at 70 °C for 10 min. The dansylated samples were evaporated to dryness and dissolved in 2 ml of acetonitrile. To derivatise the standards, we used 50 ml of each amine (stock solutions with 500 mg amine in 1 ml of solution) and the same procedure as for the samples with the exception of the last step, when the standards were dissolved in 50 ml acetonitrile [2].

To analyse BA in food samples, we used TLC plates. Derivatised standard mixtures of BA (from 5 to 40 ml) and extracts of food samples (20 ml) were applied to the TLC plates. The plate was first put into a TLC chamber saturated with Ist mobile phase: chloroform: diethylether: triethylamine (6 : 4 : 1). After the development to a height of 14 cm, the plate was allowed to dry, transferred to another chamber with IInd mobile phase: chloroform : triethylamine (6 : 1) and allowed to develop up to 14 cm. After drying, the separated spots of BA were compared with the standard spots under a UV lamp at 365 nm [4].

RESULTS AND DISCUSSION

The results of the chromatographic separation of standards and some food samples are presented in Fig. 1. Qualitative analysis was performed on the basis of the position of the individual spots with regard to the base level and quantitative analysis was based on comparison of the intensity of the spot colouration with the spots of the standards of known concentration. The concentrations of BA in the analysed samples are shown in Tables 1 to 3.

The method used in our study is suitable for both samples with higher concentrations and foods with low level of BA. The highest concentrations of BA, particularly tyramine, were found in long aged Gouda (the Netherlands), Olomoucké syrečky (the Czech Republic) and in byndza (aged sheep cheese, Slovakia). On the other hand, biogenic amines levels were low in certain cheeses: Halloumi (Cyprus), Camembert (France) and Niva (Slovakia).

High levels of tyramine was found in the samples of smoked meat and pickled olives containing a lot of histamine. The levels of biogenic amines in the samples of fish, beverages, fruit and vegetable were relatively low. Our results were in accordance with published data (1, 3).

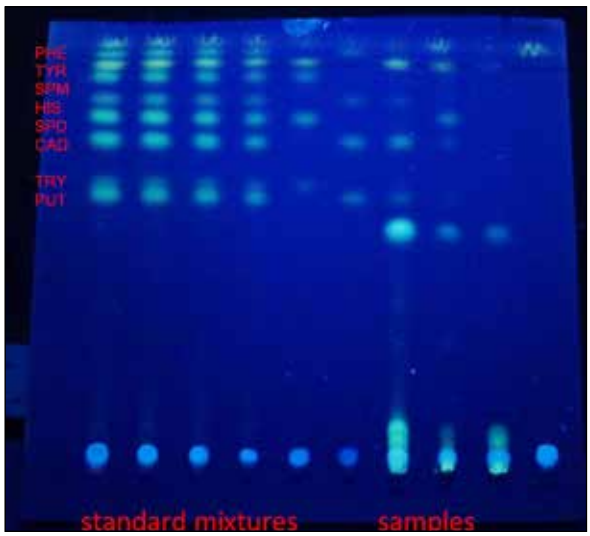


Fig. 1. Example of chromatographic separation of biogenic amines

ACKNOWLEDGEMENTS

The study was supported by the projects APVV SK-CN-0028-12 and Kega No. 011UVLF4/2012.

REFERENCES

1. Askar, A., Treptow, H., 1986: B*iogene Amine in Lebensmitteln. Vorkommen, Bedeutung und Bestimmung.* Ulmer, Stuttgart, 198 pp.

2. Dičáková, Z., Máška, L., 2011: TLC chromatography used in rapid detection of biogenic amines in dairy products (In Slovak). In P*roceedings of the 6th European Symposium (agriculture-commerce-services)*, Tatranské Matliare, March 23—25, Elsewa, Košice, 1—7.

3. Křížek, M., Kalač, P., 1998: Review. Biogenic amines and their role in nutrition (In Czech). *Czech J. Food Sci.*, 16, 151—159.

4. Lapa-Guimarães, J., Pickova, J., 2004: New solvent systems for thin-layer chromatographic determination of nine biogenic amines in fish and squid. *J. Chromatogr. A.*,1045, 223—232.

*

Selected papers from the 56th STUDENT SCIENTIFIC CONFERENCE, Section III, held at the University of Veterinary Medicine and Pharmacy in Košice on April 17, 2013.

FOLIA VETERINARIA, 57, 2: 86—87, 2013

RAPID SCREENING OF RESIDUES OF ANTIMICROBIALS IN TISSUES OF FOOD PRODUCING ANIMALS BY PREMI®TEST AND TOTAL ANTIBIOTICS TEST — COMPARATIVE STUDY

Kubacková, J., Kožárová, I.

University of Veterinary Medicine and Pharmacy, Komenského 73, 041 81 Košice
The Slovak Republic

jana.kubackova@gmail.com

ABSTRACT

This study deals with the screening of residues of antimicrobial substances in the tissues of food producing animals using Premi®Test and Total antibiotics test. The aim of the study was to compare the detection sensitivity of the test strain *Bacillus stearothermophilus* var. *calidolactis* of both screening tests for residues. Of the food matrices, we examined muscles, liver and kidneys of cattle, pigs and poultry. Our results allowed us to state that the Total antibiotics test provided more positive results, indicating potential residues of antimicrobials in the examined matrices. Positive results were obtained for pig liver; the kidneys and muscles of cattle; and the muscles of poultry. With regard to the fact that the screened matrices were obtained from food shops, the positive results should be confirmed by an appropriate confirmation method.

Key words: antimicrobials; Premi®Test; residues; tissues; Total antibiotics test

INTRODUCTION

Antimicrobials are widely used in food producing animals to treat infectious diseases. Despite the evident of advantages, the extensive use of these pharmacologically active substances can lead to their residues in animal products, such as meat, milk, eggs or honey. The presence of antibiotic residues in animal products can lead to: allergic responses in sensitive individuals; negatively affect the immune system, and intestinal microflora of food consumers. The major problems are the transfer of resistant bacteria from animals to humans or interference with technological processes during industrial processing of food.

The production of the food of animal origin and their products is subject to obligatory inspection involving the potential presence of residues [1]. The authorized method of primary screening of residues in animal tissues uses Premi®Test [2]. Currently there is also available the Total antibiotics test [3]. Although the principle of the detection of this test, based on inhibition of the growth of the test strain, is the same as with the Premi®Test, there are differences in the determination of residues which offer the possibility of comparison of both tests.

The aim of our study was to examine tissues of food animals obtained from food shops for the presence of antimicrobial residues using the authorised Premi®Test and its new alternative, Total antibiotics test and to compare detection effectiveness of both tests intended for rapid screening of antibiotic residues in tissues of food producing animals.

MATERIALS AND METHODS

Microbiological methods and test strains

a) Premi®Test; *Bacillus stearothermophilus* var. *calidolactis* (DSM Food Specialties, the Netherlands);
b) Total antibiotics test: *Bacillus stearothermophilus* var. *calidolactis* (Euroclone S. p. A, Italy).

Preparation of samples

Tissue samples (muscle, kidney, liver) were thawed before examination and finely cut with a scalpel.

a) Premi®Test: in order to obtain sufficient volume of tissue fluid, the samples were heated in a microwave oven at 500 W for 10 minutes.
b) Total antibiotics test: 3 g of sample were weighed into a test tube and 10 ml of a working extraction solution (prepared by dilution of concentrated extraction solution with sterile distilled water 1 : 10 v/v) were added. The content was mixed thoroughly and the tube was allowed to stand at 37 °C for 2 hours.

Test procedure

a) Premi®Test: 100 µl of tissue fluid was measured into a vial and covered with foil. With organs, the inactivation of the samples was carried out at 80 °C for 10 min. The vials were incubated in a thermo-block at 64 ± 0.5 °C for 3 hours.
b) Total antibiotics test: 200 µl of clear supernatant were transferred into a vial and covered with a plastic cover. The vials were incubated in a thermo-block at 65 ± 2 °C for about 3 hours.

Evaluation of results

a) Premi®Test: a yellow colour of the agar, indicated the absence of antibiotics; a violet colour, indicated the presence of antibiotics; and a yellow/violet colour, the presence of antibiotics at the level of the test detectability.
b) Total antibiotics test: a negative result was indicated by a change in colour of the agar from violet to yellow, while with positive samples, the colour of the agar (violet) remained unchanged.

RESULTS AND DISCUSSION

The results for the determination of antimicrobial residues in the tissues of food producing animals by Premi®Test and Total antibiotics test are shown in Table 1.

Table 1. Positive and negative results of examination of tissue of food producing animals by Premi®Test and Total antibiotics test

Animal species	Tissue	Premi®Test	Total antibiotics test
Pigs	liver	–	+
	kidney	–	–
	muscle	–	–
Cattle	liver	–	±
	kidney	+	+
	muscle	+	+
Poultry	liver	–	–
	kidney	–	–
	muscle	–	+

+ — positive result; ± — dubious result; – —negative result

The presence or absence of antimicrobial residues in the tissues of food producing animals was evaluated on the basis of colour change of the agar medium or absence of the change. The Total antibiotics test detected residues of antimicrobial substances in four examined samples: in the pig liver; the kidney and muscle of cattle; and the muscle of poultry. Premi®Test provided positive result only for two samples; the kidney and muscles of cattle. A dubious result was obtained after examination of the liver of cattle by the Total antibiotics test.

CONCLUSIONS

Euroclone S.p.A. (Italy), the developer of the Total antibiotics test, declares sensitivity to the test strain *Bacillus stearothermophilus* var. *calidolactis and* to many important antimicrobial substances used in veterinary medicine. Our results showed the higher detection sensitivity of the test strain *Bacillus stearothermophilus* var. *calidolactis* in the Total antibiotics test to the residues present in the examined tissues. On the basis of these results, we recommend this new alternative to Premi®Test for the rapid screening of residues of antimicrobial substances in tissues of food producing animals.

ACKNOWLEDGEMENTS

This study was supported by VEGA Grant No. 1/0939/12.

REFERENCES

1. European Commission, 1996: Council Directive 96/23/EC on measures to monitor certain substances and residues thereof in live animals and animal products. *Official Journal of the European Communities*, L 125, 10—32.

2. CH 12.18., 2006: Detection of residues of inhibitory substances in meat by the Premi®Test. *Bulletin of the Ministry of Agriculture of the Slovak Republic*, 38, 64—67.

3. TOTAL ANTIBIOTICS. Instruction manual. Available at: http://www.euroclonegroup.it/

*

Selected papers from the 56th STUDENT SCIENTIFIC CONFERENCE, Section III, held at the University of Veterinary Medicine and Pharmacy in Košice on April 17, 2013.

FOLIA VETERINARIA, 57, 2: 88—90, 2013

THE EFFECT OF WOLLY FOXGLOVE AND COMMON FOXGLOVE ON *ARTEMIA FRANCISCANA*

Špaková, M., Beňová, K., Špalková, M.

Department of Biology and Genetics, University of Veterinary Medicine and Pharmacy
Komenského 73, 041 81 Košice
The Slovak Republic

benova@uvm.sk

ABSTRACT

This study investigated the effect of Wolly Foxglove (*Digitalis lanata)* and Common Foxglove (*Digitalis purpurea*) on the brine shrimp *Artemia franciscana*. In the experiments we used 450 nauplius larvae. The tests were carried out with macerates of the drugs prepared at ambient and elevated temperatures. The highest lethality was recorded after 96 hours of action of the macerate of *Digitalis purpureae folium* with concentration of 15 g.l^{-1} which was obtained at increased temperatures.

Key words: *Artemia franciscana*; biotest; *Digitalis lanata*; *Digitalis purpurea*

INTRODUCTION

A long time ago, people found that plants could serve not only as a source of food, but also for the treatment of various diseases. Owing to some lucky chances, but also tragic mistakes, they formed a group of medicinal plants [7] that provided relief in all kinds of diseases. Cardiovascular diseases are the most frequent cause of death and the number of people suffering from them continues to increase. The discovery of plants containing cardiac glycosides, particularly foxgloves (*D. purpurea* and *D. lanata*), was a big contribution to the therapy of heart diseases, particularly heart failure [3].

The development of pharmacology, immunology, virology and other scientific disciplines resulted in an increased use of laboratory animals. With regard to the concern about the welfare of these animals, the use of alternative biotests on invertebrates including *Artemia franciscana* has been increasing.

The aim of our study was to investigate the effect of Wolly Foxglove (*Digitalis lanata)* and Common Foxglove (*Digitalis purpurea)* on the brine shrimp *Artemia franciscana*.

MATERIALS AND METHODS

We used a 5-day test on 450 nauplius larvae of *Artemia franciscana* (*A. salina*) [1], [2].

For this test, we prepared macerates of plant drugs of concentrations 2.5 and 15 g.l^{-1} by one-step maceration at ambient and elevated temperatures using water with 4.7 % salinity for soaking of the plant material. To prepare the macerate at elevated temperatures, the drug was crumbled to required size, wetted, and boiling water was poured over the drug and allowed to stand at ambient temperature with occasional mixing until cooling. The macerate at ambient temperature was prepared with a water temperature of approximately 20 °C. By means of the test of acute toxicity, we recorded the number of dead artemia after 24, 48, 72, 96 and 120 hours.

We formed 9 groups with 50 homogeneous nauplius larvae in each, with one of them serving as the control. Individuals in the same group were divided into 5 separate subgroups (dishes), 10 in each, preserving homogeneity of conditions. Gross errors were excluded by testing with Dean-Dixon test at the level of significance $\alpha = 0.05$.

Table 1. Lethality (%) in *Artemia franciscana* after exposure to macerates (M_A and M_B) of *Digitalis lanatae folium* of different concentrations

Group	24 h			48 h			72 h			96 h		
	X	N	SD	x	n	SD	x	n	SD	x	n	SD
Control	2	5	4.3	2	5	4.3	2	5	4.3	10	5	8.6
M_A 2.5 g.l^{-1}	0	5	0	2	5	4.3	2	5	4.3	26*	5	4.3
M_A 15 g.l^{-1}	0	5	0	2	5	4.3	6	5	12.9	36*	5	4.3
M_B 2.5 g.l^{-1}	0	5	0	0	5	0	2	5	4.3	4	5	4.3
M_B 15 g.l^{-1}	0	5	0	2	5	4.3	4	5	4.3	10	5	4.3

x — mean lethality in %; n— number of tested sub-groups; SD — standard deviation
M_A — macerate prepared at ambient temperature; M_B — macerate prepared at elevated temperature; * —P < 0.05

Table 2. Lethality (%) in *Artemia franciscana* after exposure to macerates (M_A and M_B) of *Digitalis purpureae folium* of different concentrations

Group	24 h.			48 h			72 h			96 h		
	X	N	SD	x	n	SD	x	n	SD	x	n	SD
Control	2	5	4.3	2	5	4.3	2	5	4.3	10	5	8.6
M_A 2.5 g.l^{-1}	0	5	0	2	5	4.3	4	5	4.3	22*	5	8.6
M_A 15 g.l^{-1}	0	5	0	4	5	4.3	20*	5	8.6	58*	5	12.9
M_B 2.5 g.l^{-1}	0	5	0	4	5	4.3	14*	5	4.3	24*	5	8.6
M_B 15 g.l^{-1}	2	5	4.3	20*	5	8.6	48*	5	8.6	82*	5	8.6

x — mean lethality in %; n — number of tested sub-groups; SD — standard deviation;
M_A — macerate prepared at ambient temperature; M_B — macerate prepared at elevated temperature; * — P < 0.05

The differences between individual concentrations, toxic substances or their combinations were tested according to Wayland and Hayes [8]. Live artemias were counted after 24, 48, 72 and 96 hours and the results were compared with the control and processed statistically.

RESULTS

The results of the study are presented in Tables1 and 2.

In the control group, we observed maximum lethality of *Artemia franciscana* at the level of 10 % after 96 hours. After 96 hours there was a significant increase in lethality in the groups exposed to the effect of macerates of both concentrations prepared at ambient temperature (M_A) compared to the control and macerates of both concentrations prepared at elevated temperatures (Table 1).

After 48 hours of exposure to macerates of *Digitalis purpureae folium* (Table 2), we observed a significantly increased lethality in the group exposed to macerate prepared at elevated temperatures (M_B) of concentration 15 g.l^{-1}, compared to the control and the other three experimental groups. After 72 hours there was a significant increase in lethality in group M_A (concentration 15 g.l^{-1}) and M_B (both concentrations) in comparison with control and M_A group exposed to concentration 2.5 g.l^{-1}. After 96 hours there was a significant increase in lethality in all experimental groups in comparison with the control.

DISCUSSION

Currently the problems with digoxin become less important owing to observation of recently recommended

therapeutic doses (0.5 – 0.9 ng.ml^{-1} = 0.64 – 1.05 nmol.l^{-1}) [5]. Patočka [4] compared the toxicity of digoxin and digitoxin. He observed considerable differences in LD_{50} in these substances, moreover, their acute toxicity depended on the method of administration and the animal species to which they were administered. For example, acute toxicity in people is induced with almost 4-fold lower dose of digoxin, than digitoxin, when administered *per os*.

When comparing the toxicity of drugs, *Digitalis lanatae folium* and *Digitalis purpureae folium,* we observed that after the exposure to macerates of Common Foxglove we recorded higher lethality in artemia than after exposure to Wolly Foxglove under the same condition. Generally, glycosides of the drug *Digitalis lanatae folium*, when administered *per os*, are better tolerated than purpurea glycosides [6], act more rapidly, are better absorbed, more rapidly eliminated, and their accumulation in the body is lower [5], [7]. For this reason it is the drug *Digitalis lanatae folium* that is used for isolation of pure compounds [6].

CONCLUSION

Our results indicate that the drug *Digitalis lanatae folium* with 1 % content of cardiac glycosides (contrary to *Digitalis purpureae folium* with 0.15 – 0.4 % cardiac glycosides) had a stimulative effect on *Artemia franciscana*. Nauplius larvae exposed to this drug showed higher motility and viability already after 24 hours in comparison with the control group which indicated that the concentrations used in our study were low for the tested *Artemia franciscana*.

REFERENCES

1. Dvořák, P., 1995: Modified test with *A. salina* for observation of the influence of interactions with xenobiotics (In Czech). In *Toxicity and Biodegradability of Wastes and Substances Important in the Aquatic Environment,* Research Institute for Fishery, Hydrobiology and Aquachemistry, Milenovice, 25—29.

2. **Dvořák, P., Beňová, K**., **2002:** The investigation of interaction of low doses of ionizing radiation and risk factors by means of Artemia salina biotes. *Folia Veterinaria,* 46, 195—197.

3. Mann, J., 1996: *Poisons, Drugs, Medicines* (In Czech). 1st edn., Academia, Prague, 203 pp.

4. Patočka, J., 2008: Cardiotoxic glycosides (In Czech). *Vojenské zdravotnické listy* (Military Medical Letters), 4, 160—168.

5. Riečanský, I., 2007: Digitalis — past, presence and prospective of the old drug (in Slovak). *Cardiol*., 16, 53—63.

6. Suchý, V., 1994: *Farmacognosy* (In Czech). Special part I, 2nd edn., Komensky University, Bratislava, 178 pp.

7. Tomko, J. et al., 1999: *Farmacognosy* (In Slovak), 2nd edn., Osveta, Martin 422 pp.

8. Wayland, J., Hayes, J., 1991: Dosage and other factors influencing toxicity. In *Pesticide toxicology*, Academic Press, 39—97.

*

Selected papers from the 56th STUDENT SCIENTIFIC CONFERENCE, Section III, held at the University of Veterinary Medicine and Pharmacy in Košice on April 17, 2013.

FOLIA VETERINARIA, 57, 2: 91—93, 2013

CONTENT OF RADIONUCLIDES IN ROCKS

Strečanská, A., Beňová, K., Čipáková, A*., Renčko, A.

Department of Biology and Genetics, University of Veterinary Medicine and Pharmacy
Komenského 73, 04181 Košice
***Regional Office of Public Health, Košice**
The Slovak Republic

benova@uvm.sk

ABSTRACT

In this study we determined the content of radionuclides in rocks from selected locations in Slovakia. The activities of: natural radionuclides ^{40}K, ^{226}Ra and ^{232}Th ; and the artificial radionuclide ^{137}Cs,in samples of rocks, was measured by gamma spectroscopy. The highest activity of ^{40}K was determined in a granite sample from mountain Chopok; the highest activity of ^{226}Ra, in a dolomite sample from Chočské mountains; of ^{232}Th in a granite samples from mountain Ďumbier; and of ^{137}Cs in a granite sample from Great Hincovo Pleso.

Key words: gamma spectrometry; radioactivity; rocks

INTRODUCTION

Radiation is a normal component of our environment. Various substrates, such as rocks or soil, but also air and water, contain some radioactive material. The most frequent radionuclides are ^{238}U and ^{232}Th, and products of their radioactive decay, as well as the radioactive isotope ^{40}K. Radiation emitted by radioactive isotopes found in rocks is responsible for more than half of the level of natural radioactive background. When investigating the exposure pathways, one must consider the fact that rocks and soil are a source of external gamma radiation and the radionuclides released from them, enter water, air and food chains. A separate issue is the radioactivity of construction materials that originate frequently from rocks and secondary raw materials.

The aim of this study was to determine the content of natural radionuclides ^{40}K, ^{226}Ra and ^{232}Th and artificial radionuclide ^{137}Cs, in samples of rocks from selected locations of Slovakia.

MATERIALS AND METHODS

Samples of rocks were collected from various locations of central and eastern Slovakia. The samples were rinsed with water to remove residues of contaminating materials and dried out. Then the samples were crushed with a hammer and allowed to pass through a sieve of mesh size 5 × 5 mm in order to obtain gravel with particle sizes of less than 5 mm.

Before determination, the processed samples were stored in Marinelli vessels for a minimum of two months in order to reach particle equilibrium. The activity of natural radionuclides ^{40}K, ^{226}Ra and ^{232}Th and artificial radionuclide ^{137}Cs were measured by the gamma spectrometry method.

RESULTS

The results of measurements of the activity of radionuclides in rock samples from various locations of Slovakia are presented in Table 1.

Table 1. Activity of radionuclides in rock samples from selected locations of central and eastern Slovakia

Location	Type of rock	Activity [Bq.kg^{-1}]			
		^{137}Cs	^{40}K	^{226}Ra	^{232}Th
Heaps after mining — Dubník	Andesite	1.11 ± 0.26	412.06 ± 10.76	17.98 ± 0.53	17.27 ± 0.40
Chočské mountains — Choč	Dolomite	3.08 ± 0.26	126.27 ± 5.98	**25.86 ± 0.47**	1.08 ± 0.21
Small Quarry, Kvetnica pri Poprade	Chromite	< 0.50	820.18 ± 15.18	14.89 ± 0.39	14.76 ± 0.31
Hincovo Pleso (mountain lake), Mengusov valley	Granite	**45.22 ± 0.77**	655.91 ± 14.12	17.95 ± 0.51	23.46 ± 0.46
Heaps from magnesite mine, Košice	Magnetite	< 0.18	35.96 ± 2.04	–	–
Lomnický peak	Granite	11.78 ± 0.50	750.42 ± 16.11	9.55 ± 0.48	17.62 ± 0.56
River Čiernanka, Svrčinovec	Sandstone	1.35 ± 0.22	335.12 ± 9.84	15.26 ± 0.51	15.52 ± 0.50
Rysy mountains	Granite	8.34 ± 0.54	592.99 ± 15.78	11.77 ± 0.58	18.33 ± 0.70
Great Žabie Pleso (mountain lake)	Granite	32.15 ± 0.71	753.59 ± 16.39	16.98 ± 0.57	21.32 ± 0.60
Mountain Kriváň	Granite	7.29 ± 0.11	681.16 ± 10.88	18.99 ± 0.21	20.17 ± 0.21
Krížová cave	Limestone	2.65 ± 0.28	160.12 ± 7.30	3.62 ± 0.36	2.57 ± 0.42
Jahodná mountain	Granodiorite	5.04 ± 0.24	195.28 ± 5.95	2.09 ± 0.26	< 0.01
Oravská dam, Trstená	Sandstone	0.81 ± 0.32	380.97 ± 10.48	18.13 ± 0.52	20.45 ± 0.72
Peaks Baranie rohy, High Tatras	Granite	12.84 ± 0.61	707.15 ± 18.53	9.73 ± 0.67	18.85 ± 0.82
Jahňací peak	Granite	4.58 ± 0.35	757.97 ± 15.61	10.98 ± 0.44	17.46 ± 0.56
Suchá Belá, Slovak paradise	Limestone	4.48 ± 0.38	210.14 ± 10.11	6.38 ± 0.60	2.33 ± 0.53
Španie pole, Špaňopoľská cave, Slovak carst	Mineral	4.37 ± 0.36	170.63 ± 9.10	5.29 ± 0.51	2.23 ± 0.49
Great ružinská cave	Dolomite	2.20 ± 0.33	212.64 ± 9.10	3.76 ± 0.44	1.77 ± 0.42
Podbanište, location Slizké, Slovak carst	Mineral	3.16 ± 0.39	150.54 ± 10.49	4.50 ± 0.59	1.06 ± 0.53
Jánošíkove holes, Terchová, Malá Fatra	Limestone	6.20 ± 0.35	269.14 ± 9.15	14.44 ± 0.48	10.19 ± 0.36
Ďumbier mountain	Granite	8.20 ± 0.42	794.94 ± 16.29	11.08 ± 0.49	**27.02 ± 0.63**
Vrbické Pleso (mountain lake)	Granodiorite	4.26 ± 0.34	679.35 ± 14.24	20.67 ± 0.51	23.92 ± 0.45
Chopok mountain	Granite	2.26 ± 0.39	**884.20 ± 18.67**	19.96 ± 0.59	25.27 ± 0.79
UVMP premises, Ardo square	Slate	0.84 ± 0.15	588.27 ± 10.65	19.34 ± 0.31	24.56 ± 0.33
Východná, High Tatras	Gneiss	9.07 ± 0.47	670.41 ± 15.78	10.84 ± 0.53	21.61 ± 0.68

Our measurements showed that the highest activity of ^{137}Cs (45.22 ± 0.77 Bq.kg^{-1}) was determined in a granite sample from the location of Great Hincovo Pleso (Great Hincovo mountain lake), the highest activity of ^{40}K (884.20 ± 18.67 Bq.kg^{-1}) in a granite sample from mountain Chopok, the highest activity of ^{226}Ra (25.86 ± 0.47 Bq.kg^{-1}) in a dolomite sample from mountain Veľký Choč and the highest activity of ^{232}Th (27.02 ± 0.63 Bq.kg^{-1}) in a granite sample from mountain Ďumbier.

DISCUSSION

Generally, approximately 85 % of the total annual radiation dose received by any person comes from natural radionuclides of terrestrial and cosmogenic origins. A considerable number of decay products of Th and U series and ^{40}K are mainly parts of external gamma radiation [1]. During various production activities and at processing technologies related to nuclear research, energy industry and health services, the level of natural background radiation may increase, or artificial radionuclides may be released [3]. Especially serious danger arises due to intensive contamination of the environment with radioactive products after nuclear explosion. When analysing exposure pathways, one should consider the fact that rocks and soil are a source of external gamma radiation and the radionuclides released from them enter water, air and food chains.

A separate issue is the radioactivity of construction materials that are frequently manufactured from rocks and secondary raw materials [2]. Regulation No. 528/2007 of the Ministry of health of the Slovak Republic specifies the maximum allowed levels of natural radionuclides in construction materials/products intended for public and residential buildings and the index of mass activity (gamma index) for the content of natural radionuclides in construction products. Our study revealed that this index was not exceeded in any of the investigated rock samples.

ACKNOWLEDGEMENTS

The study was supported by the project VEGA 1/0415/13.

REFERENCES

1. Belivermis, M., Kiliç, Ö., Çotuk, Y., Topcuoglu, S., 2010: The effects of physicochemical properties on gamma emitting natural radionuclide levels in the soil profile of Istanbul. *Environmental Monitoring and Assessment*, 163, 15—26.

2. Holý, K., 2009: *Natural Sources of Ionising Radiation* (In Slovak). Slovak Nuclear Society, 29 pp.

3. Húšťava, Š., 2011: Monitoring radiation situation in town Trnava and its surroundings (In Slovak). In *Creative Physic Teacher IV*, April 12—15, Faculty of Construction Engineering of STU, Bratislava, 123—128.

*

Selected papers from the 56th STUDENT SCIENTIFIC CONFERENCE, Section III, held at the University of Veterinary Medicine and Pharmacy in Košice on April 17, 2013.

FOLIA VETERINARIA, 57, 2: 94—96, 2013

CONTAMINATION OF SELECTED HERBS WITH RADIONUCLIDES IN SLOVAKIA

Tóthová, M., Beňová, K., Čipáková, A.*

Department of Biology and Genetics, University of Veterinary Medicine and Pharmacy Komenského 73, 04181 Košice
***Regional Office of Public Health, Košice**
The Slovak Republic

benova@uvm.sk

ABSTRACT

This study investigated the contamination of selected herbs with radionuclides in various locations in Slovakia. Samples of 5 different herbs (common dandelion, common daisy, common ivy, stinging nettle, and white nettle) were collected together with samples of soil. Radionuclides were determined by the gamma-spectrometric method. The herbs were examined for the presence of ^{137}Cs and ^{40}K and soil for the presence of ^{137}Cs, ^{40}K, ^{226}Ra and ^{232}Th. The highest activity of ^{137}Cs was measured in the sample of stinging nettles (*Urtica dioica*) from village Sikenička and the highest activity of ^{40}K in the sample of common daisies (*Bellis perennis*) from Hronovce. Of the soil samples, the highest activity of ^{137}Cs, ^{40}K and ^{226}Ra were found in samples of soil substrate of stinging nettles from Sereď, while the substrate of stinging nettles from Hronovce, showed the highest activity of ^{232}Th.

Key words: herbs; radionuclides; soil

INTRODUCTION

Central Europe, particularly the Slovak Republic, is extremely rich in flora. Of about 3000 species of vascular plants many are known for their curative effects. Plants obtain nutrients from soil and water and thus become enriched with radionuclides. We decided to investigate two herbs that are collected and used because of their medicinal effects. However, they are beneficial to health only if they are grown under normal conditions. If they are affected by some contaminants from their environment they may become harmful to health.

The aim of our study was to find out the extent of contamination of selected herbs in Slovakia either by natural or artificial radioactivity.

MATERIALS AND METHODS

Samples of 5 herbs (common dandelion — *Taraxacum officinale*; common daisy — *Bellis perennis*; common ivy — *Hedera helix,* L.; stinging nettle — *Urtica dioica*; and white nettle — *Lamium album*) were collected from six locations of Slovakia: Hronovce, Giraltovce, Košice, Lučenec, Sereď and Sikenička. When collecting herbs, we also took samples of the surface layer of soil to a maximum depth of 10 cm. The herbs were air dried at ambient temperature, homogenised and passed through a sieve. Samples of soil were also air dried and sifted. The specific activity of ^{137}Cs, ^{40}K, ^{232}Th and ^{226}Ra in our samples were determined by the gamma-spectrometric method. For this determination we used a multi channel analyser DSA 1000 Canberra, equipped with a semiconductor detector GC 3520 with 35 % effectiveness.

RESULTS

Our study showed that the highest activity of ^{137}Cs was measured in the sample of stinging nettles from village

Sikenička (4.59 ± 0.27 Bq.kg^{-1}) and of ^{40}K in that of stinging nettles from Hronovce (12700.28 ± 196.31 Bq.kg^{-1}). The highest activity of ^{137}Cs, ^{40}K and ^{226}Ra was measured in the stinging nettles substrate from Sereď (^{137}Cs = 55.74 ± 2.20 Bq.kg^{-1}; ^{40}K = 1247.37 ± 41.76 Bq.kg^{-1}; ^{226}Ra = 40.40 ± 2.03 Bq.kg^{-1}). The highest activity of ^{232}Th was measured in soil that served as a substrate for stinging nettles in Hronovce (37.89 ± 1.06 Bq.kg^{-1}). The comparison of samples collected in village Sikenička showed that the activity of ^{40}K and ^{137}Cs was higher in stinging nettles than in common ivy. In samples from Košice, the activity of ^{137}Cs was higher in white nettle than in the common dandelion but the opposite relationship was observed for radionuclide ^{40}K. In Hronovce, the highest activity of ^{40}K and ^{137}Cs was measured in common daisies.

When comparing the samples from Sikenička and Hronovce, the activity of ^{40}K and ^{137}Cs in common ivy was low in both locations but the activity of ^{40}K and ^{137}Cs in the common dandelion, was higher in samples from Hronovce. On the other hand, the activity of ^{137}Cs in stinging nettles collected in Sikenička was the highest. In Hronovce, the samples of stinging nettles were collected not only in 2012 but also in 2013. The activity of radionuclides was higher in 2012.

Soil samples from Sereď showed the highest activity of ^{40}K, 137Cs and ^{226}Ra and the soil from Hronovce had the highest activity of ^{232}Th. When comparing the soil samples from Hronovce, the highest activity of ^{137}Cs was measured in the soil substrate of common daisies and of ^{226}Ra, ^{232}Th and ^{40}K in the soil substrate of stinging nettles.

DISCUSSION

Herbs are used by the pharmaceutical industry and also as home remedies. Frequently their curative effects are such that cannot sometime be reached with modern synthetic drugs and, more important, the majority of them have no negative side effects [10]. Herbs as a part of human life must fulfil certain conditions. As they obtain nutrients from the soil, its quality and potential contaminants are very important. Currently, the most frequent way of contamination of plants with radionuclides is contamination from the soil [5]. Some plants accumulate radionuclides in higher quantity, for example moss and mushrooms. Moss can accumulate as many as 93 % of ^{137}Cs [8], [9].

We investigated the contamination of herbs from six locations of Slovakia. Generally, samples of stinging nettle showed higher activity of radiocesium than samples of other herbs. We can assume that this herb has better ability to absorb this radionuclide and thus can serve as better bioindicator of radiocesium. The highest activity of ^{40}K was measured in stinging nettle from Hronovce. Because ^{40}K is a natural radionuclide, no maximum allowed level was established. Examination of soil samples showed the highest activity of ^{137}Cs, ^{40}K and ^{226}Ra in soil substrate of stinging nettles from Sereď. Higher proportions of ^{40}K in the soil blocks the absorption of ^{137}Cs by plants so the absorption of potassium ions by plants is higher [6]. The highest activity of ^{232}Th was measured in soil from Hronovce which served as a substrate for stinging nettles. However, this activity was low and did not pose any danger to humans. With regard to ^{137}Cs, the highest measured activity in our samples reached 4.59 ± 0.27 Bq.kg^{-1} which is much lower than the dangerous level. Our results agree with those reported by other authors. Bresťáková [3] reported that the activity of ^{137}Cs in moss in Slovakia in 2008 was low and reached 127 Bq.kg^{-1}. In the study of Abukawa *et al.* [1], the activity of this radionuclide in the leaves of black tea, was 0.23 – 1.4 Bq.kg^{-1}, while in coffee beans it was only 0.025 – 0.19 Bq.kg^{-1}. In tea leaves in Italy, the level of ^{137}Cs reached 2.6 Bq.kg^{-1} [7]. In the period from 2000 to 2009, the activity of radiocesium in moss in Slovakia ranged between 0.7 and 103 Bq.kg^{-1} [2].

CONCLUSION

Our investigations showed that the health of people in Slovakia is not endangered by the use of the investigated herbs. However, because radionuclides are still detected in forest ecosystems, their constant monitoring is very important [4].

ACKNOWLEDGEMENTS

The study as supported by the project VEGA 1/0415/13.

REFERENCES

1. Abukawa J., Tsubuku, Ch., Hayano, K., Hirano, K., 1998: A survey of ^{90}Sr and ^{137}Cs activity levels of retail foods in Japan. *Journal of Environmental Radioactivity,* 41, 287—305.

2. Aleksiayenak, Yu. V., Frontasyeva, M. V., Florek, M., Sykora, I., Holy, K., Masarik, J. et al., 2013: Distributions of ^{137}Cs and ^{210}Pb in moss collected from Belarus and Slovakia. *Journal of Environmental Radioactivity,* 117, 19—24.

3. Bresťáková, L., 2010: Determination of concentration of radionuclide ^{137}Cs in moss collected from Belarus and Slovakia (In Slovak). In *Student Scientific Conference FMFI UK*, Bratislava, 196—201.

4. Bulko, N. I., Shabaleva, M. A., Kozlov, A. K., Tolkacheva, N. V., Mashkov, I. A., 2013: The ^{137}Cs accumulation by forest-derived products in the Gomel region. *Journal of Environmental Radioactivity,* In Press, 1—5.

5. Curini, M., Rosali, O., Borio, R., Saetta D. M. S., Cicioni, R., Forini, N. et al., 1995: Evaluation of ^{137}Cs activity in plant drugs and in some phytoderivatives from Chernobyl accident up to present (1986—1994). *Pharmacol. Res.*,32, 69—74.

6. Čipáková, A., 2005: Migration of radiocaesium in individual parts of the environment. *Nukleonika,* 50, Suppl. 1, 19—23.

7. Desideri, D., Meli, M. A., Roselli, C., Feduri, L., 2011: Alpha and gamma spectrometry for determination of natural and artificial radionuclides in tea, herbal tea and camomile marketed in Italy. *Microchemical Journal,* 98, 170—175.

8. Dvořák, P., Ohera, P., Grolicková, M., Beňová, K., 2001: Content of ^{137}Cs in mushrooms 15 years after Chernobyl (In Czech).

In *XXVIIIrd Seminar on Quality of Food and Food Raw Materials,* MZLU Brno, 20.

9. Heinrich, G., Müller, H. J., Oswald, K., Gries, A., 1989: Natural and artificial radionuclides in selected Styrian soils and plants before and after the reactor accident in Chernobyl. *Biochemie und Physiologie der Pflanzen,* 185**,** 55—67.

10. Németh, I., 2007: *Medicinal Herbs* (In Hungarian). Esterházy Károly College. Eger, 117 pp.

*

Selected papers from the 56th STUDENT SCIENTIFIC CONFERENCE, Section III, held at the University of Veterinary Medicine and Pharmacy in Košice on April 17, 2013.

FOLIA VETERINARIA, 57, 2: 97—100, 2013

FACTORS AFFECTING MAILLARD REACTIONS

Uheláková, K., Süli, J., Sobeková, A.

Institute of Medical Chemistry, University of Veterinary Medicine and Pharmacy
Komenského 73, 041 81 Košice
The Slovak Republic

uhelakova.kristina@gmail.com

ABSTRACT

This study dealt with factors affecting the Maillard reaction *in vivo*, concerning the risks to diabetic patients. We carried out model experiments on samples of human plasma with concentrations of glucose imitating healthy, medium and strongly hyperglycaemic blood (5, 15 and 30 mmol.dm^{-3}). Sets of samples were exposed to temperatures of 37 to 42 °C for time intervals of 1 to 48 hours. Changes in the samples were detected by measuring absorbances at 294 nm and 420 nm. The most pronounced increase in absorbance at 294 nm was induced by temperatures of 39 to 40 °C. Changes in the absorbances of samples exposed to elevated temperatures were observed also at 420 nm, but these changes were less pronounced. Marked differences were observed also between samples with different glucose concentrations. The absorbances of samples with higher glucose levels were higher in comparison with plasma samples with lower glucose levels. Our results indicate the increased possibility of development of Maillard products in diabetic patients during feverish states, particularly an increase in Amadori products and the lower production of melanoidines.

Key words: AGE-products; diabetes mellitus; Maillard reactions; melanoidines

INTRODUCTION

Maillard reactions are the most important reactions that take place in food during thermal processing. They have positive and negative effects on food properties and also decrease the nutritional value of foods. Some final products of these reactions have toxic, mutagenic and carcinogenic properties [5]. The intricate complex of glycation reactions starts with reaction between the carbonyl group of reducing sugar and amino group of amino acids.

Maillard reactions take place also in living organisms. The products of *in vivo* Maillard reactions are unambiguously harmful to live organisms. Intermediary and end products of these reactions can considerably contribute to organ and vascular complications, such as retinopathy, nephropathy and arteriosclerosis [3]. This involves polymerization of proteins and the loss of their functional properties. They play a role also in ageing of living organisms [4]. Due to the increased level of glucose in the blood of diabetic patients, they are a particularly endangered group [1]. In diabetic patients, Maillard reactions lead to increased formation of the so-called AGE-products (advanced glycation end-products).

Because increased temperatures accelerate Maillard reactions, we intended to find out whether even small changes in body temperature (fever to maximally 42 °C) can result in changes in the blood of diabetes patients.

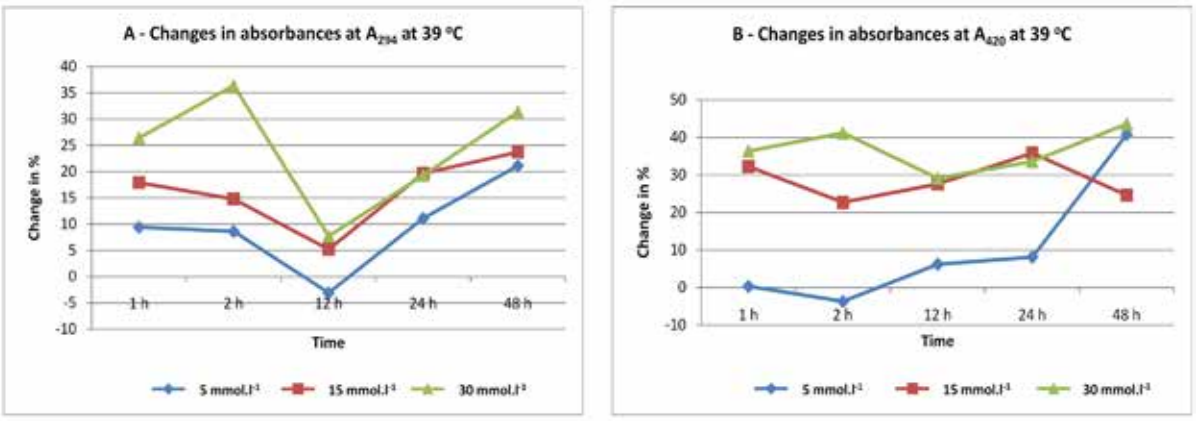


Fig. 1. Changes in absorbances at A_{294} (A) and A_{420} (B) in relation to the time of heating in plasma samples exposed to 39 °C. The change is expressed as % of the initial value (0 h; 36 °C)

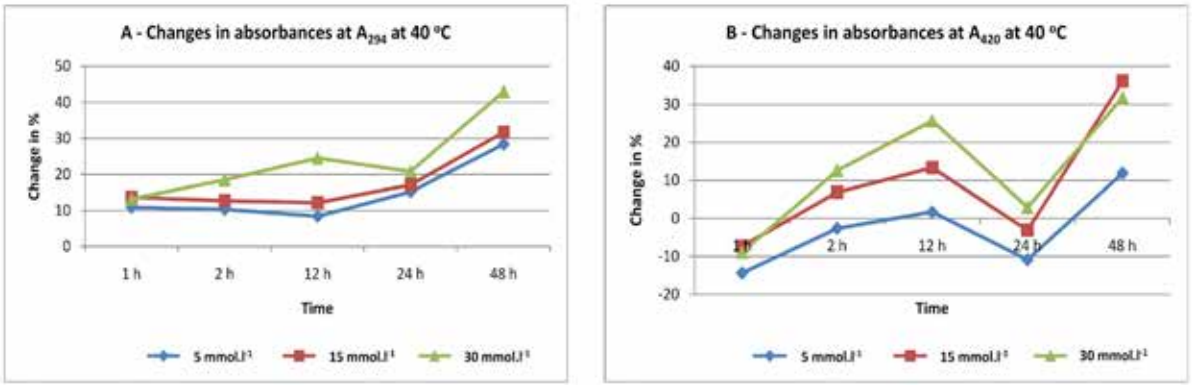


Fig. 2. Changes in absorbances at A_{294} (A) and A_{420} (B) in relation to the time of heating in plasma samples exposed to 40 °C. The change is expressed as % of the initial value (0 h; 36 °C)

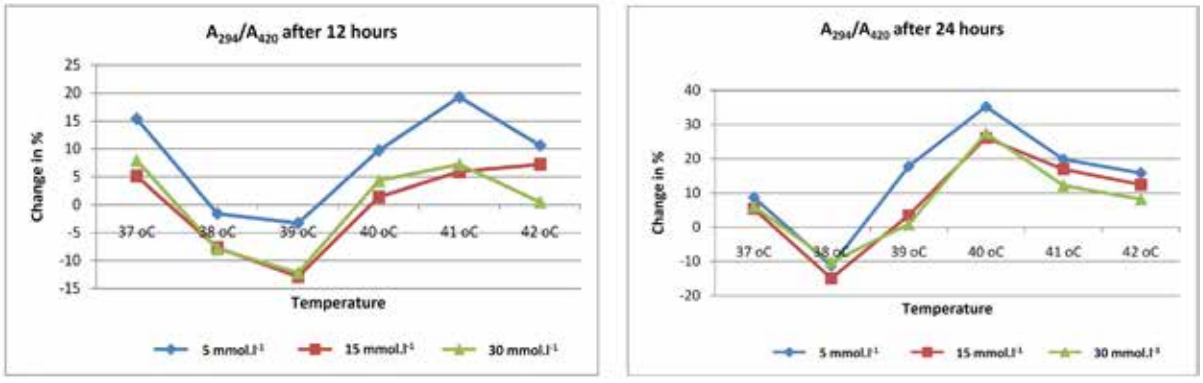


Fig. 3. Changes in the ratio of absorbances A_{294}/A_{420} at changing temperatures in plasma samples exposed to 12 and 24-hour thermal intervention. The change is expressed as % of the initial value (0 h; 36 °C)

MATERIALS AND METHODS

The influence of various factors (temperature, length of action, glucose level) on potential Maillard reactions in diabetic patients was investigated in model experiments on human plasma with different concentrations of glucose which were produced by adding relevant amounts of glucose to the plasma. We selected the following concentrations of glucose: 5 mmol.dm^{-3} (healthy individual); 15 mmol.dm^{3} (diabetic patient); and 30 mmol.dm^{-3} (diabetic patient with very high plasma glucose) to add to the plasma. We had at our disposal 6 samples of plasma (marked A—F) of volume 230—250 cm^{3} each, that were kindly provided by National transfusion service at Faculty hospital of L. Pasteur in Košice. These samples were exposed to temperatures imitating feverish states (from 37 °C up to 42 °C). The length of thermal intervention imitated real fever (from 1 hour to 48 hours). All examinations were carried out in duplicate. Changes in blood plasma were observed spectrophotometrically using an instrument λ-Helios (GB) by measuring absorbance at wavelengths 294 nm and 420 nm. At 294 nm, we assumed absorbance of Amadori products of the initial and medium phases of Maillard reactions, at 420 nm we expected absorbance of melanoidines, brown pigments produced in the final stage of the reactions [6]. The ratio A_{294}/A_{420} provides information about potential polymerization of proteins due to Maillard reactions [2].

Results were processed statistically using Student paired *t*-test.

RESULTS AND DISCUSSION

Absorbances A_{294} and A_{420} of individual plasma samples were in a relatively wide range (A_{294}: 0.885—1.664; A_{420}: 0.091—0.598; A_{294}/A_{420} : 2.78—11.08) and therefore the results after thermal intervention were compared with the base values of the original plasmas. The trend of changes for the respective parameters including all plasma samples, i. e. 12 values, was expressed as the mean value of the percentage change for each plasma compared to its base value (0 h; 36 °C).

Significant changes in the absorbance were observed at each investigated temperatures and time intervals. Individual samples of plasma did not respond in the same way to temperature exposure, e. g. "A" showed more pronounced changes at higher temperatures (41 and 42 °C), while plasma "F" responded already to lower temperatures (37 and 38 °C), but showed no significant changes at higher temperatures. The majority of significant changes occurred at 39 and 40 °C, particularly after 48-hours of exposure to elevated temperatures. Changes in diabetic plasmas were more pronounced than in the healthy plasmas and there were obvious differences between samples with medium and very high concentration of glucose (Fig. 1 and 2). The decrease in absorbance at 12 h of thermal exposure at 294 nm was associated with an increase at 420 nm which suggested that Amadori products could gradually be converted to AGE-products.

The decrease in absorbance A_{420} at 24 hours of action of 40 °C can be ascribed to the activation of protective mechanisms, i.e. various enzymes found in plasma, that can contribute to the splitting of crosslinked proteins. The splitting is supported by the values of A_{294}/A_{420} ratio suggesting polymerization of proteins (Fig. 3). A decrease in this value indicates potential splitting of Maillard products. Twelve-hour exposure to temperature of 39 °C resulted in the lowest ratios of A_{294}/A_{420}. When the exposure was extended to 24 hours, the temperature of 38 °C sufficed to produce similar effects in all plasma samples. At lower temperatures the healthy plasma did not differ from diabetic plasma. Decreased levels of A_{294}/A_{420} indicated a higher degree of polymerization in strongly diabetic plasma after 12-hours of action.

As far as the length of exposure was concerned, our results showed that higher temperatures increased the rate of changes. While at 39—41 °C, the changes were pronounced already after 2—12 hours, at 37—38 °C similar changes occurred only after 24—48 hours. However, after approximately 12—24 hours, we observed an onset of protective mechanisms that occur naturally in living organisms. They involve chemical and biochemical processes including enzymatic and immune reactions [3]. Suppression of glycation and reparation of glycated proteins in physiological systems is ensured by a group of enzymes which includes glyoxalases, aldehydreductases and dehydrogenases, amadoriase and fructosamine-3-phosphokinase. An important role in delaying the aging of tissues is attributed to the enzyme system of glyoxalase I and glyoxalase II which metabolises glyoxal and methylglyoxal, the principal precursors of AGE, and prevent glycation of cellular and extracellular proteins [5]. The enzymes responsible for suppression of glycation and restoration of glycated proteins may be present in plasma. Temperature optimum of enzymatic activity is mostly in the range of 20—40 °C, while higher physiological temperatures may accelerate the course of the reactions. Therefore, it is comprehensible that we observed a decrease in absorbances after exposure to increased temperatures, suggesting formation of Maillard products. The protective mechanisms include immune reactions, i. e. antibodies to changed proteins could be present in plasma samples. In blood *in vivo,* one can assume the presence of various substances acting for and against the development of Maillard products. Also, correctly stored plasma can retain the activity of these substances and protective mechanisms, even in experiments *in vitro*. Because we lacked information about relevant blood donors we did not know their age or past disease histories. Blood donors are healthy people so we could assume that their protective mechanisms were fully functional. In older healthy people we can expect higher levels of physiological AGE compared to younger ones. The differences between individual plasma samples observed in our experiments could be attributed also to this fact.

Our experiments confirmed the hypothesis that long lasting feverish states can increase the formation of Maillard products. The danger to diabetic patients is directly proportional to their blood glucose levels. It is well known that besides many drugs, also some vitamins, for example thiamine (vitamin B_1) or pyridoxamine (vitamin B_6), can slow down development or even split the already developed AGE products [3]. Thus foods with adequate content of these vitamins are especially important for diabetic patients, especially those with fever.

ACKNOWLEDGEMENT

This study was supported by the project KEGA No. 020UVLF-4/2012.

REFERENCES

1. Brownlee, M., 2001: Biochemistry and molecular cell biology of diabetic complications. *Nature*, 414, 813—820.

2. Lerici, C. R., Barbanti, D., Manzano, M., 1990: Early indicators of chemical changes in foods due to enzymatic or non-enzymatic browning reaction. 1: Study on heat-treated model systems. *Lebensm.-Wiss. Techn.*, 23, 289—294.

3. Monnier, V. M., 2003: Intervention against the Maillard reaction *in vivo*. *Archives of Biochemistry and Biophysics*, 419, 1—15.

4. Nagai, R., Jinno, M., Ichihashi, M., Koyama, H., Yamamoto, Y., Yonei, Y., 2012: Advanced glycation end products and their receptors as risk factors for aging. *J. Anti-Aging Med.*, 9, 108—113.

5. Velíšek, J., Hajšlová, J., 2009: *Food Chemistry I* (In Czech). 3rd edn., OSSIS, Tábor, CR, 580 pp.

6. Yu, X., Zhao, M., Hu, J., Zeng, S., Bai, X., 2012: Correspondence analysis of antioxidant activity and UVeVis absorbance of Maillard reaction products as related to reactants. *LWT — Food Sci. Technol.*, 46, 1—9.

FOLIA VETERINARIA, 57, 2: 101—103, 2013

PHYSICO-CHEMICAL CHARACTERISTICS OF VARIOUS SNAKE VENOMS

Rohaľová, V., Sobeková, A.

Institute of Medical Chemistry, University of Veterinary Medicine and Pharmacy
Komenského 73, 041 81 Košice
The Slovak Republic

sobekova@uvlf.sk

ABSTRACT

Cobra *Naja ashei* is a new species of snake that was originally considered to be a brown coloured form of cobra *Naja nigricollis*. *The b*asic organic analysis of venom of the cobra *Naja ashei* provided qualitative proof of carbonyl carbon, nitrogen bound in the form of amide and the presence of sulphur. The presence of halogen elements was excluded. Thin-layer chromatography confirmed the presence of saccharide components. Lipid component was not proven. Subsequently, some physico-chemical characteristics of the venom of selected species of snakes from the family Elapidae were determined for comparative purposes. The venom of snakes of the genus *Naja* differ in pH from that of snakes of the genus *Dendroaspis*. Neither the content of saccharides nor the content of proteins in venom is suitable for differentiation of snakes that produced them. The image after isoelectrophoretic (IEF) separation, showed marked differences in the protein composition of venoms not only between snakes of the genera *Naja* and *Dendroaspis,* but also, visible differences in the composition of snake venom within the same genus.

Key words: *Naja ashei;* qualitative analysis; snake venom; spitting cobra

INTRODUCTION

Spitting cobras use a unique way of defence against their enemies. When endangered, these snakes spit venom. They have the ability to eject it to considerable distances aiming it usually at the eyes [9]. The venom causes temporary blindness which, without treatment, can become permanent. After entering the body through the eyes, it causes systemic problems [6]. The venom of spitting cobras is mostly cytotoxic [7]. For a long time, the cobra *Naja ashei* was considered a brown coloured form of cobra *Naja nigricollis.* Only DNA analysis proved that it is a new cobra species [8]. This snake is the largest spitting cobra. In comparison with other species, *Naja ashei* is capable of ejecting so much venom that could cause death of 20 adult people [3].

This study was aimed at the detailed investigation of some physical and chemical characteristics of *Naja ashei* venom and potential use of these characteristics for distinguishing venom of individual snake species.

MATERIALS AND METHODS

In our experiments, we examined samples of snakes of the genera *Naja* and *Dendroaspis* (Table 1). The venom was collected by allowing the snake fangs to bite into a graduated vessel with a plastic foil. Then the venom was transported in cryo-Eppendorf tubes (–195 °C) to a laboratory and stored at –70 °C. Individual determi-

Table 1. Selected physico-chemical parameters of snake venoms

	Snake species	pH	Refraction index	Saccharides [%]	Proteins [mg.ml^{-1}]
1.	***Naja nigricollis* (Tanzania)**	6.3	1.3991	39.7	0.6986
2.	***Naja mosambica***	6.0	1.3990	39.6	0.6911
3.	***Naja haje***	6.1	1.4051	42.7	0.6884
4.	***Naja ashei***	6.3	1.3980	39.0	0.9817
5.	***Naja nigricollis* (Ghana)**	6.3	1.4146	47.4	0.8971
6.	***Dendroaspis jamesoni kaimosea***	5.3	1.3827	30.1	0.2108
7.	***Dendroaspis polylepis***	5.5	1.3815	30.1	-
8.	***Naja melanoleuca***	6.2	1.3960	38.1	0.9684

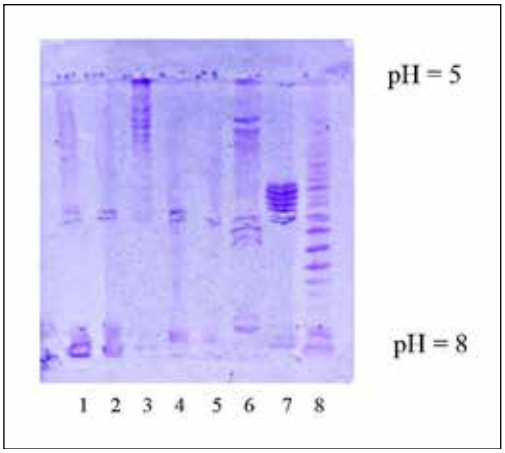


Fig. 1: IEF image of selected snake venoms (pH gradient 5.0—8.0)
1 — *Naja nigricollis* **(Tanzánia)**; 2 — *Naja mosambica*; 3 — *Naja haje*; 4 — *Naja ashei*; 5 — *Naja nigricollis* (Ghana)
6 — *Dendroaspis jamesoni kaimosea*; 7 — *Dendroaspis polylepis*; 8 — *Naja melanoleuca*

nations were carried out using either native undiluted or suitably diluted samples of venom.

The level of the pH was determined potentiometrically in undiluted samples. The refraction index and subsequently the content of saccharides was determined refractometrically. The basic organic qualitative analysis of elements and functional groups was carried out according to Křenek *et al.* [4]. Thin-layer chromatography (TLC) employing specific development systems and detection reagents was used to distinguish between lipids and saccharides [2]. Proteins were determined by the method of Bradford [1].

Isoelectrophoretic focusing (IEF) of the proteins or the peptides of snake venoms was carried out using Phast-system (Pharmacia). Separation took place in commercial gels with pH gradient 5.0—8.0 using optimised technique. The conditions of separation were as follows: 2000 V; 2.5 mA; 3.5 W; 15 °C, pre-electrophoresis 74 Vh; electrophoresis 510 Vh.

RESULTS AND DISCUSSION

The basic organic analysis of venom of the cobra *Naja ashei* proved qualitatively the presence of carbonyl carbon, nitrogen in the form of amide and the presence of sulphur. The Belstein test unambiguously excluded the presence of

halogens. The TLC analysis of *Naja ashei* venom confirmed the presence of saccharides. In order to compare venom of selected snake species of the family Elapidae, some physical and chemical characteristics of these venoms were determined. The pH level of individual undiluted venom samples (Table 1) showed considerable differences between the genera of *Naja* and *Dendroaspis*. The pH values within the genus were similar. The venom of cobras, *Naja ashei* and *Naja nigricollis,* was identical (6.3) which could support the already repulsed opinion that *Naja ashei* is a subspecies of *Naja nigricollis.* The contents of saccharides and proteins were not suitable for distinguishing the snakes that produced the respective venoms (Table 1).

Isoelectric focusing is a method that can separate ampholytes on the basis of their different isoelectric points. The results obtained in our study are shown in Fig. 1.

This method was also used to distinguish between male and female venom of one snake species [5]. Despite similar content of proteins in the investigated samples, the IEF method accomplished considerable separation of individual venom fractions. The IEF image showed very different compositions of venom of the genera *Naja* and *Dendroaspis.*

An unexpected finding was the considerable differences in the composition of the venom within the genius. While the venom of *Naja haje* contained components with pI values mostly in the acidic range, the venom of *Naja melanoleuca* consisted of components with pI values predominantly in the alkaline range. On the other hand, two components with pI value around 6 were found in all venom samples regardless of the species (Fig. 1). Different IEF image of venoms of *Naja ashei* and *Naja nigricollis* cobras can be considered another proof of the fact that *Naja ashei* is not a subspecies of cobra *Naja nigricollis*.

CONCLUSION

Snake venom has a great potential for use in medicine and pharmacy. The knowledge of properties and composition of venom of individual snake species allows one to prepare a specific anti-venom and forms a basis of potential use of cytotoxic properties of venom in the treatment of many serious diseases. The measurement of the pH and isoelectrofocusing appear as a suitable method for partially distinguishing snake venoms in the future.

REFERENCES

1. Bradford, M. M., 1979: A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem*., 72, 248—254.

2. Holovská, K., Sobeková, A., Lenártová, V., 2007: *Laboratory Practicals in Chemistry* (In Slovak). UVMP in Košice, 195 pp.

3. Johnson, P., 2011: *Cobra Facts.* Available on internet: http://www.buzzle.com/articles/spitting-cobra-facts.html (Feb. 25, 2013)

4. Křenek, P., Dvořáková, E., Muchová, A., 1992: *Laboratory Practicals in Analytical Chemistry 1: Qualitative Analysis of Inorganic and Organic Substances* (In Slovak). Komensky University, Bratislava, 151 pp.

5. Mackessy, S. P., 2009: *Handbook of Venoms and Toxins of Reptiles.* CRC Press, Boca Raton, 528 pp.

6. Nair, S., 2010: *Spitting Cobra*. Available on internet: http://www.buzzle.com/articles/spitting-cobra.html (Nov. 11, 2012)

7. Valenta, J., 2008: Poisonous Snakes: Intoxication, Therapy. 3. Snake Venom (In Czech). Galén, Prague, 45—66.

8. Wüster, W., Broadley, D., 2007: Get an eyeful of this: a new species of giant spitting cobra from eastern and north-eastern Africa (Squamata: Serpentes: Elapidae: Naja). *Zootaxa*, 1532, 51—68.

9. Young, B. A., Boetig, M., Westhoff, G., 2009: *Functional bases of the Spatial Dispersal of Venom During Cobra "Spitting"*. The University of Chicago Press, 82, 80—89.

*

Selected papers from the 56th STUDENT SCIENTIFIC CONFERENCE, Section IV, held at the University of Veterinary Medicine and Pharmacy in Košice on April 17, 2013.

FOLIA VETERINARIA, 57, 2: 104—106, 2013

INFLUENCE OF SOYBEAN DIET ON AMINOPEPTIDASE ACTIVITY OF HOUSEFLY LARVAE

Dobríková, I., Kostecká, Z., Heinová, D.

Department of Chemistry, Biochemistry and Biophysics
University of Veterinary Medicine and Pharmacy, Komenského 73, 041 81 Košice
The Slovak Republic

kostecka@uvm.sk

ABSTRACT

The mechanism of maggot therapy of chronic wounds is not completely clear. It seems that proteolytic enzymes play an important role in this process. Proteases (peptidases) are enzymes, which cleave peptide bonds between two amino acids with the release of water molecules. The aim of our study was to observe the influence of soybean diet (2% and 4% of total diet) on leucine aminopeptidase and lysine aminopeptidase activities in individual stages (48, 72, 96 and 120 hours) of development of the housefly (*Musca domestica*) larvae. Peptidase activities were measured as specific activities (U), defined as the enzyme amount producing 1µmol of p-nitroaniline per minute per mg of proteins (U.mg^{-1}). Both concentrations of soybean flour in the diet increased the aminopeptidase activity in comparison to controls. The lower concentration (2% soybean flour) caused a higher activity of the observed enzymes at 72 and 120 hours.

Key words: aminopeptidase; housefly; maggot therapy; soybean diet

INTRODUCTION

Leucine aminopeptidase (EC 3.4.11.1) is a hydrolytic enzyme which contains zinc and catalyses the removal of N-terminal amino acid of most L-peptide, mainly with the leucine terminal residue. The activity of leucine aminopeptidase is analysed by leucine-aryl-amide chromogenic substrate and by leucyl-beta-naphthylamide. Its presence was confirmed in the gut of larvae *Cephalopina titillator Clark (Diptera: Oestridae)* and *Morimus funereus (Coleoptera: Cerambycidae*).

Lysine aminopeptidase (EC 3.4.11.15), also called aminopeptidase Y, is activated by cobalt (Co) and inhibited by Zn^{2+} and Mn^{2+}. This hydrolytic enzyme releases N-terminal lysine from proteins. It was isolated from *Saccharomyces cerevisiae* [4]. Lysine aminopeptidase hydrolyses L-Lys-pNA and L-Arg-pNA.

Insect larvae need many free amino acids and nitrogen during their development. These components are products of peptidolytic activity of larval proteases, hydrolysing food proteins into smaller peptides and free amino acids. The dominant peptidolytic activity was described in the group of trypsin-like and chymotrypsin-like serine proteases [3]. Soybean is a natural inhibitor of proteases which inhibit enzyme activity during the protein degradation process.

The aim of our study was to compare the influence of soybean diet (2% and 4% of total diet) on leucine aminopeptidase and lysine aminopeptidase activities in different stages of larval development (48, 72, 96 and 120 hours) in the larvae of the housefly *(Musca domestica*).

MATERIAL AND METHODS

Housefly larvae were fed with: (a) a standard diet containing agar (20 g), dried milk (100 g), yeast (100 g) and water (up to 1000 ml); and (b) an experimental diet that contained, beside these

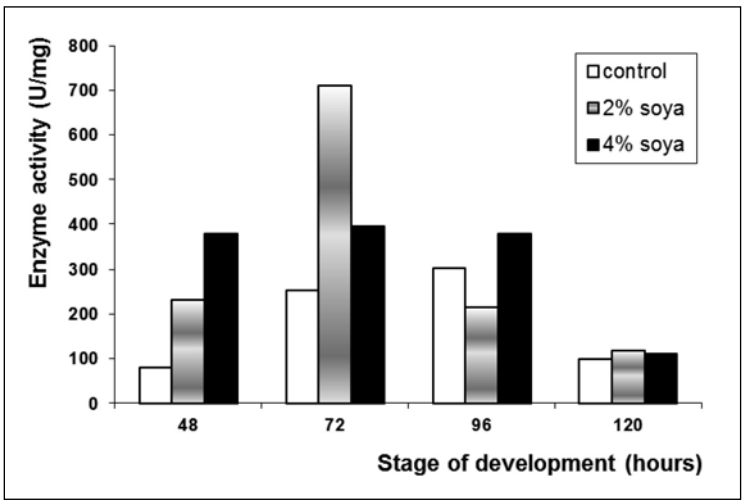


Fig. 1. Effects of the addition of 2 % and 4 % soybean flour to the diet on leucine aminopeptidase activities of housefly larvae in different stages of larval development

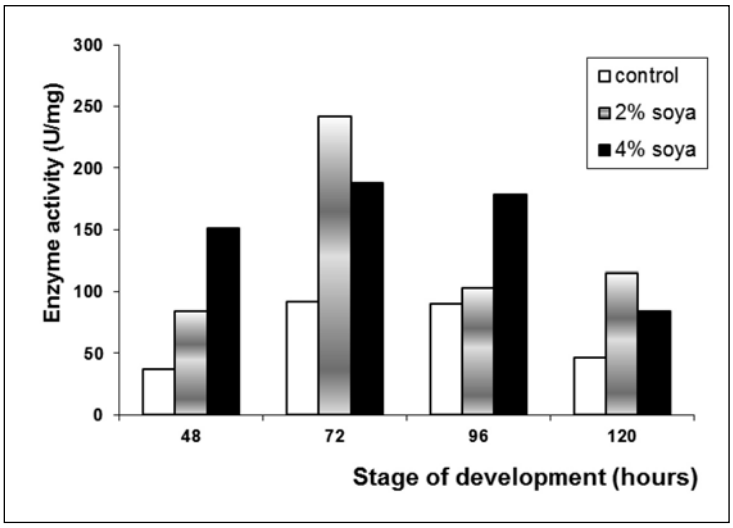


Fig. 2. Effects of the addition of 2 % and 4 % of soybean flour to the diet on lysine aminopeptidase activities of housefly larvae in different stages of larval development

components, 20 g and 40 g of soybean flour, respectively. Larvae in larval stages of 48, 72, 96 and 120 hours were homogenized and the enzymes were extracted with deionized water. The insoluble cellular fragments were removed by centrifugation. Then the supernatant was filtered and the enzyme activity was determined against two synthetic chromogenic substrates, L-leucyl-p-nitroanilide and L-lysyl-p-nitroanilide. The incubation mixture contained, 640 µl of 0.1mol.l^{-1} Tris/HCl buffer, pH = 8.3, 30 µl of supernatant and 30 µl of substrate. After incubation at 37 °C for 10 minutes, the reaction was stopped by 300 µl of 10 % trichloroacetic acid. The reaction rate was estimated by reading the reaction product, p-nitroaniline, at 405 nm. The protein concentration was determined by the Bradford's method [2]. Enzyme activity was expressed as U, which is defined as the enzyme amount which produces 1 µmol p-nitroaniline per min per mg of proteins.

RESULTS AND DISCUSSION

Generally, the addition of a soybean inhibitor significantly influences the activity of the studied peptidases. The addition of 2 % of soybean flour increased the leucine aminopeptidase activity in developmental stages of 48, 72 and 120 hours. Diet supplementation by 4 % of soybean flour increased the activity of the observed enzyme in all stages in comparison to the control group. However, the increase

in enzyme activity was lower in the stages of the 72 and 120 hours than in the groups supplemented with 2% soybean flour (Fig. 1).

Both concentrations of soybean flour in the diet increased the lysine aminopeptidase activities in comparison to controls. The lower concentration (2% soybean flour) caused higher activities of the observed enzyme in the stages of 72 and 120 hours (Fig. 2). The decreasing tendency in activities of observed aminopeptidases of larvae in stages at 48—72 hours agrees with the influence of soybean inhibitor not only on aminopeptidases but also on endopeptidases, such as trypsin and chymotrypsin [1], [3].

In our study we determined the activity of leucine aminopeptidase and lysine aminopeptidase influenced by different concentrations of soybean flour in the diet used for feeding housefly larvae in dependence on developmental stages.

ACKNOWLEDGEMENTS

The authors thank for financial support to VEGA grant agency project No. 1/0098/2011.

REFERENCES

1. Blahovec, J., Kostecká, Z., 2007: *Proteases and their Inhibitors in Fly Larvae, Possibilities of their Therapeutic Use* (In Slovak). University of veterinary medicine and pharmacy in Košice, Institute of social sciences and health services of P. P. Gojdič in Prešov, Prešov, 84 pp.

2. Bradford, M. M., 1976: A rapid and sensitive method for quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.*, 72, 248—254.

3. Muharsini, S., Sukarsih, R. G., Partoutomo, S., Hamilton, S., Willadsen, P., Wijffels, G., 2000: Identification and characterisation of the excreted/secreted serine proteases of larvae of the old world screw-worm fly, *Chrysomia bezziana*. *Int. J. Parasitol.,* 30, 705—14.

4. Yasuhara, T., Nakai, T., Ohashi A., 1994: Aminopeptidase Y, a new aminopeptidase from *Saccharomyces cerevisiae*. Purification properties, localization and processing by protease B. *J. Biol. Chem.,* 269, 13 644—13 650.

*

Selected papers from the 56th STUDENT SCIENTIFIC CONFERENCE, Section IV, held at the University of Veterinary Medicine and Pharmacy in Košice on April 17, 2013.

FOLIA VETERINARIA, 57, 2: 107—109, 2013

HISTAMINE AND HISTAMINE INTOLERANCE

Nezbedová, Z., Dičáková, Z., Dudriková, E.

Department of Food Hygiene and Technology, University of Veterinary Medicine and Pharmacy
Komenského 73, 041 81 Košice
The Slovak Republic

dicakova@uvm.sk

ABSTRACT

The aim of this study was to gather information on the relatively little known condition of histamine intolerance (HIT), its: health manifestations; difference from food allergy; and suggest ways of treatment of this relatively rare disorder. Histamine is a biological amine present in many tissues which has complex effects on physiological and pathological processes in an organism. It develops by carboxylation of the amino acid L-histidine. It is distributed very unevenly in the body, most of it bound in granules of mastocytes (fat cells) or basophils. Histamine is found in some species of fish, meat, canned food, smoked meat, cabbage, cheeses, beer, wine, tomatoes, spinach and other vegetable and fruit. Histamine intolerance (HIT) is a disorder that can develop, due to the disproportion between supply of histamine and the ability to degrade it and results in allergic manifestations. The disbalance may result from the increased content of histamine in food, supply of substances that block the enzyme responsible for histamine degradation or excessive elimination of the body's own histamine from mastocytes. Clinical symptoms can lead to suspicion of HIT or allergy, but this condition should be confirmed or excluded by special examination. Doctors make the diagnosis on the basis of repeated blood tests that can be accompanied with dermatologic, gastroenterologic or neurologic examinations.

Key words: allergy; histamine; histamine intolerance

HISTAMINE

The name histamine originates from the Greek ιστός, meaning tissue. Thus, it's very name indicates its occurrence. It was synthesised for the first time in 1907 and 3 years later it was identified in ergot alkaloids by British researcher Henry Dale. Later, together with Laidlaw, they found out that histamine affects smooth muscles of the digestive and respiratory tract, causes vasodilatation and stimulated cardiac contractility. In the thirties of the 20th century the scientists discovered that histamine is an important mediator of anaphylactic shock [1], [5].

Histamine is a biological amine present in many tissues which has complex effects on physiological and pathological processes in an organism. The first hypotheses on the potential physiological role of histamine in tissues were based on the similarity between the effects of histamine and the manifestation of anaphylactic shock and tissue damage. Histamine is an important mediator of early allergic and inflammatory reactions and has an important role in the control of gastric secretion [2].

Histamine develops by decarboxylation of the amino acid L-histidine. After its development, histamine is either stored or rapidly inactivated. It is distributed very unevenly in the body, most of it is bound in granules of mastocytes (fat cells) or basophils. The bound form is biologically inactive. Degranulation is the only mechanism of histamine release that occurs after chemical or mechanical damage to the cells. It is released from granules upon the action of sodium ions present in extracellular fluid which rapidly displace this amine from the respective complex. Histamine can be released also

by some specific mechanisms. Its method of its release after immunological stimuli is important. The binding of IgE antibodies to the surface of mastocytes or basophils causes sensitisation of these cells and after contact with the relevant antigens, the cells release the content of their granules. This type of release requires energy and calcium and degranulated mastocytes again accumulate histamine but this process lasts several days to weeks. This way, released histamine is a mediator of early allergic reactions (type I). Mastocytes and basophils can also release histamine in the course of immune reactions mediated by immunoglobulins IgG and IgM. Most of histamine manifestations depend on its action on smooth muscles that carry on its surface many H1-histamine receptors. Histamine affects muscles of vessels and causes their dilatation resulting in the escape of fluid from vessels and a decrease in blood pressure. Dilatation of minute skin capillaries is observed as reddening of skin. The escape of fluid causes urticaria in the uppermost layer of skin while in the deeper layers, it results in swelling. The consequences of escape of vascular fluid in the nose and stimulation of glandules are manifestations of rhinitis (watery discharge, swelling of mucosa, and lacrimation). Dilatation of certain vessels in the central nervous system area causes migrainous headaches or dizziness [2].

Histamine can be degraded in two ways:

1. Oxidative deamination by means of the enzyme diaminooxidase (DAO)
2. Methylation of the ring by histamine-N-methyltransferase (HNMT)

The DAO plays a role in the metabolism of extracellular histamine, e.g. after consumption of food rich in histamine, because it is stored in plasmatic membrane of epithelial cells and upon stimulus is released into the circulation. The highest concentration of DAO was observed in the digestive tract and relatively high concentrations were detected also in kidneys. HNMT is a cytosol enzyme capable of processing histamine inside of the cells. It is found in almost all tissues and the highest concentrations are detected in the kidneys, liver, spleen and intestines [3].

HISTAMINE INTOLERANCE

Histamine intolerance (HIT) is a condition which develops due to a disproportion between the supply of histamine and the abilities of the body to degrade it. This disbalance may result from the: increased content of histamine in food; supply of substances that block DAO; or excessive elimination of the body's own histamine from mastocyte granules. Activity of DAO in individual people differs. Increased levels of histamine in the body results in various clinical manifestations. The typical manifestations include pruritus and reddening of skin, popular rash (urticaria), but also flatulence, abdominal cramps and diarrhoea. However, histamine can induce manifestations that are not commonly associated with HIT, such as congested nose, migraine and chronic exhaustion. Frequency of problems and the manifestation depend on the severity of the enzymatic disorder and the quantity of histamine accepted in food [1], [3]. Histamine intolerance is a "sub-chapter" of food intolerances. Although it does not shorten one's life, it makes it considerably less pleasant. It can manifest itself as allergy and cause similar problems as food intolerance. HIT is a metabolic disorder, enzymatic insufficiency, which is still relatively little known even among medical practitioners because up to the recent past there were not available either examinations for the determination of this disorder or means of the replacement of the missing enzyme. Allergy and intolerance are not the same. Allergy is an inadequate response of the immune system to common substances which do not induce response in non-allergic individuals. The allergy-inducing substances are then called allergens. The diagnosis of intolerance is based on examination of specific IgG antibodies. HIT is determined by blood tests but also examinations by dermatologist, gastroenterologist or neurologist is often necessary. The affected person should also exclude foods that contain higher levels of histamine and slow down patient's metabolism. Histamine is found in many common foods, beverages and spices. This includes: some species of fish, ripened meat, all conserved and fermented food, smoked products, cabbage, gherkins, some cheeses, beer, wine, fruit juices, tomatoes, spinach and other vegetables and fruit. Some foods do not directly contain much histamine but cause its excessive release, for example pineapple, bananas, citrus fruit, strawberries, kiwi, nuts, papaya, tomatoes, legumes, spinach, various spices, cocoa, chocolate, alcohol, fish, crustaceans, egg white and various additives. Some foods contribute to the production of histamine, particularly those containing viable yeasts, such as fresh, insufficiently baked pastry, bread and fermented dough. Problems may arise from some substances present in alcohol, cocoa, chocolate and black and green tea that decrease activity of DAO [3], [5].

CONCLUSIONS

The reliable treatment of HIT is based on a low-histamine diet and change in lifestyle of patients that excludes stress and allergens [5]. It is essential to exclude from the diet all foods after consumption of which the patient has some difficulties. When the diet brings significant improvement, the patients can gradually, one by one, consume individual "forbidden" foods. Patients with HIT should always consume only fresh food. If even strict diets fails to provide relief from all the problems, it is suitable to turn to pharmacological nutrient supplement DAOSIN. One capsule consumed before meal degrades histamine present in the food and minimizes its absorption. In patients with more serious form of HIT, the histamine reactions should be prevented by antihistaminics and some of them should also be administered a medication cromoglycate which prevents the release of histamine from mastocytes. It was observed in some experiments that high doses of vitamin C, decrease production of histamine in human body and accelerate its degradation. A proof was provided that vitamin B_6 increases the activity of DAO in the intestine and thus improves degradation of histamine in food [4].

Our further studies on this subject will supply additional knowledge on histamine from the chemical point of view with a focus on food products available on our market.

ACKNOWLEDGEMENTS

The study was supported by the projects APVV SK-CN-0028-12 and Kega No. 011UVLF4/2012.

REFERENCES

1. Hrubiško, M., 2011: *Histamine Intolerance* (In Slovak). Available on internet <http://www.daosin.sk/Daosin-brozura-350DPI.pdf> (accessed 03.06.2012, 28 pp).

2. Katzung, B. G., 1995: *Basic and Clinical Pharmacology* (In Czech). Jinočany, H & H, 240—244.

3. Maintz, L., Novak, N., 2007: Histamine and histamine intolerance. *Am. J. Clin. Nutr.,* 85, 1185—1196.

4. <http://primar.sme.sk/c/6401527/ked-sa-z-jedla-stava-nepriatel.html> (March 22, 2013).

5. <http://histaminovakasulka.com/histaminova-intolerance-hit/terapie/> (March 22, 2013).

*

Selected papers from the 56th STUDENT SCIENTIFIC CONFERENCE, Section IV, held at the University of Veterinary Medicine and Pharmacy in Košice on April 17, 2013.

FOLIA VETERINARIA, 57, 2: 110—113, 2013

TREATMENT OF CORNEAL ULCER BY USE OF HORSE AMNIOTIC MEMBRANE

Husebø, C.

Department of Surgery, Orthopaedics and Radiology
University of Veterinary Medicine and Pharmacy, Komenského 73, 041 81 Košice
The Slovak Republic

christina.husebo@gmail.com

ABSTRACT

This study was performed to evaluate the use of equine amniotic membrane (AM) for the surgical repair of corneal ulcers of different aetiology and severity in dogs. Five dogs (six eyes) of various breeds and ages were treated with a donor implant of equine AM. Five of the six eyes showed minimal corneal scarring and good transparency, with only slight visual impairment at the end of the study. One eye showed a relatively large corneal scar, covering approximately 80 % of the cornea, resulting in a great degree of visual impairment. Amniotic membrane transplantation (AMT) was a valid procedure for surgical treatment of corneal ulcers in dogs. This procedure resulted in a great degree of corneal clarity and functional vision in five of six eyes.

Key words: cornea; corneal ulcer; dog; equine amniotic membrane

INTRODUCTION

Corneal ulceration is one of the most common ocular conditions leading to pain and vision loss in dogs and immediate and effective treatment is important to reduce the pain and maintain or restore the vision [12]. Several types of tissue grafts have been used in reconstruction of the ocular surfaces, including; the conjunctiva [9], [10], amniotic membrane [3], [4], [12], porcine small intestinal submucosa [7], renal capsule [1], [17], [18], [19], pericardium [6], and corneal grafts [14]. The use of AM in reconstruction of ocular surfaces has been shown to: promote epithelial cell growth and differentiation [14]; suppress myofibroblast differentiation of normal corneal; limbal and conjunctival fibroblasts [20]; reduce scarring [2] and corneal haze [8]. Other positive effects of the amniotic membrane transplant (AMT) are; reduced stromal inflammation [14] and corneal neovascularisation [13]. The aim of this study was to evaluate the general outcome of the corneal surgery with use of air-dried equine AM in the treatment of corneal ulcers in dogs, and to compare this treatment to other treatment regimes in use for corneal ulcers.

MATERIALS AND METHODS

Five dogs (six eyes) of different breed, gender and age, diagnosed with corneal ulcers were surgically treated with AMT (Table 1). All of the dogs were anesthetized using xylazin (Rometar®, Bioveta, 1 mg.kg^{-1}, IM) and butorphanol (Butomidor®, Richter Pharma AG, 0.2 mg.kg^{-1}, IM) for premedication, diazepam (Apaurin®, Krka, 0.3 mg.kg^{-1}, IV) and ketamine (Narketan®, Vétoquinol, 5 mg.kg^{-1}, IV) for induction, and isoflurane (Isoflurane®, Torrex Chiesi) for maintenance of anaesthesia. Following epithelial debridement and a lamellar keratectomy, the AM was carefully placed with the epithelial side down over the entire keratectomy bed and then sutured to the margin of the keratectomy using 8-0 Vicryl (Vicryl®, Ethicon) in an interrupted suture pattern. The patients were controlled on day 1, 3, 7, 14, 21, 28 and 60 post surgery. The cornea was examined for healing, epithelialisation, oedema, vascu-

larisation, and stability of the AM graft. The sutures were removed under general anaesthesia three to four weeks post surgery.

RESULTS

Following surgery there was no aqueous humour leakage or flare noticed. The globe maintained its shape; with a mild protrusion of the graft membrane the first few days post surgery. Conjunctival oedema and blepharospasm disappeared within one to two weeks after surgery. Corneal oedema was present close to the implant the first day, but absent or greatly reduced after one week. The clinical results are summarised in Table 2. Figures 1a and 1b show the appearance of one of the eyes after placing the AM and four weeks post surgery after suture removal, respectively.

DISCUSSION

In the present study, air-dried equine AMT was performed in five dogs (six eyes) diagnosed with corneal ulcers, with results that encourage its use in such cases. A successful outcome, considering corneal clarity and functional vision, was obtained with AMT in five out of six eyes. The corneas undergo epithelialisation quickly and had good transparency; although in all cases the AMT resulted in some degree of corneal opacity and thereby slight visual impairment at the end of the study.

There were no complications such as; uveitis, glaucoma, or breakdown of sutures seen in these patients. In one case, the outcome was not considered successful at the end of the study. The preservation technique of the AM may influence its properties, and the optimal preservation method should

Table 1. Summary of clinical cases

Breed	Sex	Age	Etiology	Eye affected	Diagnosis
Cavalier King Charles Spaniel	Male	7 years	Unknown	OD	Melting ulcer with perforation
German Shepherd	Male	9 months	Foreign body	OD	Perforating ulcer
Pug	Female	2 years	Possible nasal fold trichiasis	OS	Perforating ulcer
Lhasa Apso	Male	3 years	Unknown	OD	Melting ulcer
French Bulldog	Female	4 years	Thermal injury	OS & OD	OS: melting ulcer OD: Melting ulcer with keratoconus

OD — oculus dexter; OS — oculus sinister

Table 2. Clinical results

Patient	Vascularisation	Pigmentation	Fibrosis	Clinical outcome
Cavalier King Charles Spaniel	**3**	**2**	**80 %**	**Poor**
German Shepherd	**1**	**1**	**5 %**	**Very good**
Pug	**2**	**0**	**25 %**	**Very good**
Lhasa Apso	**1**	**0**	**20 %**	**Very good**
French Bulldog OD	**1**	**0**	**20 %**	**Very good**
French Bulldog OS	**1**	**0**	**5%**	**Very good**

0 — no changes; 1 — slight changes
2 — moderate changes; 3 — severe changes

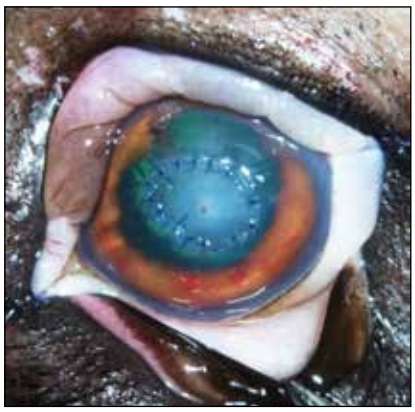


Fig. 1a

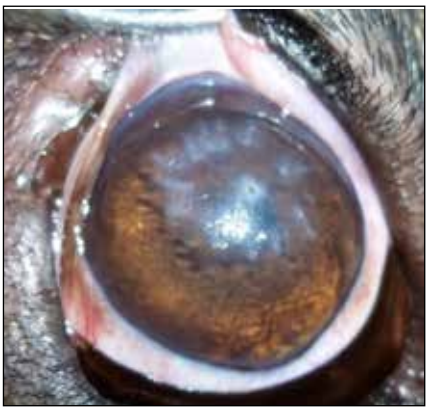


Fig. 1b

Fig 1. Appearance of the eye after placing the AM (1a) and four weeks post surgery after suture removal (1b)

maintain the important biological features of the AM, while ensuring easy handling and clinical safety [16].

Fresh AM is not used in clinical practice today, as there is a risk of transferring serious blood-borne infections. Previous works have demonstrated that some of the AM properties may be lost or altered due to dehydration when preparing air-dried AM [19]. Freeze-dried AM (–50 to –80 °C), on the other hand, has shown to induce minimal changes in the properties of the AM, but this technique is more complex and expensive than air-drying, making it less desirable in veterinary medicine [15]. However, Von Versen-Hoeynck *et al* [21] demonstrated that air dried AM possesses similar biophysical properties to AM preserved by storage in glycerol at 4 °C and by deep freezing at –80 °C. When compared with other surgical techniques described in the literature for the treatment of corneal ulcers, AMT can be considered among those with optimal results.

Conjunctival grafts are widely used as: they are readily available; relatively easy to harvest; need no preservation; seem to facilitate healing by providing a direct blood supply to the lesion; and by providing tectonic support. However, they have been associated with secondary corneal scarring of varying density, which may impair the vision. In addition, there is a risk of dehiscence of the conjunctival graft [10].

Other surgical methods used in the treatment of corneal ulcers include: the use of body membranes such as porcine small intestinal submucosa (SIS) [7]; equine renal capsule [1], [17], [18], [19]; equine pericardium [5]; and corneal grafts [11]. The use of porcine SIS seems to generate similar effects regarding corneal clarity as with AMT, and has the advantage of being easier to obtain than other transplant materials, due to a high availability of slaughter animals. Hansen and Guandalini [11] used corneal grafts with similar success, but found this technique to provide better results in corneas with non-perforating corneal defects. In comparison, the use of renal capsules seems to be associated with a higher degree of corneal opacity and thereby reduced vision, and complications such as breakdown of graft sutures, uveitis, corneal oedema and vascularisation [1], [17], [18], [19].

Epithelisation is the first step in corneal repair, and in order to fully evaluate the extent of epithelial healing and the number of proliferating cells after AMT, a more detailed investigation involving histological examination of the cornea is needed. Kim *et al.* [12] compared the use of bovine freeze-dried AM to a nictitating membrane flap or a contact lens in the treatment of surgically created corneal ulcers. The proportion of the corneal wound that healed was calculated two days later by measuring the fluorescein uptake in the cornea, before all eyes were enucleated and histological sections of the cornea were assessed using proliferating cell nuclear antigen assay. Their results showed that both the healing rate and epithelial cell proliferation were greater with the AMT compared to the other treatment regimes.

Barros *et al.* [3] performed histological evaluations of the cornea after equine AMT in canine eyes with corneal ulcers, and found some remnants of amniotic tissue 60 days post surgery with completely restored corneal architecture at day 180 after the experiment. Although histological examination of the cornea is preferred to fully evaluate the effects of AMT, or any other surgical treatment, this was not possible in our cases, as these patients were companion-dogs, and a histological evaluation requires enucleation. AMT has been successfully used in several procedures for restoration of the ocular surface and indications for its use are steadily growing. More research is needed in this field, to fully understand the mechanism of action of the AM. In this way its application will become more refined, with better usage of this valuable technique. The full potential of this technique is yet to be discovered, thus more randomised prospective studies are needed in the future.

CONCLUSION

Air-dried equine AMT can be considered a valid method for reconstruction of the ocular surface in dogs with corneal

ulcers, with good repair of the cornea, minimal scarring and a great degree of corneal clarity. The outcome of this procedure performed on canine patients presented to UVMP in Košice shows similar success as in other AMT described in the literature. When compared to other surgical techniques in use for the treatment of corneal ulcers, AMT can be considered among those with optimal results.

ACKNOWLEDGEMENT

This study was supported by the Scientific Grant Agency of the Ministry of Education of the Slovak Republic and the Slovak Academy of Sciences No. 1/1192/12.

REFERENCES

1. Andrade, A. L., Laus, J. L., Figueiredo, F., Batista, C. M., 1999: The use of preserved equine renal capsule to repair lamellar corneal lesions in normal dogs. *Veterinary Ophthalmology,* 2, 79—82.

2. Azuara-Blanco, A., Pillai, C. T., Dua, H. S., 1999: Amniotic membrane transplantation for ocular surface reconstruction. *Br. J. Ophthalmol.,* 83, 399—402.

3. Barros, P. S. M., Garcia, J. A., Laus, J. L., Ferreira, A. L., Salles-Gomes, T. L., 1998: The use of xenologous amniotic membrane to repair canine corneal perforation created by penetrating keratectomy. *Veterinary Ophthalmology,* 1, 119—123.

4. Barros, P. S. M., Safatle, A. M. V., Godoy, C. A., Souza, M. S. B., Barros, L. F. M., Brooks, D. E., 2005: Amniotic membrane transplantation for the reconstruction of the ocular surface in three cases. *Veterinary Ophthalmology,* 8, 189—192.

5. Barros, P. S. M., Safatle, A. M. V., Rigueiro, M., 1999: Experimental lamellar corneal graft in dogs using preserved equine pericardium. *Brazilian Journal of Veterinary Research and Animal Science,* 36, 304—307.

6. Bourne, G. L., 1966: The anatomy of the human amnion and chorion. In *Proceedings of the Royal Society of Medicine,* Nov. 59, Part 1, 1127—1128.

7. Bussieres, M., Krhone, S. G., Stiles, J., Townsend, W. M., 2004: The use of porcine small intestinal submucosa for the repair of full-thickness corneal defects in dogs, cats and horses. *Veterinary Ophthalmology,* 7, 352—359.

8. Choi, Y. S., Kim, J. Y., Wee, W. R., Lee, J. H., 1998: Effect of the application of human amniotic membrane on rabbit corneal wound healing after excimer laser photorefractive keratectomy. *Cornea,* 17, 389—395.

9. Hakanson, N., Lorimer, D., Merideth, R., 1987: Further comments on conjunctival pedicle grafting in the treatment of corneal ulcers in the dog and cat. *Journal of American Hospital Association,* 24, 602—605.

10. Hakanson, N., Merideth, R., 1987: Conjunctival pedicle grafting in the treatment of corneal ulcers in the dog and cat. *Journal of American Hospital Association,* 23, 641—648.

11. Hansen, P. A., Guandalini, A., 1999: A retrospective study of 30 cases of frozen lamellar corneal graft in dogs and cats. *Veterinary Ophthalmology,* 2, 233—241.

12. Kim, J. Y., Choi, Y. M., Jeong, S. W., Williams, D. L., 2009: Effect of bovine freeze-dried amniotic membrane (Amnisite-BA) on uncomplicated canine corneal erosion. *Veterinary Ophthalmology,* 12, 36—42.

13. Kim, J. C., Tseng, S. C. G., 1995: Transplantation of preserved human amniotic membrane for surface reconstruction in severely damaged rabbit corneas. *Cornea,* 14, 473—484.

14. Lee, S. H., Tseng, S. C., 1997: Amniotic membrane transplantation for persistent epithelial defects with ulceration. *Am. J. Ophthalmol.,* 123, 303—312.

15. Nakamura, T., Yoshitani, M., Rigby, H., 2004: Sterilized freeze-dried amniotic membrane: a useful substrate for ocular surface reconstruction. *Investigative Ophthalmology and Visual Sciences,* 45, 93—99.

16. Shingh, R., Gupta, P., Kumar, P., 2003: Properties of air dried radiation processed amniotic membranes under different storage condition. *Cell Tissue Banking,* 4, 95—100.

17. Trbolová, A., Balicki, I., 2007: Treatment of deep corneal lesion in dogs with the use of renal capsule. *Veterinary Ophthalmology,* 10, 328—334.

18. Trbolová, A., Balicki, I., Mihalova, M., 2011: Verwendung von Nierenkapselgewebe zur Therapie einer Keratitis profunda. *Kleintierpraxis,* 56, 57—66.

19. Trbolová, A., Kozák, I., Balicki, I., Ledecký, V., 2003: Renal capsule transplantation in treating perforated corneal ulcer. *Medycyna Weterynaryjna,* 59, 68—69.

20. Tseng, S. C. G., Li, D. Q., Ma, X., 1999: Suppression of Transforming Growth Factor isoforms, TGF-β receptor II, and myofibroblast differentiation in cultured human corneal and limbal fibroblasts by amniotic membrane matrix. *J. Cell. Physiol.,* 179, 325—335.

21. Von Versen-Hoeynck, F., Steinfeld, A. P., Becker, J., Hermel, M., Rath, W., Hesselbarth, U., 2008: Sterilization and preservation influence the biophysical properties of human amnion grafts. *Biologicals,* 36, 248—255.

*

Selected papers from the 56th STUDENT SCIENTIFIC CONFERENCE, Section VI, held at the University of Veterinary Medicine and Pharmacy in Košice on April 17, 2013.

FOLIA VETERINARIA, 57, 2: 114—116, 2013

PARADILEPIS SCOLECINA (RUDOLPHI, 1819) TAPEWORM OF CORMORANTS *(PHALACROCORAX CARBO)* IN SLOVAKIA

Melzer, R., Kočišová, A.

Institute of Parasitology, University of Veterinary Medicine and Pharmacy
Komenského 73, 041 81 Košice
The Slovak Republic

kocisova@uvlf.sk

ABSTRACT

In this study, 39 cormorants were examined for the presence of tapeworms between 2011 and 2012. A total of 89.47 % and 80 % of the cormorants were found infected with tapeworms in 2011 and 2012, respectively. One species of tapeworm was identified, i.ve. *Paradilepis scolecina* (Cyclophyllidea: Gryporhynchidae). The mean intensity of infection in 2011 was 316.5 with a range of 10—1888, while the mean intensity in 2012 was 199.3 with a range of 3—1083. Randomly chosen tapeworms were analyzed for their morphometric characteristics. The worm's length ranged from 2.1 to 4.9 mm (3.48 ± 1.089). Scolexes were 263—445 µm long (354.95 ± 56.9) and 212—414 µm wide (349.05 ± 50.59). Rostellum was 102—270 µm long (178.3 ± 53.49) and 78—182 µm wide (128.15 ± 26.25). The suckers' length was 105—149 µm (122.35 ± 11.01) and width 86—122 µm (107.75 ± 11.45). Strobilae with proglottids consisting of immature proglottids were 152—331 µm long (237.15 ± 51.34) and 12.4—19.6 µm wide (16.49 ± 1.99).

Key words: morphological analysis; occurrence; *Paradilepis scolecina*; prevalence

INTRODUCTION

Cormorants are piscivorous predators, both in marine and freshwater environments. They feed exclusively on a wide range of sea and freshwater fish, which are caught by means of dives from the surface of the water. The rapid increase in the numbers of the cormorants had a significant effect not only on the fish population, but also on the parasites composition in the ecosystem [6]. It is likely that the range expansion and the rapid increase in numbers of great cormorants has caused a spread of helminths [2]. Cormorants may harbour infectious agents and transmit it further to other birds, fish and even to humans. Cormorants may transmit parasites to fish that can harm their fitness and behaviour. *Paradilepis scolecina* is a specific parasite of the cormorant [4], its larval stages harbour 2 intermediate hosts: the first is the crustacean *Eudiapromus graciloides* (copepod), in which cercoscolex develops, and the second is mainly Cyprinidae, harbouring the developed plerocercoid [3].

The aim of this study was to determine the occurrence, prevalence and morphological analysis of the tapeworms in cormorants.

MATERIALS AND METHODS

In the winter season of the years 2011 and 2012, a total number of 39 Great cormorants (23 males and 16 females) were obtained. The birds were shot randomly at 3 different rivers (Hornád, Váh and Torysa) located in Kysak, Dubná Skala, Budatín, Košická polianka, Piešťany, Kostoľany, Lodina, Žilina, Gyňov, Košické Olšany, Bokša, Veľká Lodina, Malá Lodina, Geča and Piešťanská Sĺňava. The tapeworms found in the small intestine were isolated and fixed in 80 % alcohol. The morphometric data were obtained using a microscope ZEISS AXIO Lab.A1 with software of AxioCam ERC5S.

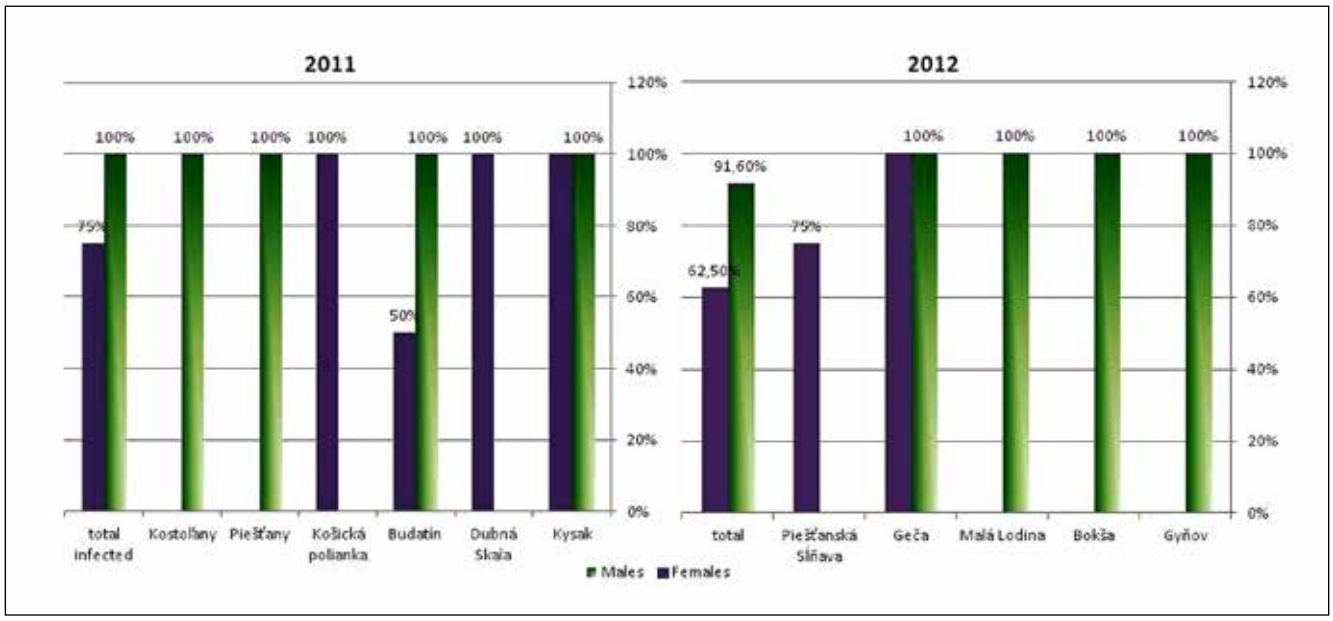


Fig. 1. Prevalence (%) of tapeworms in male and female cormorants

Fig. 2. *Paradilepis scolecina* scolex, strobilae and mature proglottids

RESULTS AND DISCUSSION

A total of 89.47 % and 80 % of the cormorants were found to be infected with tapeworms in 2011 and 2012, respectively (Fig. 1). The intensity of tapeworm infestation in the cormorants can be very high. The mean intensity in 2011 was 316.5 with a range of 10—1888, while the mean intensity in 2012 was 199.3 with a range of 3—1083. The mean abundance in 2011 was 283.2 with a range of 0—1888 and 159.5 in 2012, with a range of 0—1083. All the tapeworms were found in

the small intestine. One species of tapeworm, *Paradilepis scolecina,* was identified (Fig. 2).

Most of the tapeworms were adult individuals more than 2 mm long. The worm's length ranged from 2.1 to 4.9 mm (3.48 ± 1.089). Scolexes were 263—445 µm long (354.95 ± 56.9) and 212—414 µm wide (349.05 ± 50.59). Rostellum was 102—270 µm long (178.3 ± 53.49) and 78—182 µm wide (128.15 ± 26.25). The characteristics of the genus *Paradilepis* consist of a double row of hooks, of urceus or scolecina pattern found on the rostellum, unilateral genital pores, genital ducts dorsal to excretory vessels, genital organs without accessory structures, vagina ventral to cirrus sac, uterus sacciform, four testes and cirrus with rosethorn spines [1]. The highest intensity of infection with tapeworms was found in the cormorants shot in Budatín (1888 per bird) and Malá Lodina (1083 per bird). The high intensity indicates the occurrence of the cestode larvae in the fish that the cormorants consume. The pathogenicity of this tapeworm towards the cormorants has not been found; however, it may cause hypertrophy of the tissue surrounding the parasite within the fish's body [5] and facilitate the development of secondary diseases [4].

CONCLUSION

A high number of *P. scolescina* may cause extensive damage to the small intestine, caused by the deep penetration of the scolexes through the intestinal wall after ingestion of the infected fish. However, no visible harmful effects were observed in the cormorants. They were in good condition and may survive well with high intensity of parasitic infection.

REFERENCES

1. Bona, F. V., 1994: Family Dilepididae Railliet & Henry. In **Khalil, L. F., Jones, A., Bray, R. A.** (Eds.): *Keys to the Cestode Parasites of Vertebrates*. CAB International, Wallingford, Oxon, UK, 445—554.

2. Chukalova, N., 2008: First record of *Paradilepis scolecina* (Cestoda: Dilepididae) and *Contracaecum rudolphii* (Nematoda: Anisakidae) in bream (*Abramis brama*) in the Curonian Lagoon of the Baltic Sea (In Russian). *Parazitologiya*, 42, 533—536.

3. Dziekonska-Rynko, J., Dzika, E., 2011: The tapeworm *Paradilepis scolecina* (Rudolphi, 1819) (Cestoda: Cyclophyllidea) invasion in Great Cormorant [*Phalacrocorax carbo sinensis* (Blumenbach, 1798)] from the breeding colony in Lake Selment Wielki (northern Poland). *Helminthologia*, 48, 23—28.

4. Murai, E., Molnar, K., Gubanyi, A. 1997: Occurrence of the adult and plerocercus forms of *Paradilepis Scolecina* (Rudolphi, 1819) (Cestoda: Dilepididae) in Lake Balaton, Hungary. *Parasitology Hungary*, 29, 33—38.

5. Prost, M., 1994: *Diseases of fish* (In Polish). Polish Society of Veterinary Sciences, Lublin, 551 pp.

6. Shwiff, S., Kirkpatrick, K., 2009: The economic impact of Double-crested cormorants to central New York. *Economic project*, 37 pp. Online: <http://www.flintsteelheaders.com/cormorant_control_file/impact_dcc.pdf>

*

Selected papers from the 56th STUDENT SCIENTIFIC CONFERENCE, Section VI, held at the University of Veterinary Medicine and Pharmacy in Košice on April 17, 2013.

FOLIA VETERINARIA, 57, 2: 117—119, 2013

PREVALENCE OF FLUKE WORMS IN RUMINANTS IN THE WEST OF IRELAND

Gordon, M., Halán, M.

University of Veterinary Medicine and Pharmacy, Komenského 73, 041 81 Košice
The Slovak Republic

halan@uvlf.sk

ABSTRACT

The prevalence of fluke infection among cattle of various breeds was investigated on ten different farms in an area of western Ireland. Faecal samples were collected at each farm over several weeks during late August 2012 and were analysed for the presence of *Fasciola hepatica*, *Paramphistomum* spp. and *Dicrocoelium dendriticum* eggs. An abundance of *Paramphistomum* spp. was detected on all but two farms with a concurrent low level detection of mature infections of *Fasciola hepatica* on four farms, while *Dicrocoelium dendriticum* was an incidental finding on two farms. The management factors, such as farm pasture management, regular treatment schedules, and yearly control programs, were noted on farms with lower counts of *Paramphistomum* spp. and an absence of *Fasciola hepatica.* Environmental factors, such as flooding, the type of pasture used, such as wetland and lowland areas, and the out wintering of animals, were noted on farms with significant burdens of *Paramphistomum* spp. and mature infections of *Fasciola hepatica.* The timing and regularity of treatments, and in particular, the products used, were noted with significant variation in levels of infections found on each farm. In the results of the study, *hepatica* was found on 9 farms, *Paramphistomum* spp. was found on all 10 farms, while *D. dendriticum* was found only on 3 farms. The highest incidence of *F. hepatica* was noted on Farm 6, while for both *Paramphistomum* spp. and *D. Dendriticum,* the highest incidence was found on Farm 9. Overall, Farm 9 had the highest number of positive samples, while Farm 1 had the lowest number of positive samples.

Key words: *Dicrocoelium dendriticum*; *Fasciola hepatica;* fluke worm; Ireland; *Paramphistomum* spp.

INTRODUCTION

On the north-west edge of Europe, Ireland enjoys a temperate climate and abundant rain water [6]. Fasciolosis is a very common parasitic disease of ruminants caused by *Fasciola hepatica* in Ireland. In Ireland alone, the Department of Agriculture, Food and the Marine calculates that liver fluke costs in the region of € 25 million every year [2]. In previous years *Paramphistomum* spp. caused little or no production losses and was considered of minimal importance, in recent years however, a surge in clinical illnesses has renewed its significance. According to Animal Health Ireland, *Dicrocoelium dendriticum* is significantly less prominent than *Paramphistomiasis* and *F. hepatica* infections on Irish farms [1]. The life cycle of *Paramphistomum* spp. is broadly similar to that of *F. hepatica*, they both require two hosts to complete their life cycle; a mammalian definitive host and a snail intermediate host [7]. In this study, 10 farms in the west of Ireland were selected on the basis of, the availability of suitable samples and locations within the target area, to gain an insight into the prevalence of fluke infections in the area.

MATERIALS AND METHODS

Materials: disposable gloves, small plastic containers, plastic 'ziplock' bags, plastic pipettes, 2 ml Eppendorf tubes, formalin, watch glass, and a microscope.

Ten farms were chosen for study in east Galway, an area in the west of Ireland. In late August 2012, faecal samples were collected from two groups of animals on each farm, providing 100 samples in total. The faecal sedimentation technique was completed on each sample in Ireland. A sample of the sediment was then placed in an Eppendorf tube with an equal amount of formalin to ensure preservation of the material.

These were then transported back to the Department of Epizootiology and Parasitology at the University of Veterinary Medicine and Pharmacy in Košice. The samples were examined using a microscope and any *Fasciola hepatica*, *Paramphistomum* spp. and *Dicrocoelium dendriticum* eggs, if present, were recorded. Data on treatments, pasture quality and control programs were noted for each farm.

RESULTS

The results showing the prevalence of fluke infection among cattle of various breeds obtained from 10 farms in the west of Ireland are presented in Table 1.

In Table 1, the mean numbers of each of *Fasciola hepatica, Paramphistomum* spp. and *D. dendriticum,* on each farm are combined to obtain a total average fluke egg number of all three species on each farm. The best farm was Farm 1 and the worst was Farm 9. *Paramphistomum* spp. was found on all 10 farms while *Fasciola hepatica* was found on 9 of the 10 farms and *D. dendriticum* was found on 3 farms.

Fig. 2 shows the mean numbers of *Paramphistomum* spp., *Fasciola hepatica* and *D. dendriticum* eggs recorded over all 10 farms. This shows that *Paramphistomum* spp. far outweighted both *Fasciola hepatica* and *Dicrocoelium dendriticum.*

DISCUSSION

Paramphistomum spp. was found in all samples and was more common than expected, a finding that has been noted by DeWaal [4]. The average figure for *F. hepatica* found over all the farms was almost half that of *Paramphistomum* spp., However, the presence of mature *F. hepatica* infection in the late August samples, despite regular treatment with flukicidal drugs on all farms over the previous year, suggests that the seasonality of *F. hepatica* is changing as has been reported by Fox *et al.* [5]. Two farms with the highest incidence of *Paramphistomum* spp. were both subject to flooding on pasture. Of the 10 farms, just 3 used a treatment product effective against *Paramphistomum* spp., demonstrating the importance of flooding and lack of effective treatment in the prevalence of *Paramphistomum* spp. Farms 1, 3 and 8, had a fluke control plan in place and had the lowest overall averages of all three fluke species, supporting the finding by Agriculture and Food Development Authority, Ireland_(3), that control plans can result in lower levels of liver and rumen fluke infections.

Table 1. Mean number of all fluke eggs per farm

	Fasciola hepatica	Paramphistomum spp.	Dicrocoelium dendriticum	Total	Rank (low to high)
Farm 1	2	3	–	5	1
Farm 2	3	4	–	7	4
Farm 3	–	4.5	1	5.5	2
Farm 4	2.5	8	–	10.5	5
Farm 5	6	19	–	25	8
Farm 6	13	10	–	23	7
Farm 7	11	16	1	28	9
Farm 8	3	3.5	–	6.5	3
Farm 9	8	22	2	32	10
Farm 10	4	13	–	17	6
Total	**52.5**	**103**	**4**	**159.5**	

CONCLUSIONS

Good control plans on farms can significantly reduce the incidence of infection regardless of the type of farming, although farms with pasture in highland or mixed high and low land pastures with good control plans have significantly lower infection levels. The west of Ireland has long been reported to be a location of regular fluke infections with *F. hepatica* and in recent years *Paramphistomum* spp. infections. The prevalence of *Paramphistomum* spp. infections is higher than was thought and may be a result of progressive climate change causing heavier rainfall and flooding combined with a lack of effective treatment and understanding of its pathogenicity and treatment among farmers. The usually low seasonal risk for mature *F. hepatica* infections in summer, has been dispelled by the presence of a consistent level of *F. hepatica* infection on nine of the ten farms, despite regular treatments throughout the year, indicating a loss of seasonality of infection.

REFERENCES

1. Animal Health Ireland, 2009, 2011: Liver Fluke Guide 2009, Rumen Fluke Guide 2011.

2. Department of Agriculture, Food and the Marine, Ireland, 2011: <http://www.agriculture.gov.ie>

3. Dept. of Research and Innovation, Teagasc, Agriculture and Food Development Authority, Ireland, 2012: <http://www.teagasc.ie/research>

4. De Waal, T., 2010: Paramphistomum infection in cattle, UCD School of Veterinary Medicine. *Vet. Ireland Journal*, 63, 313—315.

5. Fox, N. J., White, P. C. L., McClean, C. J., Marion, G., Evans, A., 2011: *Predicting Impacts of Climate Change on Fasciola hepatica Risk. PLoS ONE,* 6, e16126.

6. Met Eireann, Irish Weather Service, 2012: <http://www.met.ie>

7. U rquhart, G. M., Armour, J., Duncan, J. L., Dunn, A. M., Jennings F. W., 1996: *Veterinary Parasitology.* 2nd edn., Blackwell Sci., Cambridge, Mass., 307 pp.

*

Selected papers from the 56th STUDENT SCIENTIFIC CONFERENCE, Section VI, held at the University of Veterinary Medicine and Pharmacy in Košice on April 17, 2013.

FOLIA VETERINARIA, 57, 2: 120—123, 2013

PINWORMS (OXYURIDA) OF LIZARDS KEPT IN TERRARIUMS IN SLOVAKIA

Butler, M., Halán, M.

University of Veterinary Medicine and Pharmacy, Komenského 73, 04181 Košice
The Slovak Republic

halan@uvlf.sk

ABSTRACT

This study was carried out in order to ascertain both the prevalence and intensity of parasitic infection with Oxyurid nematodes in terrarium kept lizards in Slovakia. A total of 204 samples were collected over a 2-year period, from the 1st of January 2011 until the 31st of December 2012, and examined for the presence of endoparasites. The specimens originated from 15 species of lizards ranging across 6 families. All positive cases were recorded, but only those with Oxyurid eggs were fully documented. Records were made regarding the species from which the sample was derived, the month and year that the sample was examined and the intensity of the infections present. Eighty-five of the 204 samples were found to be positive, with the most significant findings seen in Bearded Dragons, Leopard Geckos and Green Iguanas. Leopard Geckos were the most commonly infected with 82 % positive. Bearded Dragons had the most comprehensive results, as samples from this species accounted for over 30 % of the total sample pool, with 58 % positive.

Key words: lizards; Oxyurida; pinworm; Slovakia

INTRODUCTION

Oxyurida in reptiles are surprisingly common but are not usually a cause for concern as their numbers are usually maintained at a safe level by the bacteria in the gut. Problems arise when the immune system is compromised, e.g. following periods of stress. Only when numbers reach intense levels, will they be presented diagnostically.

Oxyurida comprise approximately 850 known species which can be found in the intestine of arthropods and vertebrates. This gives rise to the belief that arthropod hosts can be a source of infection to the reptile species that they prey upon. Of these known species, Pharyngodon appears to be the most regularly isolated species in lizard faecal samples [3].

Infection rarely manifests with clinical symptoms in the lizard, however, signs of infection can include a decreased activity level and appetite, weight loss, a change in faecal consistency and a poor body and skin condition [1]. With higher levels of infections, their impaction may occur due to the sheer number of Oxyurids present in the intestine. Therefore, vomiting and tenesmus may be seen [4].

The aim of this study was to ascertain the prevalence and intensity of parasitic infection with Oxyurid nematodes in terrarium kept lizards in Slovakia.

MATERIALS AND METHODS

Oxyurid infection was investigated in 204 faecal samples over a 2-year period from January 2011 to December 2012. The samples were mainly from private owners, but also from the local zoo. The sample pool, as regard to species, was random and families included *Chamaeleonidae, Gekkonidae, Polychrotidae, Iguanidae, Varanidae, Scincidae* and *Agamidae* (Table 1).

Table 1. Summary of investigated subjects

	Scientific Name	Common Name	No. examined
Gekkos	*Eublepharis macularius*	Leopard Gecko	17
	Gekko gecko	Giant/Tokay Gecko	8
	Phelsuma madagascariensis	Madagascan Day Gecko	4
	Terratoscincus scincus	Common Wonder Gecko	2
Iguanas	*Iguana iguana*	Green Iguana	35
	Chalarodon madagascariensis	Madagascar Iguana	4
Dragons	*Pogona vitticeps*	Bearded Dragons	62
	Physignathus cocincinus	Chinese Water Dragon	6
Chameleons	*Chamaeleo calyptratus*	Veiled Chameleon	16
	Furcifer pardalis	Panther Chameleon	7
	Chameleo chamleon	Common Chameleon	22
Anolis	*Anolis caroliensis*	Carolina Anole	9
Skinks	*Cryptoblepharus egeriae*	Blue Tailed Skink	2
	Chalcides ocellatus	Ocellated Skink	2
Monitors	*Varanus exanthematicus*	Bosc Monitor	8

Excrements were collected and a simple flotation method was performed. Most were obtained from private breeders/owners carrying out routine testing as normal active animal husbandry and health maintenance. Samples from the zoo were collected by veterinarians during routine visits. They were kept at 4 °C until examinations could be carried out. For this study, Breza's solution (3 parts $MgSO_4$, 3 parts $Na_2S_2O_3$ and 1 part H_2O) was used as the flotation solution [5].

Following preparation, samples were placed on a glass slide using a parasitological loop and submitted for microscopic examination.

RESULTS

Six lizard families from the class Reptilia were examined; with samples from a total of 15 species belonging to these families. The samples were first divided according to Family (*Geckonidae*, *Iguanidae*, etc.) with each of these groups then listed according to the species investigated.

The first set of crude results showed that 85 of the total 204 samples were positive. The Bearded Dragons accounted for the highest representation of positive samples at 17.65 %, however they also comprised 30 % of the total samples investigated.

The most commonly encountered species throughout this study were Bearded Dragons, Green Iguanas, Common Chameleons, and Leopard Geckos. Interestingly, these species also had the highest percentage of positive results within their own species; Leopard Gecko 82 %, Bearded Dragons 58 %, Common Chameleon 36 % and Green Iguana 29 %.

The intensity of infections was either light or heavy. Very few species fell into the "moderate" category. Light infections gave rise to the thought that lizards were either treated prophylactically with anti-helmintics or the infection was relatively new and numbers hadn't reached problematic levels. Heavy infections were possibly incited by a lack of owner knowledge on basic care and husbandry, lack of anti-helmintics or a lizard that had been exposed to stress.

DISCUSSION

Faecal samples were collected from a variety of lizard species ranging across 6 different lizard families, in an attempt to fully estimate the pervasiveness of Oxyurid infec-

tion within privately owned as well as zoo kept species. Lizards from which the samples were derived, were not submitted for clinical examination, so no other data such as age, sex or health status, were collected.

Animals subjected to stress such as transportation, adverse environmental conditions or the presence of different species, can lead to the multiplication and spread of parasites which in nature live in cohabitation with their hosts [7]. For this reason, the health status of the animal is of importance when considering the parasitic burden encountered in faecal samples.

Another parameter of note is that of the number of each species that were examined. The percentage of positive results will have differing significance depending on the actual number of animals investigated when looking at the results relative to species, family and that of the total number of samples in the study. The number of each species examined was random, dependent on the samples sent to the laboratory for routine examination and the numbers of each species are reflected in the lizards that are most commonly kept as pets.

In this study, 41.66 % of all samples tested positive for the presence of Oxyurids. While a higher percentage would have been recorded if all parasites found were taken into consideration, it is clear that Oxyurids were the most prevalent. No significant correlation was noted regarding the results from year to year and the number of positive samples. The numbers merely reflected the number of samples investigated in each year, i.e. more samples were processed in 2011 than 2012 and subsequently more positive samples were seen in 2011 also.

The most commonly encountered species throughout this study were Bearded Dragons, Green Iguanas, Common Chameleons and Leopard Geckos. While other species were also examined and their results recorded, it is difficult to gauge the significance of the numbers found to be positive due to only a small numbers of these species being investigated. The percentage of positive results from some of these species were high; however it is impossible to say that this is a true representation of all lizards of that particular species that are kept in terrariums in Slovakia. Of the species that are outlined above, the Leopard Gecko proved to be the species most commonly infected by Oxyurids, with 82 % positive. Bearded Dragons had possibly the most comprehensive results, as samples from this species accounted for 30 % of the total samples examined and 58 % were positive. Oxyurid nematodes were also confirmed most significantly in the Common Chameleon (36 %) and the Green Iguana (29 %).

Oxyurids (Pinworms) represent the largest group of parasites of the caudal intestine of lizards. All genera that are parasitic in reptiles are classed in the family *Pharyngodonidae* [2]. This fact has been confounded by numerous studies investigating various species of lizards in many countries where Pharyngodon species were the most commonly reported pinworm to be isolated from samples [3], [6], [8]. While Pharyngodon have been identified as the most common species isolated from lizard faecal samples, species identification cannot always be made and requires further investigation. Throughout the course of this study, Pharyngodon species were identified but this was not always possible in all positive cases.

Pinworm infections can often go unnoticed as they may not always be visible in the faeces of the lizards. In carrying out this study it became very apparent that infestation levels are either light or heavy, with very few cases falling into the moderate category. Figures practically matched in these cases with light infections accounting for 35 of the samples and heavy infections reaching 36. Only 11 samples were within the range considered to be moderate infections and these were only seen in the species most frequently encountered.

One possibility for light infection is that owners prophylactically treat their lizards so numbers are low or that it is a new infection, thus numbers have not yet reached their potential. This could be due to a variety of factors including the introduction of new animals into the terrarium or they had only just become infected through ingestion of infected feeder rodents or insects and the parasites have not increased to problematic numbers. Heavy infections can also have a number of contributory causes which may include a lack of knowledge by the owner or substandard anti-helminthic routines.

CONCLUSION

Oxyurids (Pinworms) represent the largest group of intestinal parasites in the lizard, a hypothesis which appears to have been confirmed in this study. It is also of note to say that many of these infections are an incidental finding at routine faecal examination as clinical signs are usually not apparent. Recommendations concluded from this study are that further parameters need to be taken into account such as age, gender and health status of each test subject. A bigger sample pool should be established to account for all species that were examined, in order to adequately ascertain their significance in terms of levels of positive cases together with level of the intensity of the infections. More studies could be conducted as to the species of Oxyurids that are encountered, namely Pharyngodon species.

This will invariably lead to a greater understanding of the species involved in Oxyurid infections in lizards which in turn will lead to better care and maintenance in general, which will enhance animal husbandry, health and welfare.

REFERENCES

1. Baker, M. R., 1984: Nematode parasitism in amphibians and reptiles. *Canadian Journal of Zoology,* 62, 747–757.

2. Fitzsimmons, W. M., 1961: A new nematode Pharyngodon morgani sp. nov., intestinal parasite of a lizard, Mabuya striata, in Nyasaland. *Parasitology*, 51, 395–399.

3. Goldberg, S. R., Bursey, C. R., Cheam, H., 1998: Gastrointestinal helminths of four gekkonid lizards, Gehyra mutilata, Gehyra oceanica, Hemidactylus frenatus and Lepidodactylus lugubris from the Mariana Islands, Micronesia. *J. Parasitol.*, 1295–1298.

4. Knotek, Z., 1999: *Diseases of Reptiles* (In Czech). Association of Czech Veterinarians of Small Animals, Palackeho 1–3, Brno CZ, T.S. Print.

5. Letková, V., Goldová, M., Csizsmárová, G., 1998: *Handbook for the Diagnosis of Parasites.* Part I, M&M Presov, 18–19.

6. Papini, R., Manetti, C., Mancianti, F., 2011: Coprological survey in pet reptiles in Italy. *Vet. Rec.,* 169, 207.

7. Rataj, A. V., Lindtner-Knific, R., Vlahović, K., Mavri, U., Dovč, A., 2011: Parasites in pet reptiles. *Acta Vet. Scand.*, 53, 1–21.

8. Saehoong, P., Wongsawad, C., 1997: Helminths in house lizards (Reptilia: Gekkonidae). *Southeast Asian J. Trop. Med. Public Health*, 28, 184–189.

*

Selected papers from the 56th STUDENT SCIENTIFIC CONFERENCE, Section VI, held at the University of Veterinary Medicine and Pharmacy in Košice on April 17, 2013.