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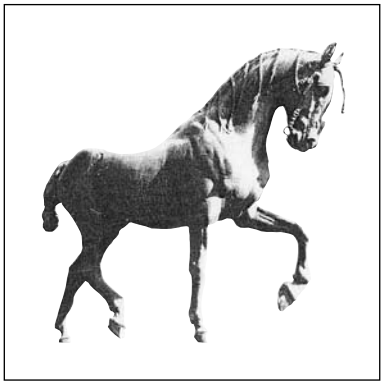

51st STUDENT SCIENTIFIC CONFERENCE

April 23rd, 2008

The aim of the 51st Student scientific conference (ŠVOČ) organised in the academic year 2007/2008 was to present results fo scientific investigations carried out by undergraduate and PhD. students. The papers were presented in the following four sections:

1. Pre-clinical – 2. Clinical
3. Hygiene of food and the environment
4. Post-graduate students

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COMPARISON OF TTA AND BAL FOR DIAGNOSIS OF LOWER RESPIRATORY AIRWAYS INFECTIOUS AGENTS

Ulbrík, M., Boldižár, M.*, Kasenčák, M.**

Clinic For Horses, University of Veterinary Medicine, Komenského 73, 041 81 Košice
*Department of Pathological Anatomy, State Veterinary and Food Institute Dolný Kubín
**Testing Laboratory, Bajkalská 28, 080 01 Prešov
The Slovak Republic

ABSTRACT

Bronchoalveolar lavage (BAL) and transtracheal aspiration (TTA) are special methods most frequently used to diagnose diseases of lower respiratory airways in horses. BAL is a blind, non-sterile method of sampling of bronchiolar secretion by means of a catheter which is passed through upper respiratory airways and trachea always to one of the principal bronchi. The obtained bronchiolar aspiration is suitable for cytological examination. TTA is a blind, sterile method of sampling of the tracheobronchiolar secretion. In comparison with BAL, the TTA is an invasive method eliminating completely contamination of lower respiratory airways with micro-organisms of the upper respiratory tract. The ventral section of trachea is reached by means of a catheter inserted through *lig. intertracheale* the end of which reaches the level before *bifurcatio tracheae*. The obtained transtracheal aspiration is suitable for microbiological examination. The present study compares the two sampling methods, BAL and TTA, with regard to potential microbiological culturing of pathogens from lower respiratory airways.

Key words: *Aspergillus* sp.; bronchoalveolar lavage; horse; *Mucor* sp.; *Streptococcus equi;* transtracheal aspiration;

INTRODUCTION

Infectious and allergic inflammatory diseases of respiratory airways in horses are a constantly discussed issue in the field of horse industry. Intolerance of load and poor performance are terms used by owners and trainers of horses when describing failure of these animals to reach the maximum performance potential (5). After diseases of the musculoskeletal system the respiratory diseases rate as the second most frequent cause limiting performance of horses. Early diagnosis of respiratory diseases is a basic precondition of rapid recovery of performance and return to the training process. Even more important is the prevention of secondary complications which may terminate early the successful athletic career of the horse (1). The current studies indicate a gradual decrease in performance level in young horses suffering from infectious and non-infectious inflammatory diseases of the respiratory tract (2).

MATERIAL AND METHODS

The experiment compared bacterial contamination of lower respiratory airways detected in the same horse by simultaneous collection of samples (aspirations) by TTA and BAL methods. TTA and BAL aspirations were collected under total sedation from a standing patient. The puncture site for TTA was located by palpation in the ventral part of the neck, in median line between *m. sternothyrohyoideus.* Cutis and subcutis in the puncture site was desensitized by infiltration. A hypodermic needle 1.6 × 40 mm was inserted into the tracheal lumen through a puncture and a sterile catheter was passed through the needle lumen in the direction of bifurcation. After application of about 40 ml of sterile saline, the tracheobronchial secretion was collected by aspiration (4).

Bronchoalveolar lavage was performed with BAL catheter, 240 cm long and 10 mm in diameter, with inflatable cuff at

its distal end. The catheter was inserted through a nostril and ventral meatus into the hypopharynx. From there it was passed blindly through *rima vocalis* into lower respiratory airways and its end was located in left or right bronchial stroma. After inflating the sealing cuff, we infused 500 ml of sterile saline and aspired the bronchiolar lavage (3). Samples collected by both methods were transported for microbiological diagnosis on sterile swabs placed in a Stuart's medium. Microbiological recovery for the two sampling techniques was compared by examining the matched samples.

RESULTS

The experimental set of animals consisted of 4 mares of Slovak Warm-blooded breed, 8 to 22 years old. Collection of simultaneous samples by TTA and BAL methods and subsequent microbiological examination allowed us to diagnose the following: *Streptococcus equi* ssp. *zooepidemicus, Staphylococcus coagulase* negative, *Aspergillus flavus, Aspergillus parasiticus, Mucor* sp., *Penicillium* sp., *Rhizopus* sp., *Candida* sp., *Serratia rubidea,* saprophytic microflora, aerobic sporulates. Comparison of individual microbiological findings for the matched samples by standard microbiological culturing under aerobic conditions showed coincidence in two cases and in case of mycological culturing in three cases. A more massive finding of microbiological colonies was observed in a sample obtained by TTA. In all examined cases the microbiological findings corresponded to the clinical status of the patients.

DISCUSSION

TTA and BAL are valuable supplementary methods for diagnosis and monitoring of infectious and non-infectious inflammatory diseases of lower respiratory airways in horses. The studies performed on a bigger experimental set of animals showed insignificant differences in microbiological recovery for the two tested sampling techniques (2). The present study confirmed conclusions of H e w s o n and V i e l (2) who indicated better recovery of pure microbiological culture from samples collected by the TTA technique.

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DETECTION OF FUNGAL PATHOGENS

Neriah, O., Hričinová, M., Chomová, Z.

University of Veterinary Medicine in Košice, Department of Microbiology and Immunology and Centre for Analysis of DNA, Komenského 73, 041 83 Košice
The Slovak Republic

ABSTRACT

For successful PCR detection of fungal pathogen several different methods of DNA extraction have been compared. The primers ITS1, ITS3 and ITS4 were used for identification of *Malassezia* spp. and *Candida* spp. to the genera level. We received positive result in all cases with the primers ITS 3 and ITS 4 including, when we used a mix of samples.

Key words: dermatomycosis; DNA extraction; PCR

INTRODUCTION

Early identification of pathogenic fungi has been necessary in a variety of fields (e.g. food analysis, environmental monitoring, clinical diagnosis). A few decades ago, detection of pathogenic microorganisms required laborious culture methods. Since the ability to amplify specific DNA sequences was developed, molecular methods have replaced conventional assay based on classical microbiology. In the last 20 years, PCR has become a useful tool for rapid detection or identification of many pathogenic microorganisms.

Dermatomycosis are mycotic diseases of skin caused by a few mycetes: dermatophytes, and some opportunistic fungi (yeast and moulds) as *Malassezia, Candida, Trichosporon, Rhodutorula, Cryptococcus* or *Aspergillus, Geotrichum, Alternaria,* etc. Since on the skin of animals there are many saprobic organisms (*Malassezia*) and many fungi may infect the fur, it is important to make an accurate diagnosis. Direct microscopy, although false negative up to 50 % of cases, is a highly efficient screening technique. Serological approaches have revealed difficulties. Many kinds of molecular biologic techniques have been made available for clinical diagnosis recently; almost all of these techniques involve the polymerase chain reaction (21).

MATERIAL NAD METHODS

Cultivation: Fungal isolates recovered from the clinical specimens were cultured for 2–5 days on Sabouraud agar plates at 30 °C in the stationary condition.

DNA extraction: The genomic DNA was isolated from each colony using:

a) DNA extraction kit (PureLink™-Genomic DNA Mini Kit, Invitrogen).

b) Colony (approximately 15 mm in diameter) was collected from the culture and:

- resuspended in 500 µl of distillate water and vortexed;
- resuspended in 500 µl of distillate water and vortexed with sea sand;
- resuspended in 500 µl of lysis buffer (from PureLink™-Genomic DNA Mini Kit, Invitrogen) and vortexed;
- resuspended in 500 µl of lysis buffer (from PureLink™-Genomic DNA Mini Kit, Invitrogen) and vortexed, enzymatic digestion with Proteinase K (37 °C 1 h);
- resuspended in 500 µl of solution physiological and vortexed;
- resuspended in 500 µl of solution physiological and vortexed with sea sand;

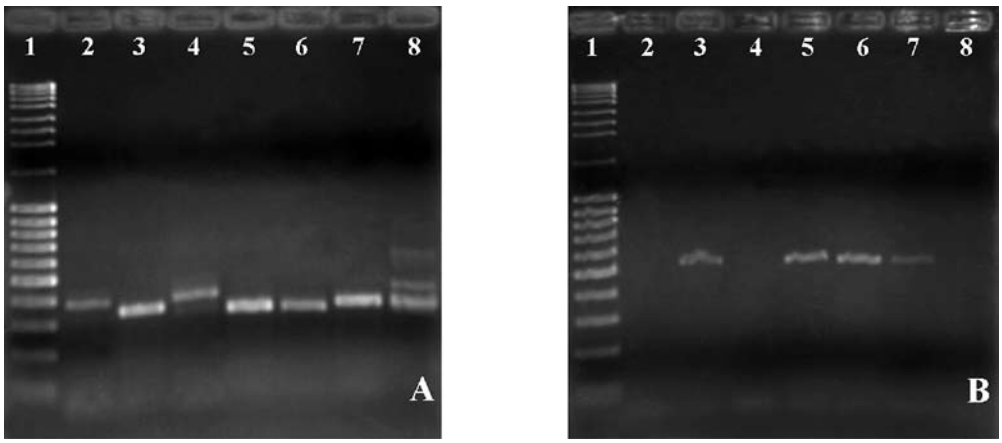

Fig. 1. Agarose gel electrophoresis of the PCR products

Legend (from the left):
A (primers ITS 3, ITS4): 1 – DNA Ladder (100 bp); 2–8 – PCR products: 2 – *Malassezia pachydermatis*; 3 – *Candida albicans*; 4 – *Candida albicans*; 5 – *Malassezia pachydermatis; 6 – Malassezia pachydermatis*; 7 – *Candida albicans*; 8 – *Malassezia pachydermatis* and *Candida albicans;*
B (primers ITS 1, ITS4): 1 – DNA Ladder (100 bp); 2–8 – PCR PCR products: 2 – *Malassezia pachydermatis;* 3 – *Candida albicans*; 4 – *Candida albicans*; 5 – *Malassezia pachydermatis*; 6 – *Malassezia pachydermatis*; 7 – *Candida albicans*; 8 – *Malassezia pachydermatis* and *Candida albicans*

After boiling all samples at 100 °C from 5 to 20 min following the centrifugation for 5 min at 12 000 *g* (twice), the supernatants were used for PCR. All supernatants (DNAs) used for PCR amplification were kept at +4 °C until use.

PCR conditions were performed according to Gupta *et al.* (10).

PCR Master Mix was performed in 50 µl reaction volume containing AmpliTaq DNA polymerase (Perkin Elmer) 2.5 U, 75 µmol of each dATP, dCTP, dGTP, dTTP, PCR buffered solution Perkin Elmer with 1.5 mmol $MgCl_{2,}$ 2 µl of DNA template and 0.5 µmol of each primer

a) ITS 1/ ITS4 for *Malassezia* and *Candida* species according (10);

b) ITS 3/ ITS4 for Malassezia species according Gaitanis *et al.* (9);

Agarose Gel Electrophoresis: Electrophoresis of PCR products was performed with 1 % or 1.5 % agarose gel in TAE electrophoretic solution during 1 hour at 80 V using Bio-Rad Gel electrophoretic system. DNA was colourised with ethidium bromid (0.5 $\mu g.ml^{-1}$) and visualized by UV light (320 nm). Gels were evaluated according "Kodak GelLogic 100" system.

RESULTS AND DISCUSSION

Fungi have cell walls that impede cell lysis and the recovery of DNA using conventional extraction methods (15). Simple lysis procedures, such as the use of sequential freeze-thaw cycles or incubation with hot detergent and proteases, have not produced high yields of DNA from many fungal species. Alternative approaches for the lysis of fungal cells include the agitation of tissue samples with microspheres or particulates within a sealed tube for physical disruption and the enzymatic digestion of cell wall polysaccharides to form spheroplasts followed by conventional membrane lysis procedures (14). Some DNA extraction methods for fungi, such as grinding cells frozen with liquid nitrogen using a mortar and pestle and disrupting cell walls with a probe sonicator, work well for the large-scale preparation of fungal DNA from cultures. However, these methods are not practical for use in a clinical microbiology laboratory (8).

We compared several different methods for DNA preparation. The basic requirement for a good yield of DNA, however, is freshly grown (2- to 5-day-old) yeast cells. When we resuspended colonies in 500 µl of distillate water, vortexed and boiled, this method was found to be most simplest, fastest and provided good results. The recovery of DNA from the kit was the fastest but we needed to use a special enzyme Proteinase K (With this kit, extraction of DNA from bacteria gave very good results).

Applied DNA was isolated from reference strains of *Mallassezia* spp. and *Candida* spp. (general gift by Dr. Eva Čonková, University of Veterinary Medicine in Košice, The Slovak Republic).

After obtaining of the PCR results we continued with agarose electrophoresis and we obtained following results:

- **Positive result** in all cases with the primers ITS 3 and ITS 4 including, when we used a mix of samples.
- The same samples were used with the primers ITS 1 and ITS 4, but in some cases **negative results** were received (see Fig. 1).

Consequently the same PCR conditions were used for a "mixture of *Malassezia* and *Candida* spp.". During PCR we used DNA samples of positive control obtained from reference strain and negative control. Positive result

was obtained only in with the primers ITS 3 and ITS 4 (Fig. 1A, lane 8).

CONCLUSIONS

All methods for DNA extraction which we have used are applicable for the purpose to obtain fungal-DNA for PCR detection. We received **positive result** in all cases with the primers ITS 3 and ITS 4 including, when we used a mix of samples. But still, it seems to be necessarily to use enzyme (e.g. zymolase) for better lysis of fungal cells. The time required for obtaining results by PCR detection of *Malassezia* and *Candida* spp. (include time for preparing of samples) was 4–5 hours. For more precise diagnosis the usage of restriction enzymes must be tested.

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DETECTION OF CHROMOSOMAL DAMAGE AFTER EXPOSURE TO TOLYLFLUANID IN BOVINE PERIPHERAL LYMPHOCYTES *in vitro*

Pulzová, L., Šivíková, K.

Institute of Genetics, University of Veterinary Medicine
Komenského 73, 041 81 Košice
The Slovak Republic

ABSTRACT

A technical fungicide EUPAREN MULTI (Bayer AG, Germany) containing tolylfluanid was tested for the induction of sister chromatid exchanges (SCE) in cultured bovine peripheral lymphocytes. Cultures of lymphocytes were exposed to tolylfluanid at concentrations ranging from 1.7 to 17.5 μg.ml^{-1} for the last 24 and 48 h of incubation. A statistically significant elevation in the SCE frequency was observed after exposure to tolylfluanid for 48 h. Exposure to tolylfluanid for 24 h caused a statistically significant elevation in the SCE frequency only at the highest concentrations. The reduction of proliferation indices was also seen.

Key words: proliferation indices; sister chromatid exchanges; tolylfluanid

INTRODUCTION

Tolylfluanid is fungicide widely used in agriculture, against fungal diseases in strawberries, raspberries, apples, vine and other plants. It is one of the most frequently detected pesticides in vegetables and fruit. It was rapidly and extensively absorbed and almost completely excreted within 48 h after the oral administration (4). In mice and rats tolylfluanid was regarded as a low toxic agent. In comparison with other pesticides, tolylfluanid was lower or higher toxic than chlorothalonil and ingarol for the embryos and larvae of marine invertebrates (2).

The aim of this study was to detect early effects of the exposure to tolylfluanid in cultured bovine peripheral lymphocytes using cytogenetic biomarkers, as sister chromatid exchanges (SCE) and proliferation indices (PI).

MATERIAL AND METHODS

The technical tolylfluanid (Euparen MULTI, Bayer, AG, Germany, active compound 50%) was dissolved in dimethyl sulphoxide (DMSO) and applied into culture flasks at the concentrations of 1.7, 3.5, 8.75 and 17.5 μg.ml^{-1} for 24 and 48 h of cultivation. Mitomycin (MMC, Sigma, St. Louis, MO, USA, 4 μmol) and DMSO (Sigma, St. Louis, MO, USA) were used as a positive and negative control. Experiments were carried out with healthy calves (Slovak spotted cattle, 6–8 months old).

Chromosome preparations were obtained by the standard cytogenetic method. For SCE assay and cell cycle kinetics, bromodeoxyuridine (BUdR, Sigma, St. Louis, USA) at a final dose 8 μg.ml^{-1} was added to all cultures 24 h after initiation of division. Slides were stained with FPG method. Fifty differentially stained and well-spread metaphases per concentration were examined for SCE, and 100 metaphases were analyzed for the determination of M1, M2 and M3 mitotic division. The statistical analysis of results was performed using a simple analysis of variance (ANOVA) and then the Student's *t* test was applied to compare SCE occurrence. For the PI a χ^2 test was used.

RESULTS AND DISCUSSION

The frequencies of SCE induced by the technical tolylfluanid and lymphocyte proliferation kinetics after 24 h and 48 h treatments are shown in Table 1.

Table 1. Frequencies of SCE and proliferation indices in cultured peripheral bovine lymphocytes exposed to tolylfluanid for 24 h and 48 h

Dose	SCE/ ell	PI	SCE/cell	PI
DMSO	**6.98 ± 2.92**	**1.76**	**6.40 ± 2.41**	**1.76**
tolylfluanid (μg.ml^{-1})	**24 h**		**48 h**	
1.7	7.33 ± 1.21^{a}	1.66	7.05 ± 2.64^{a}	1.76
3.5	7.70 ± 1.20^{a}	1.61*	7.94 ± 3.41**	1.73^{a}
8.75	8.28 ± 1.45***	1.59*	8.46 ± 2.76***	1.73^{a}
17.5	8.84 ± 1.24***	1.53**	9.25 ± 3.29^{***,b}	1.68^{a}
MMC (0.4 μmol)	9.74 ± 2.76***	1.65^{a}	23.2 ± 3.9***	1.54**

50 second-division metaphases were analyzed per concentration for SCE
*, **, *** – statistical significant data ($p < 0.05$, $p < 0.01$ and $p < 0.001$)
^{a} – without statistical significance, ^{b} – insufficient number of cells

For 24 h, a significant increase in the SCE frequency was obtained only at the highest concentrations (8.75 and 17.5 μg.ml^{-1}, $p < 0.001$). This concentrations are also reflected in the reduction of the PI (χ^2 test, $p < 0.01$).

Treatment for 48 h caused a statistically significant elevation in SCE frequencies at the concentrations ranging from 3.5 to 17,5 μg.ml^{-1} ($p < 0.01$ and $p < 0.001$) with dose-dependence. This elevation was associated with cell cycle delay.

References regarding the cytogenetic effect of tolylfluanid in farm animals are quite scant. In the mammalian chromosome aberration assay *in vitro* the cytotoxic effect was shown after the exposure to tolylfluanid at the highest concentration of 10 μg.ml^{-1} (3). Significant increases in the number of chromosomal aberrations and micronuclei were found after the subchronic exposure to the technical tolylfluanid in sheep (5). The concentration-dependent increases in the SCE after the prolonged time of exposure (48 h) to tolylfluanid indicated a potential of the fungicide to induce chromosomal damage (replication injuries). SCEs are considered as a measure of the dose and they detect the early effects of exposure as well (1).

CONCLUSION

Based on our results it is suggested that tolylfluanid acts as a multi-site inhibitor (microtubule and topoisomerase inhibitor) and in high concentration could block cell division.

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INFLUENCE OF INSULIN-LIKE GROWTH FACTORS OF AMNIOTIC FLUID ON LYMPHOCYTE PROLIFERATION

Šteffeková, Z.

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

ABSTRACT

Different substances of amniotic fluid (hormones, cytokines, polypeptide growth factors) influence the cell proliferation and differentiation of developing animal foetus. The immunomodulatory properties of amniotic fluid were demonstrated in different animal species. The aim of this study was to isolate the insulin-like growth factors and their binding proteins from sheep amniotic fluid and to determine their mitogenic effect on bovine peripheral blood lymphocytes and also to find out if concanavanin A potenciates this effect. The active mitogenic fraction coincidated with molecular weights of IGF-I and the activity enhaced after supplementation of concanavalin A. These results suggest, that IGF-I has positive mitogenic effect on bovine lymphocytes and concanavalin A potenciates this effect.

Key words: amniotic fluid; grouth factors; insulin-like factors; lymphocyte

INTRODUCTION

Insulin–like growth factors system (IGF system) consists of IGFs (IGF-I and -II), their receptors and their binding proteins (1). IGF-I stimulates the proliferation and differentiation of many cell types. In the case of T-cells, IGF-I potentiated not only mitogen-induced DNA synthesis, but also directly DNA synthesis of human T-cells (2).

MATERIAL AND METHODS

Amniotic fluid was obtained of sheep in last week before birth. Delipidated and lyophylised amniotic fluid was loaded into a Sephadex G-50 column and the content of peptides was determined by measuring of the absorbance at 280 nm. Isolated bovine lymfocytes were supplemented by fractions of amniotic fluid and by concanavalin A. For detection of proliferation activity we used methyl tetrazolium (MTT) colorimetric assay. The intensity of proliferation was quantificated by measuring of the absorbance at 550 nm using ELISA reader.

RESULTS AND DISCUSSION

For the separation of peptides we used the chromatography and the content of peptides was determined by measuring of the absorbance at 280 nm. We obtained two peaks of peptide substances (Fig. 1). By using of MTT colorimetric assay we determined the mitogenic activity of obtained fractions on lymphocytes. The higher peptide content peak (II), which probably represents oligopeptides smaller than 7 kDa (3) has no mitogenic activity. On the other hand, in the peak I we obtained fractions 31–32 with high proliferation activity, which is responsible to the IGF-I (7.6 kDa) (4). Concanavalin A – induced lymphocyte proliferation was potentiated (Fig. 1) by supplementation of IGF-I (fractions 31–32). By using mouse BP-A31 fibroblasts as indicator cells the biological activity separates into two peaks (3). Position of the second peak coincidates with eluted volume of

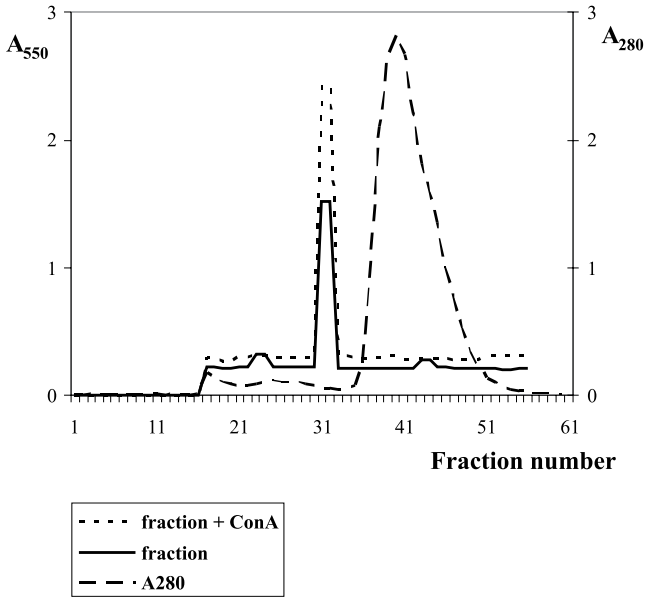

Fig. 1. Proliferation of lymphocytes in the presence of fractions of amniotic fluid obtained by gel filtration using Sephadex G-50 (A_{280}), and concanavalin A determinated by MTT test (A_{550})

IGF-I, so we can certify, that IGF-I has mitogenic effect not only on fibroblasts, but also on lymphocytes. Using MTT test we discovered, that the proliferation activity of bovine lymphocytes in the presence of concanavalin A increased. These results correlates with endings of study of phytohaemaglutinin influence and IGF-I on human peripheral mononuclear cells (5).

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PARASITIC INVESTIGATION OF HORSES IN EASTERN SLOVAKIA

Pilaker, A., Goldová, M.

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

annapilaker@yahoo.se

ABSTRACT

The purpose of this study was to investigate the present parasitic status of horses in Eastern Slovakia during 2006/2007. A total of 368 horses were examined during this period. The result showed that 255 faecal samples were positive for internal parasites, with prevalence 69 %. The severity of the infection varied between the horses, most of the horses were mono-infected (94 %) and 6 % was mixed-infected. Faecal samples were collected from rectum and examined by qualitative flotation method according Breza and McMaster quantitative method, EPG (eggs per gram) was evaluated. Identification of species composition in faecal samples: Strongylata 83.5 %, *Parascaris equorum* 13.7 %, *Anoplocephala* sp. 2.6 %. During 2006 the highest prevalence was in February and between August and October, and in 2007 the highest prevalence was also in two peaks: in January and September to October.

Key words: horses; internal parasites; parasitic status; seasonal dynamics

INTRODUCTION

With increasing numbers of performance horses in Slovakia, there is an obvious requirement for knowledge of the present parasitic situation in Slovak horse herds. This knowledge is vital for the development of sustainable parasite control of equines in Eastern Slovakia. In presented work the parasitic status in Eastern Slovakia horse herds was studied. The objectives of this project was to investigate the endoparasitic status in horses in Eastern Slovakia, state the prevalence of parasites and the most dominant species, the seasonal dynamics, and the intensity of infection of endoparasites of horses.

MATERIAL AND METHODS

Horses were randomly selected from farms in Eastern Slovakia during 2006–2007. Collection of faeces was done by horse owner or manager, and was sent for analysis.

Qualitative concentration method: Flotation method according Breza (specific gravity: 1.30) for detection of helminths eggs was used.

Quantitative method: McMaster method, standard egg count method, was used for estimation of eggs per gram (EPG). Evaluation of method: low intensity of infection 50–500 EPG (+), moderate 500–1000 EPG (++), and severe 1000–2000 EPG (+++).

RESULTS

From total 368 horses tested during 2006/07 were 254 positive (69 %). The severity of the infection varied between the horses, most of them were mono-infected (94 %) and 6 % was mixed-infected. During 2006, the most dominant parasites were Strongylata with (86 %), prevalence of *Parascaris equorum* was 13 %, and *Anoplocephala* sp. 1 %. During 2007 the most dominant parasites were also species from subpbyllum Strongylata (82 %), 14 % of horses were infected with *Parascaris equorum*, and 2,6 % with *Anoplocephala* sp.

Seasonal dynamics. During 2006 there were two peaks of parasitic infection, one in February and the second peak between August and October. In 2007 the highest prevalence was also in two peaks, in January and second in September to October. Comparison between 2006/2007, the peaks show a slight difference between the years, in 2006 the first peak begins in February while in 2007 the first peak begins in January, the second peak in 2006 starts in July/August while in 2007 it starts in August/September.

Intensity of infection of internal parasites in horses in 2006/07. Quantitative faecal egg count showed that the most frequent intensity of infection of Strongylata was low (31 %), mild (26 %), and severe (16 %). Intensity of infection of *Parascaris equorum* found in 37 horses tested during 2006/07 was low (76 %), mild (11 %), and severe (5 %). Intensity of infection of *Anoplocephala* sp., found in 7 horses was low. This low number of positive horses and low intensity of infection could be due to specific life cycle of tapeworm, since the whole proglottids can be found in the faeces and not only eggs.

DISCUSSION

Prevalence. This study showed that endoparasites were highly prevalent in horses in Eastern Slovakia. The most prevalent was group of Strongylata (86 %) second was *Parascaris equroum* (13 %) and the least *Anoplocephala* sp. (1 %). If compared to a study made in Sweden 2005 (3) the highest prevalence in Swedish horses was also the same group (Strongylata*)* (78 %). Our findings in Slovakia show a little higher prevalence. In another study made in Hungary, 55 % of the horses were infected with identical of internal parasites; 52 % of the sample contained Strongylata eggs, 15 % *Parascaris equorum*, 11 % both Strongylata and *P. equorum*, and 2 % other species (4). In our study 69 % horses were positive with gastro-intestinal parasites. The high number of Strongylata eggs may be due to the increase in resistance to antihelmintics among some species and to improper use of antihelmintics (1).

Seasonal dynamics. In seasonal dynamics we could see an obvious difference depending on the season and month. The first seasonal peak in 2006 started in early spring, February and the second in early autumn, September. During 2007 the first peak was in January and the second peak was in September and beginning of October. In Kentucky, 1998, the seasonal prevalence was the similar with the highest prevalence of Strongylata (60.5 %) (2). The seasonal dynamic is an important fact for veterinarians to be aware of, in order to give the horse the best available antihelmintic treatment.

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ANALYSIS OF OCCURRENCE OF PCB SUBSTANCES IN FRESHWATER FISH FROM ZEMPLÍNSKA ŠÍRAVA IN PERIOD 2004–2006

Poľakovský, J., Nagy, J., Popelka, P.

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

nagy@uvm.sk

ABSTRACT

Polychlorinated biphenyls (PCB) were massively used in industry and water, soil and atmosphere can be contaminated from various sources. In period 2004–2006, were analyzed 18 samples of various freshwater fish from Bodrog and Laborec rivers areas and Zemplínska Šírava for presence of PCB congeners. In 2004 all of the samples were positive (above limits established by Food code). In 2005 average values of PCB congeners were lower than in 2004, but in 2006 they increased again.

Key words: freshwater fish; polychlorinated biphenyls

INTRODUCTION

Polychlorinated biphenyls (PCB) are liquids with oil character, which can occur in 209 various combinations. In respect of their chemical composition, PCB present serious problem for human health and environment. PCB substances are strongly biocumulative and they belong among 12 persistent organic pollutants, which must be eliminated according to Stockholm convention (1).

PCB, analogous to chlorinated insecticides, stimulates activity of enzymes in microsomal fraction of liver. Substrates, sensitive to these enzymes are steroid hormones. Induced enzyme systems accelerate process of hormones modification on biological less effective polar metabolites, which are probably primary reason of reproductive disorders of animals exposed to impact of PCB.

Drugs and lipophilic vitamins can be also affected by induced enzymes with negative impact on biological function of animals (2). Polychlorinated biphenyls were massively used in industry, because of their properties – low inflammability, low electric conductivity, high resistance to temperature fluctuation and chemical agents, and high degree of chemical stability. Water, soil and atmosphere are contaminated from various sources like using of recycled oil, chemical production, and combustion of industrial and biological waste in refuse incinerating plants.

The most frequent sources of PCB intake are milk, meat, fish and milk and meat products. Even low doses of PCB can cause damage of liver, reproductive abnormalities and disorders of immunity system. International Agency for Research of Cancer classified PCB as a "evincible human carcinogen" (3).

In Slovakia, PCBs were produced in Chemko Strážske since 1984. In sediment of Laborec river and in Zemplínska Šírava (mouth of sewer from Chemko Strážske) was observed 100–2000 times higher content of PCB than in sediment from compared area (Ondava and Domaša rivers). Fish caught in contaminated water in Zemplínska Šírava and Laborec contain in average 100 times higher levels of PCB than fish from Domaša and Ondava. Levels of PCB, but also HCB and p.p.´-DDE in these regions are significantly higher than in other European countries and research proved dependence between increased levels of PCB and number of thyroid gland changes in humans (4). Nowadays, Chemko Strážske participates on the project Alternative destruction of PCB, under patronage of UNIDO, agency of OSN for industrial development and cofinancing of environmental found of the World Bank (1).

Table 1. Values of PCB congeners in freshwater fish in 2004

2004	PCB-28	PCB-52	PCB-101	PCB-180	PCB-138	PCB-153
COMMON CARP	0.672	0.856	1.130	1.246	2.330	2.767
GOLDGISH	0.364	0.327	0.315	0.805	1.050	1.297
ZANDER	1.242	2.330	4.680	5.827	9.118	10.264
PIKE PERCH	0.941	4.715	6.531	68.912	52.550	78.157
COMMON DACE	0.553	0.424	0.453	0.778	1.142	1.338
PIKE	1.233	2.199	4.710	12.382	14.477	19.072
AVERAGE	0.834	1.808	2.969	14.991	13.444	18.815

Table 2. Values of PCB congeners in freshwater fish in 2005

2005	PCB-28	PCB-52	PCB-101	PCB-180	PCB-138	PCB-153
COMMON CARP	**0.093**	**0.090**	**0.168**	0.289	0.450	0.524
GOLDGISH	**0.032**	**0.014**	**0.020**	**0.056**	**0.088**	**0.114**
ZANDER	1.504	4.234	10.584	21.182	24.120	36.605
PIKE PERCH	0.538	0.882	0.877	0.921	1.925	1.898
COMMON DACE	0.220	0.370	0.260	0.329	0.627	0.556
PIKE	0.370	1.020	1.664	4.533	6.307	6.630
AVERAGE	0.459	1.101	2.262	4.551	5.586	7.721

Table 3. Values of PCB congeners in freshwater fish in 2006

2006	PCB-28	PCB-52	PCB-101	PCB-180	PCB-138	PCB-153
COMMON CARP	1.436	4.778	7.048	11.260	16.198	16.224
GOLDGISH	0.496	1.046	0.881	1.194	2.197	2.164
ZANDER	0.765	2.764	10.286	26.293	37.147	43.515
PIKE PERCH	0.642	1.488	2.026	2.07	3.736	4.120
COMMON DACE	**0.151**	**0.137**	**0.164**	0.345	0.372	0.591
PIKE	0.288	0.399	0.245	**0.125**	0.582	0.459
AVERAGE	0.629	1.768	3.441	6.881	10.038	11.178

MATERIAL AND METHODS

Monitoring of polychlorinated biphenyls was performed on the territory of Bodrog and Laborec rivers and various parts of Zemplínska Šírava in period 2004–2006 in freshwater fish. Monitoring was realised in accordance with valid methodical instructions of State Veterinary and Food Administration of the Slovak Republic. In monitored period, on State Veterinary and Food Institute in Košice, was analyzed 18 samples of various fish for presence of PCB.

1. Freshwater fish

Common carp (*Cyprinus carpio*), Goldfish (*Carassius auratus*), Zander (*Sander lucioperca*), Pike perch (*Stizostedion lucioperca*), Common dace (*Leuciscus leuciscus*), Pike (*Esox lucius*).

2. Origin of samples

Laborec – Brekov, Zemplínska Šírava, Kaluža – Lúč, Laborec – Zemplínska Šírava, Laborec – Krivošťany (drawing sewer), Chemko Strážske (draining sewer, basin), Ondava – N. Hrušov, Laborec under Vojany, Jovsa, Laborec – Vojany.

Monitored parameters

Polychlorinated biphenyls – congeners and their MRL (maximum residue limits): PCB-28, PCB-52, PCB-101, PCB-180 (MRL – 0.2 $mg.kg^{-1}$ of fat), PCB-138, PCB-153 (MRL – 0.3 $mg.kg^{-1}$ of fat). Gas chromatography – mass spectrometry, accredited in State Veterinary and Food Institute in Košice, was used for analysis of samples. The method was validated in accordance with Governmental Regulation 320/2003 (5) establishing monitoring of certain substances and their residues in live animals and foodstuff of animal origin.

RESULTS AND DISCUSSION

Results obtained in period 2004–2006 are presented in Tables 1–3. In 2004 was recorded occurrence of over limited values of PCB in all analyzed samples and at all monitored congeners. The highest value (78.157 $mg.kg^{-1}$ of fat) was measured in sample of Pike perch from area Laborec – Zemplínska Šírava in congener PCB – 153 (Table 1). In 2005, only 2 sample were in accordance with requirements established by Food code SR (2004) (6), namely Goldfish in congeners PCB-28, PCB-52, PCB-101, and samples of Common carp were below limits from area Laborec – Krivošťany (Table 2). Similar situation was recorded in samples of Common dace and Pike in 2006. Samples of Common dace contained values below limits in three congeners (PCB-28, PCB-52, PCB-101) and one sample of Pike (PCB-180) did not exceed established limit (Table 3).

CONCLUSION

In period 2004–2006 was in accordance with valid methodical instructions of State Veterinary and Food Administration of the Slovak Republic performed monitoring of PCB congeners (PCB-28, PCB-52, PCB-101, PCB-180, PCB-138, PCB-153) in freshwater fish from area Bodrog and Laborec rivers and Zemplínska Šírava. Together, 18 samples of fish were analysed and in 2004 all of them were positive (above limits established by Food code) (6). In 2005 average values of PCB congeners were lower than in 2004, but in 2006 they increased again.

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PASTERN JOINT DISEASES IN SPORT AND WORK HORSES

Torzewski, J., Mihály, M.

Clinic for Horses University of Veterinary Medicine, Komenského 73, 041 81 Košice
The Slovak Republic

juromrak@seznam.cz

ABSTRACT

The proximal interphalangeal joint (pastern joint) is a simple trochlear joint with relatively small movement range between distal epiphysis of the proximal phalange and proximal epiphysis of middle phalange. A high load on the joint together with non-physiological movement, incorrect stance and non-physiologically formed articular facets may result in damage involving periarticular and articular tissues. Damage to both types of the structures may lead to development of cysts in subchondral bones, sclerotization or even to osteoarthritis and remodelling of bones. In more serious cases it can result in luxation and fractures of the interphalangeal joint or interphalangeal bone (Figs. 1a, b). In our study we evaluated 10 patients with suspected fracture of interphalangeal joint, examined at the Clinic for horses. X-ray examination was conducted to detect potential pathological changes and all horses were examined for signs of limping.

Key words: arthrodesis; arthrosis; desmitidis; luxation, pastern joint; sport horses; subluxation

INTRODUCTION

Acute diseases, such as luxations, fractures and desmitidis, affect particularly western and polo horses which are required to stop or accelerate abruptly, change direction and so on. In such cases the physiological limits are frequently exceeded and may result in development of diseases.

Other risk groups include horses with irregular stance or exposed to bad shoeing practices (1). Pastern bone fractures are most frequent in western horses and usually involve an acute process (2). The fractures occur with 3-fold higher frequency in hindlimbs and are comminuted in the majority of cases (3).

Osteoarthrosis as a chronic disease is known for degenerative changes in articular and periarticular structures of distal epiphysis of fetlock and proximal epiphysis of the pastern bone (4) (Figs. 2a, b).

In older horses osteoarthrosis may develop as a consequence of chronic trauma while in young horses osteochondrosis may be the primary cause (5, 6).

In case of subluxation due to a non-physiological movement and a subsequent rupture of the articular capsule and instability of joint, the damage to joint cartilage can result in arthritis (4).

MATERIAL AND METHODS

The present study focuses on patients which were examined at the Clinic for Horses, compares the cases with those published abroad and investigates the impact of the diagnosed diseases on performance of sport and work horses. We examined altogether 10 horses, of that 6 mares, 3 geldings and 1 stallion, 3 weeks to 17 years old.

Five mares were of Slovak Warm-blooded breed (SW). One 3-week old foal was with its mother, 3 mares, 2, 8 and 9 years old, were used for recreational riding and 17-year old mare was used for hipotherapy. Of the three geldings two were Slovak Warm-blooded horses, 3 and 7 years old, and one was 5 years old Norik, used for work in a forest. The only stallion was 3 years old Norik also used as a work horse in a forest.

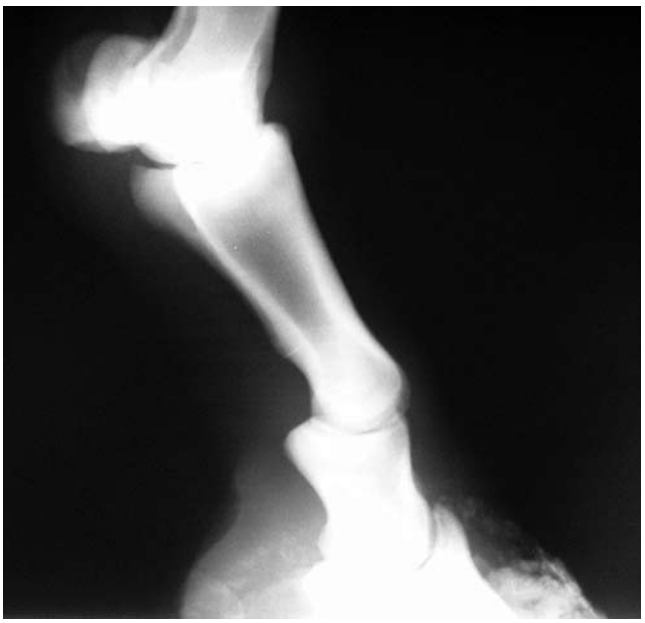

Fig. 1a. Subluxation of the interphalangeal joint

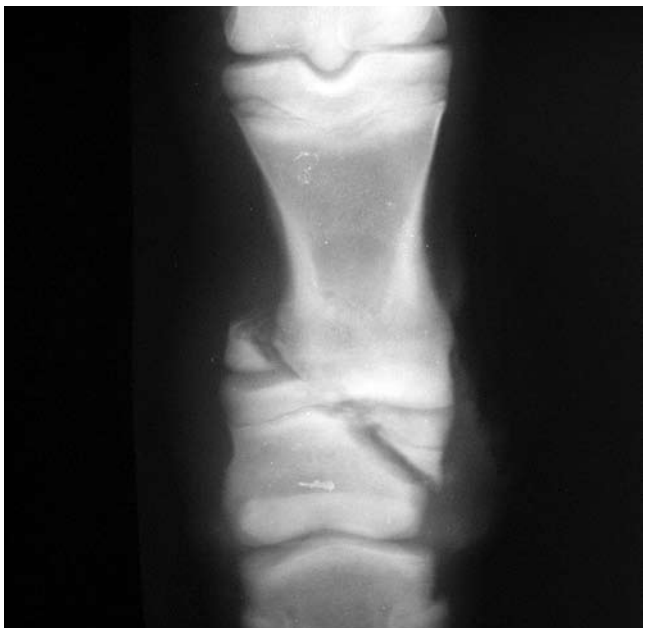

Fig. 1b. Oblique articular fracture of the interphalangeal joint

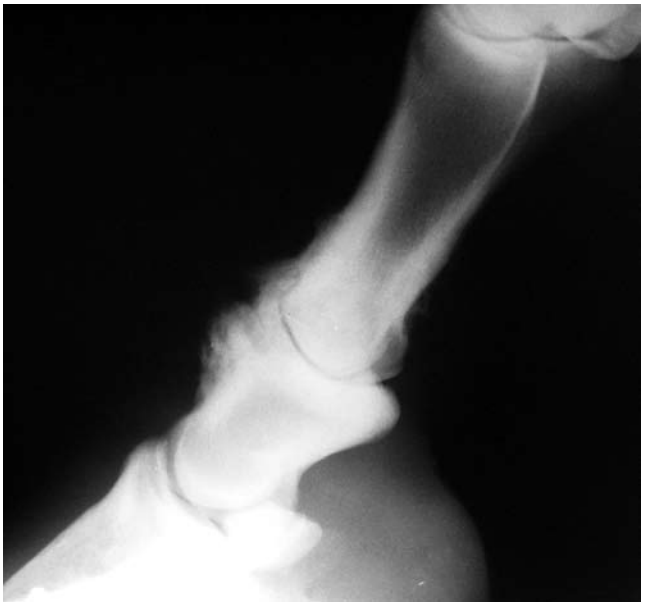

Fig. 2a. Arthrosis of the interphalangeal joint

Fig. 2b. Arthrosis of the interphalangeal joint – changes in the interphalangeal zone

Pathological changes were determined by clinical examination for the signs of limping and by subsequent X-ray examination. We used lateromedial and dorsopalmar projection of the affected and healthy limb to detect pathological changes by means of an apparatus Chirax 70, 70 kV, 10 mA; 0,3 s.

In the initial stages of arthrosis, the process is not demonstrable by X-rays if only soft tissues are affected, so the examination is repeated 3–4 weeks later when periostitis and formation of osteophytes are detectable (5).

An alternative method used in the acute stage is a nuclear scintigraphy.

RESULTS

Arthrosis of interphalangeal joint was diagnosed in five cases, namely in 5 year old Norik gelding, 16 year old Hutsul mare, which developed spontaneous ankylosis of pastern joint in the right forelimb and was shoed with orthopaedic NBS, 8 year old SW mare, which was subjected to arthrodesis treatment of the pastern

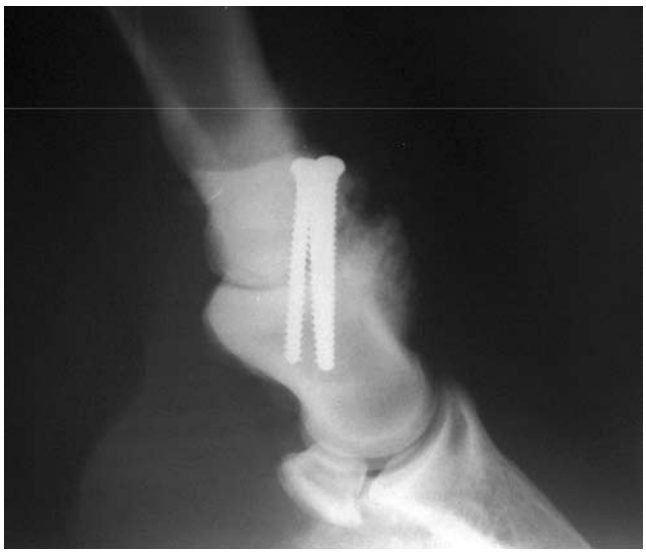

Fig. 3. Arthrodesis of the interphalangeal joint

joint of the right forelimb using three parallel cortical tensile screws and the lag method (Fig. 3), 7 year old SW gelding in the left forelimb (therapy refused by the owner) and 17 years old SW mare in the right forelimb which was treated with NSAID (non-steroid phlogistics) Equine joint health.

One 2 years old SW mare showed subluxation in dorsal direction but the owner refused any therapy.

Fixation of fracture with tensile screw was performed in one case in 3-month old SW filly with oblique intrarticular fracture affecting articular facet of the pastern joint of the right hindlimb. Pastern bone comminuted fracture was diagnosed in 3 years old SW gelding in its right forelimb, 4 years old Norik stallion in the right hindlimb and 9 years old ST mare in left hindlimb. Plaster bandage was used in all three cases.

DISCUSSION

When comparing our results with those published aboard we must agree with Adams (7) who stated:

Degenerative diseases affect horses of all age categories and are located more frequently on forelimbs (7). In our study, the age of affected horses ranged between 3 weeks and 17 years.

We diagnosed four fractures of which three were comminuted and one oblique, affecting articular facet, which is in agreement with observations of other authors:

In the majority of cases the fractures are comminuted while the simple ones are less frequent (1).

CONCLUSION

The relatively small facet of the interphalangeal joint compared to the size of horse's body must bear enormous load and therefore every abnormality in formation of articular facets, neglectful care of hoofs resulting in incorrect stance, movement on unsuitable surface or too high load in young age and, of course, unbalanced rations may initiate development of pathological processes.

Our attention should focus on correction of abnormal stance and hoofs already in the early age and on providing professional consultations which could help the owners and trainers to determine appropriate training load corresponding to the age and health status of the respective horse and, in collaboration with farriers, to ensure regular and correct shoeing.

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POISON APPARATUS IN SNAKES

Petrilla, V., Flešárová, S., Šulla, I.

University of Veterinary Medicine, Komenského 73, 04181 Košice
Department of Anatomy, Histology and Physiology, Institute of Anatomy
The Slovak Republic

skondak@azet.sk

ABSTRACT

Snakes posses four types of poison apparatus which differ from each other by size and location of the venom gland, type of poison-fangs and their location. All existing types have evolved from the oldest aglyphous (*Natrix natrix*) to the youngest solenoglyphous type (*Vipera berus*) which resulted in changes in the outer appearance of head and neck areas.

Key words: ***Natrix natrix; snakes; Vipera berus***

INTRODUCTION

Venomous snakes are members of the group of phanerotoxic animals with active toxicity, i.e. animals that have a specialised organ for production of venom and an apparatus for introducing venom into the target site. Snake venom is a highly viscous liquid of white to orange colour consisting of neurotoxins, cardiotoxins, haemorragines, haemolysines, coagulants or anticoagulants, enzymes with various action and 70 to 80 % of water (4). Cells of the venom gland are of serous type with internal and external secretion which means that the blood serum of all venomous snakes is toxic (3). Some components of snake venom are sensitive to UV radiation and because of that, to protect their venom gland and prevent detoxication of venom, some snakes, which are exposed to higher radiation, developed a protective dark coloured pattern in the head temporal zone. Melanin absorbs UV radiation and provides the necessary protection (1).

MATERIAL AND METHODS

Our study was conducted on 32 dead snakes obtained from private breeders. The dead bodies of snakes were skinned and the respective poison apparatuses were removed using a blunt procedure.

RESULTS AND DISCUSSION

The type *AGLYPH* has solid teeth in its maxilla of approximately the same size, without any grooves or channels. Such teeth are found in non-venomous species, but the first venomous species of the family Colubridae (colubrids) without poison-fangs have developed within this type, e.g. *Natrix natrix* (grass snake). *Glandula venenosa* – poison gland (the so-called Duverney's gland) developed by complete division of *glandula maxillaris,* the salivary gland, to two parts. *Glandula maxillaris* is located in the rostral part of maxilla while *glandula venenosa* is found in the caudal part and its content flows out through a separate opening into the mouth cavity where it mixes with saliva. The venom gland of the aglyphous type is not surrounded by musculature capable of active squeezing the venom from the gland and its volume is smaller than that of the salivary gland from which it separated. It has thin walls, rounded shape and is located superficially beyond orbit, underneath the skin (1).

OPISTOGLYPH – is the first type that delivers venom by means of poison-fangs which are longer than other teeth and are equipped with a groove on the rostral side through which the venom flows into the puncture where the snake sunk its fang. They are located in the caudal or central part of the maxilla under *glandula venenosa*, which is moderately enlarged compared to the aglyphous type but it is still located beyond maxillary salivary gland. The venom channel is connected to the poison-fang and opens up into its groove. The venom gland is thin-walled and located superficially under the skin. Venom is introduced by mechanical pressure on the gland when the food passes through mouth cavity into oesophagus. The process is not controlled by the respective individual. This type is found in family Colubridae, for example in *Dispholidus tipus*.

PROTEROGLYPH – is a more differentiated opistoglyph which can be observed in the Elapidae family (elapids), for example in *Naja nigricollis* (black-necked cobra). Poison-fangs in this type of snakes are strongly anchored in the rostral part of maxilla, are longer, more massive, slightly bent, with a hollow channel. The channel empties into a short section of the fang apex. *Glandula venenosa* located beyond orbit has an increased volume even compared to the salivary gland and thus displaced this gland downwards. Its rostral tapered part runs along dorsal side of the salivary gland and continues rostrally along the venom channel which empties at the basis of the poison-fang into its hollow channel. The caudal portion of the venous gland is dilated and overlaps the level of angle of the mouth. It is thick-walled, sinks deeper into the tissue and is surrounded by voluntary-controlled musculature (*musculus temporalis anterior*) which by its contractions allows the snake to empty the venom gland. The gland with its channel is located under the skin surface.

SOLENOGLYPH – is the youngest and most perfect poison apparatus. This apparatus is possessed by developmentally youngest species of the Viperidae family (vipers), such as *Bitis gabonica* (gaboon viper). Its poison-fangs are located in the rostral part of maxilla with which they are joined. This means that at rest they are kept folded back horizontally, oriented caudally and in parallel with *os pterygoideum* and at attack they are raised into maximal rostral position.

The poison-fangs are long, massive, with hollow channel, bent caudally, with a straight tip. The channel opens up on the rostral side along one third of its length and is face-truncated. Because of its increased volume, g*landula venenosa* compressed completely the salivary gland into a narrow band in the submucous connective tissue, it is located immediately beyond orbit and has triangular shape. It sinks into voluntary-controlled musculature the contractions of which produce pressure on the venom gland which then forces the venom through the venom channel, running rostrally above the salivary gland, into the basis of the poison-fang where the venom gland opens up into a hollow fang channel. The venom channel and gland are located immediately underneath the skin. The quantity of delivered venom is controlled by the respective snake by means of strength and contraction of muscles.

CONCLUSION

The aim of the study was to describe the poison apparatus in snakes from the anatomical point of view and the way they delivery their venom in order to increase the safety of handling of these poisonous animals and the manipulation during withdrawal of venom for pharmaceutical industry.

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MORPHOLOGY AND MORPHOMETRY OF BLOOD PROTOZOA OF THE GENUS *Karyolyssus* IN LIZARDS OF FAMILY Lacertidae FROM DIFFERENT LOCATIONS IN SLOVAKIA

Kredatusová, G., Račka, K., Goldová, M.

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

ABSTRACT

In the period of 2000–2007 we captured lizards of Lacertidae family, *Lacerta viridis* and *Podarcis muralis,* from four locations in Slovakia: Tajba – 27 specimens *L. viridis,* Turňa castle hill – 42 specimens *L. viridis*, Čabraď – 29 specimens *P. muralis,* Fiľakovo – 5 specimens *P. muralis*. Blood parasitoses occur mostly in reptiles kept in captivity due to disturbed balance between the parasite and the host resulting from the weakened immune system while they can hardly be detected at first sight in free living reptiles (2).

Key words: blood protozoa; *Laurta viridis; Karyolyssus; Podarcis*

INTRODUCTION

Protozoa of the genus *Karyolyssus* belong taxonomically to the family Karyolyssidae. So far only 10 species of the genus *Karyolyssus* have been described and the majority of them was described only on the basis of morphology of intraerythrocytary stages without information concerning their developmental cycles in respective carriers or any knowledge of their carriers or definite hosts (3). Blood protozoa of the genus *Karyolyssus* are heteroxenous parasites the developmental cycle of which includes vertebrates as intermediate hosts and blood-sucking mite as a vector and, at the same time, a definite host (4).

MATERIAL AND METHODS

In the period of 2000–2007 we captured and examined 69 specimens of lizard *L. viridis* and 34 specimens of lizard *P. muralis*. We captured them by means of a fish landing-net or a silon line loop in selected locations in Slovakia with permission of the Ministry of the Environment of the Slovak Republic.

Samples of blood were withdrawn from *vena coccygea ventralis* of the captured lizards. Blood smears prepared from the samples were fixed with methanol and stained according to Giemsa-Romanovsky. The stained smears were examined under a light microscope at 500× magnification. Parasitaemia was calculated per 10 000 erythrocytes and measurements of developmental stages of parasites were obtained by means of a calibrated eyepiece micrometer.

Freshly blood-fed mites were collected from body surface of lizards and smears were prepared from the mites and their eggs and were stained according to Giemsa-Romanovsky. The smears were examined under a light microscope at 500× magnification.

RESULTS

Of the 27 lizards *L. viridis,* collected from location Tajba during our study, 11 were positive for blood protozoa (40.7 % prevalence) and the mean parasitaemia reached 0.065 %. All 42 specimens *L. viridis* captured in the location Turňa castle hill tested negative. Of 29 lizards *P. muralis,* originating from location Čabraď, 16 (55.2 %) were positive with mean parasi-

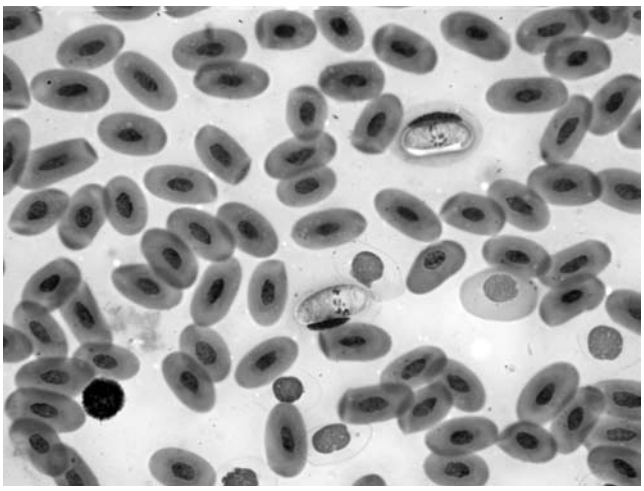

Fig. 1. Morphotype 1, location Tajba (Orig. photo)

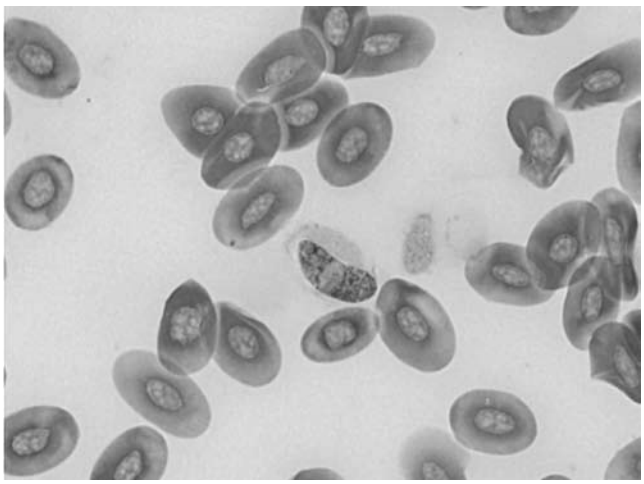

Fig. 2. Morphotype 6, location Čabraď (Orig. photo)

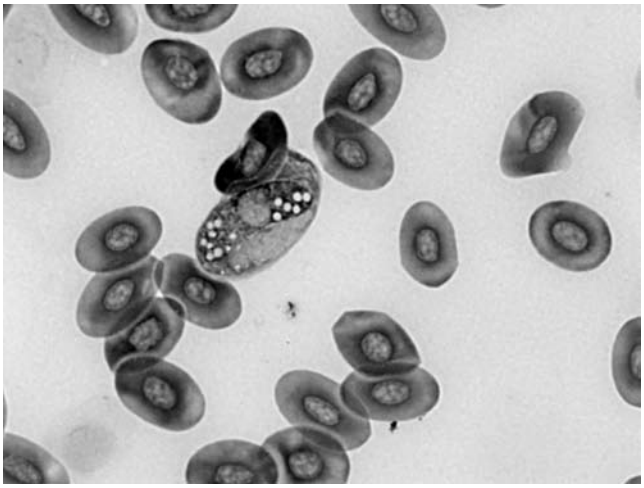

Fig. 3. Morphotype 8, location Čabraď (Orig. photo)

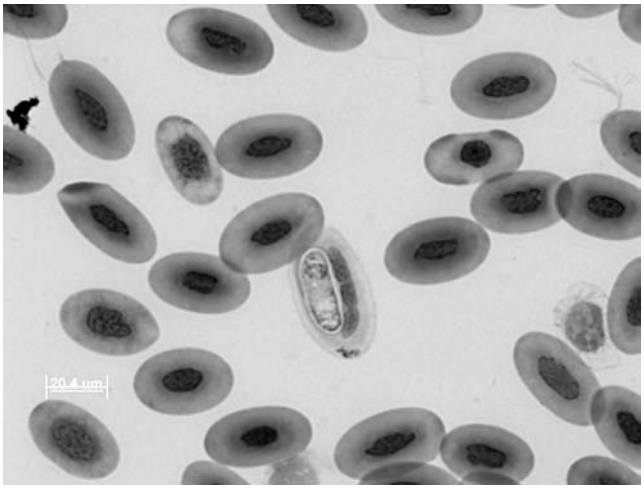

Fig. 4. Morphotype 9, location Fiľakovo (Orig. photo)

taemia equal to 0.46 %. All 5 specimens *P. muralis* captured in location Fiľakovo were positive (100 %) with mean parasitaemia reaching 2.082 %.

The morphological properties of blood parasites found in preparations from positive lizards allowed us to divide them into ten morphotypes.

Four morphological types of blood parasites occurred in preparations originating from location Tajba: morphotype 1 – bean-shaped, mean length 15.2 μm and width 6.2 μm, cytoplasm coloured blue, central location of nucleus which appeared as a light, red plane with chromatin granules (Fig. 1); morphotype 2 – elongated, oval shape, slimmer and shorter than the morphotype 1, mean length 12.4 μm and width 4.2 μm, cytoplasm was light blue to white, nucleus compact, located centrally; morphotype 3 – wide-oval to round shaped, mean length 6.1 μm and width 3.6 μm, cytoplasm was light blue and contained vacuoles, the nucleus was red, located eccentrically; morphotype 4 – slender shape, mean length 12.9 μm and width 3.1 μm, cytoplasm was coloured blue, contained vacuoles, the nucleus was compact and located centrally.

Preparations from the location Čabraď allowed us to distinguish four morphological types of blood parasites: morphotype 5 – merozoite, suspended freely in bloodstream (extraerythrocytary); dimensions: 14.1 × 1.9 μm; morphotype 6 – cigar-shaped; mean length 11.9 μm and width 4.1 μm; absence of vacuoles, blue coloured; the nucleus located centrally (Fig. 2); morphotype 7 – slender; mean length 11.9 μm and width 1.7 μm, vacuoles in cytoplasm; morphotype 8 – big and wide, mean length 17.6 μm and width 5.0 μm (Fig. 3).

In the preparations from location Fiľakovo we observed two morphological types of blood parasites: morphotype 9 – (Fig. 4) cigar-shaped; mean length 11.9 μm and width 3.9 μm; blue cytoplasm free of vacuoles; terminal location of the nucleus; morphotype 10 – slender; mean length 12.2 μm and width 3.9 μm; presence of vacuoles in cytoplasm.

DISCUSSION AND CONCLUSION

In the period of 2000–2007 we captured lizards of Lacertidae family, *L. viridis* and *P. muralis,* in four locations in Slovakia: Tajba – 27 specimens *L. viridis,* Turňa castle hill – 42 specimens *L. viridis*, Čabraď – 29 specimens *P. muralis,* Fiľakovo – 5 specimens *P. muralis.* We examined the captured lizards for parasitaemia and prevalence of blood protozoa. Parasitaemia was calculated per 10 000 erythrocytes and the blood parasites found were measured by a calibrated eyepiece micrometer. The observations proved presence of blood sporozoites (Apicomplexa) of the genus *Karyolyssus* and their morphological properties allowed us to divide them into ten morphotypes.

Comparison of morphological properties of the observed morphotypes with the results of authors from abroad showed that the morphotypes from location Tajba were coincident with the species *K. latus* (4), morphotypes from location Čabraď with the species *K. bicapsulatus* (1) and morphotypes from location Fiľakovo with the species *K. lacertae* (4).

ACKNOWLEDGEMENT

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EFFECTIVENESS OF IMMUNOPROPHYLAXIS IN ENDOPARASITE HARBOURING PUPPIES

Čobádiová, A., Smrčo, P., Mojžišová, J., Goldová, M.

Department of Epizootiology and Parasitology, University of Veterinary Medicine
Komenského 73, 041 81, Košice
The Slovak Republic

ABSTRACT

In addition to typical endoparasitoses young dogs also harbour protozoan parasites which are resistant to conventional doses of antiparasit. *Giardia intestinalis,* which occurs routinely in young and immunosuppressed individuals, may weaken their immune response and cause insufficient post-vaccination protection. Our study was conducted on puppies affected by *Giardia intestinalis* with the aim to investigate production of specific anti-CPV antibodies on days 7 and 28 post-vaccination. The results obtained were compared with the control animals that tested negative for presence of *Giardia* cysts. The positive puppies showed significantly decreased production of anti-CPV antibodies. These animals should be examined coprologically before vaccination and in the positive cases subjected to thorough antiprotozoan treatment with preparations effective against single-cell parasites.

Key words: *Giardia intestinalis*; immunoprofylaxis; immunosupresion

INTRODUCTION

Besides other measures, prevention of infectious diseases in dogs is based also on specific immunoprophylaxis. The use of vaccines in animal practice should be based on knowledge related to development of immunity in the animals to be vaccinated, transfer of immunity from mother to the young and the period of persistence of protective level of antibodies after the vaccination. Prior to vaccination one should consider, diagnose or treat therapeutically all the states that could affect negatively the efficiency of vaccination. Such pathological states include also parasitoses. *Giardia intestinalis* is the single-cell parasite most frequently harboured by 6 weeks to 6 months old puppies.

Immunosuppression is a frequent finding associated with infectious and parasitic diseases (9). Immunosuppressive states can influence the effectiveness of vaccination and subsequent protection of animals against infectious diseases (3, 5). The parvovirus disease of dogs, caused by CPV, belongs among alimentary infectious diseases. It is usually an acute (peracute) systemic viral diseases of domestic dogs and other wild canine species manifested by enteritis or myocarditis (8). Therefore it is inevitable to immunize puppies with vaccines that normally provide sufficient protection and to consider any states that could affect negatively the effectiveness of this essential vaccination. Giardiosis is protozoan infection characterized by catarrhal gastroenteritis (2).

The aim of the present study was to investigate production of specific antibodies after anti-CPV vaccination in puppies harbouring the single-cell parasite *Giardia intestinalis* in comparison with healthy dogs.

MATERIAL AND METHODS

Animals

Group E – 35 dogs of various breeds, males and females, 10 weeks old, with anticipated immunosuppression, originating from shelter for stray and abandoned dogs, harbouring *Giardia intestinalis*. The puppies were treated with antiparasitic preparation Cestal and vaccinated against canine parvovirus infection

using commercial, live, attenuated vaccine (Biocan P, Bioveta, a.s., Ivanovice na Hané, Czech Republic *Parvovírus enteritidis canis* min.105.0 TCID50 or 512 HAU).

Group K – 30 healthy puppies, originating from private owners, without clinical symptoms of immunosuppression, males and females of various breeds, 10 weeks old, with negative parasitological findings, which were vaccinated against canine parvovirus infection using commercial, live, attenuated vaccine.

Methods

Sampling of blood and faeces:

Sampling 0 – before administration of the vaccine, sampling 1 – three days post-vaccination; sampling 2 – 7 days post-vaccination; sampling 3 – 14 days post-vaccination, by puncture of *v. cephalica.*

Parasitological examination – detection of *Giardia* cysts and helminth eggs in faeces by the method of Breza and Faust (Manual of veterinary laboratory methods, 1989).

Immunological analysis – level of specific antibodies to CPV was determined by the haemagglutination-inhibition test.

Statistical analysis – significance of differences was assessed by the ANOVA-test (GraphPad InStat).

RESULTS

Cysts of *Giardia intestinalis* (Fig. 1) were found in all investigated puppies from **group E**. The affected puppies showed delayed growth, emaciation and intermittent diarrhoea. Coprological monitoring revealed presence of *Giardia* cysts throughout the experiment. The mean titre of anti-CPV antibodies determined in this group before vaccination was 1 : 20. Even on day 3 post-vaccination, the mean level of specific post-vaccination anti-CPV antibodies did not show any increase and persisted at the level of 1 : 20. Only on day 7 post-vaccination we detected an increase in the mean titre of anti-CPV antibodies to the level of 1 : 320. By day 14 post-vaccination the antibody level was unchanged and persisted at the level of 1 : 320.

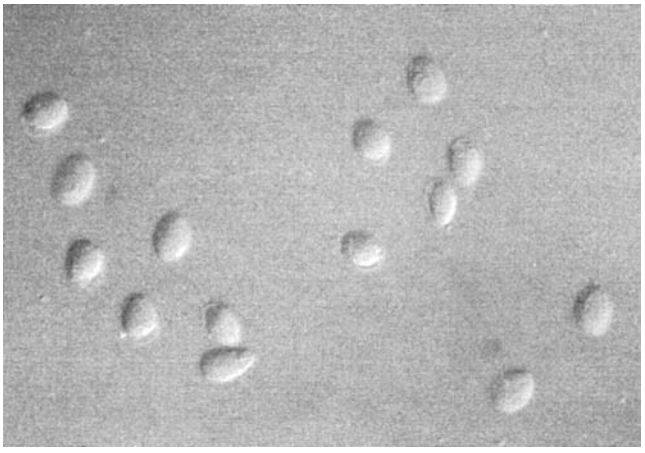

Fig. 1. Cysts of *Giardia intestinalis* in the samples of faeces of dogs. Orig. ×400

Parasitological testing of animals from the **group K** (control) provided negative results.

Before anti-CPV vaccination (sampling 0) of 4 months old puppies, their maternal anti-CPV antibodies were already degraded so we could commence the vaccination. On day 3 post-vaccination the concentration of specific post-vaccination anti-CPV antibodies was still equal to zero. On days 7 and 14 post-vaccination the mean titre of anti-CPV antibodies increased abruptly to the mean level of 1 : 640.

DISCUSSION

Secondary immunodeficiencies associated with some infectious and parasitic diseases affect negatively the specific and non-specific immune responses of animals. Any endoparasitosis may be associated with immunosuppression, either as its cause or its consequence(10). Immune response to vaccination is affected by various factors including the environment, stress, nutrition (11), chemotherapy (1), surgery (4) or long-term antibiotic treatment (6). Our results documented a marked difference in production of anti-CPV antibodies between dogs that tested negative for the presence of cysts of *Giardia intestinalis* and the dogs positive for the presence of this parasite. The titre of anti-CPV antibodies determined by HIT is considered protective at the level of 1 : 80 (1). The titre of anti-CPV antibodies reached the protective level also in dogs positive to *Giardia intestinalis* but was significantly lower in comparison with anti-CPV antibody titre in parasitologically negative dogs.

CONCLUSION

Production of specific antibodies after vaccination against canine parvovirus was recorded in both groups of dogs 7 days post-vaccination. Their titre in the group E was significantly lower ($P \leq 0.01$) in comparison with the group K and similar was observed also in other time intervals. The titre of specific antibodies reached the protective level in both groups of dogs.

Parasitological examination prior to vaccination should become a normal routine and a basis for decision whether or not to apply antiparasitic therapy.

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SEXUAL DIFFERENTIATION OF INDIVIDUAL SPECIES OF CHEWING LICE ON FARMED PHEASANTS IN SLOVAKIA

Lehotská M., Goldová M.

Department of Epizootology and Parasitology, University of Veterinary Medicine
Komenského 73, 041 81 Košice
The Slovak Republic

ABSTRACT

In the period of 2005–2007 we examined 30 farmed kommon pheasants *(Phasianus colchicus)* in Slovakia, namely from farms in Rozhanovce, Veľké Zálužie, Starý Tekov and Štitáre, for the presence of ectoparasites. We determined species abundance of individual ectoparasites and male to female ratios. We diagnosed altogether 4 species of chewing lice: *Lipeurus maculosus*, *Goniodes colchici*, *Goniocotes chrysocephalus* and *Amyrsidea perdicis*.

Key words: chewing lice; Mallophaga; *Phasianus colchicus*; sexual differentiation

INTRODUCTION

The study focused on species abundance of chewing lice (Mallophaga), the ectoparasites which decrease considerably the economy of pheasant farm rearing as they damage feathers of the birds, lower their resistance and make them nervous which eventually leads to decreased weight gain and egg yield. On farms with high density of feather game, chewing lice may decrease profitability of farm rearing and affect negatively introduction of pheasants to nature. Despite that the breeders frequently underestimate infestation with ectoparasites on their farms.

MATERIAL AND METHODS

Pheasants were examined for the presence of ectoparasites in the period of 2005–2007. For this purpose we collected 30 pheasants on which we identified 633 chewing lice. The pheasants were obtained from pheasant preserves in Rozhanovce, Veľké Zálužie, Starý Tekov and Štitáre. The ectoparasites were removed from dead pheasants directly from their bodies and feathers by means of tweezers and a magnifying glass (1). If live birds were involved, the ectoparasites were collected by means of our own modification of the ruffling method, the *fumigation-chamber method* (2, 3). The chewing lice were classified on the basis of morphological characteristics of individual species using available diagnostic keys (4, 5, 6, 7, 8, 9). We focused particularly on sexual dimorphism as this issue has been studied very little. Because sexual dimorphism was not pronounced in some species, we used chaetotaxy – differentiation by means of setae.

RESULTS

We identified 4 species of chewing lice which parasitise on the Common Pheasant in central Europe: *Amyrsidea perdicis* (1.11 %), *Goniodes colchici* (18.10 %), *Goniocotes chrysocephalus* (20.95 %) and *Lipeurus maculosus* (59.84 %). In our findings the number of females exceeded considerably the number of males. The ratio of male to female on individual pheasants but also within the farms was approximately 1 : 2. The sexual dimorphism was determined on the basis of morphological characteristics typical of the respective genera and on the basis of chaetotaxy. Details for the species *Lipeurus maculosus* are presented in Table 1.

Altogether we were able to recover 37 nymph stages

Table 1. Diagnostic key of the species *Lipeurus maculosus* (orig.)

LIPEURUS MACULOSUS 	♀			**NYMFA** Size of approx. 1 mm for the N 1. The head is elongated with parabolically rounded, temporally narrowing clypeus. All stenae located on the head are short. The  thorax consists of two parts : smaller prothorax and bigger prerothorax. The abdomen is just about as wide as the head and has five pairs of tergolateral, 9 pairs of tergocentral and 3 pairs of sternocentral setae.
		Females lack two setae that are present on pterothorax of males. Abdomen shows no pronounced genus differences.	Genera of adults is recognized according to different shape of the head. Female antennae are considerably thinner than those of males.	
	♂			
		Gender is recognized by means of two setae that are located in males on pterothorax, close to the central body line.	Adult male antennae differ considerably from those of females. They are more massive with particularly wide first segment.	

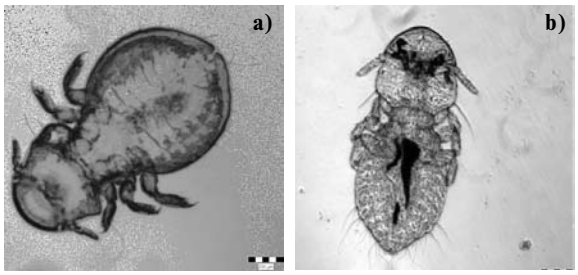

Fig. 1. Chewing lice nymphs
a) *Goniodes colchici*, b) *Goniocotes chrysocephalus*

of all four chewing lice species. This low number can be explained by very small size of the respective parasites so they could be overlooked. Morphology of nymphs is almost coincident with that of adults and the most important difference is their size. A typical characteristic of *Goniodes colchici* is that all nymphs have head of the male type (Fig. 1a) while sexual dimorphism in *Goniocotes chrysocephalus* is well discernible (Fig. 1b).

CONCLUSION

Four species of chewing lice were diagnosed on the investigated pheasant farms: *Lipeurus maculosus* (59.84 %), *Goniodes colchici* (18.10 %), *Gonicotes chrysocephalus* (20.9 %), *Amyrsidea perdicis* (1.11 %). We recorded considerable differences in the male to female ratio as the number of females was twice the number of males. We recommend to the breeders to observe strictly the principles of husbandry hygiene and regular sanitation measures.

ACKNOWLEDGEMENT

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SEROPREVALENCE OF *Neospora caninum* IN ABORTING SHEEP AND GOATS IN THE EASTERN SLOVAKIA

Špilovská, S., Reiterová, K.

Parazitological Institute SAS, Hlinkova 3, 040 01 Košice
The Slovak Republic

reiter@saske.sk

ABSTRACT

***Neospora caninum* is a major causative agent of prenatal loss in dairy cattle worldwide and also in sheep, goats and horses, with a negative economic impact on their breeding. For the presence of anti-*Neospora* antibodies 313 sera of sheep from five districts and 18 sera of goats from four districts of the Košice region and 69 sera of sheep from five districts of the Prešov region were examined. In sheep from the both regions 3.7% of animals were positive in average. Out of 18 goats examined only 3 animals were seropositive. The first survey in sheep revealed low prevalence of neosporosis in Slovak sheep flocks when compared to dairy cows post abortion. This sporadic occurrence may result in lower impact on reproduction loss in small ruminants.**

Key words: Eastern Slovakia; goat; *Neospora caninum;* seroprevalence; sheep

INTRODUCTION

Neospora caninum, a causative agent of neosporosis, is a protozoan intracellular parasite, which belongs to the phylum Apicomplexa and family Toxoplasmatidae. It was first identified as a cause of canine paralysis in Norway in 1984 (1). Similarly to other cyst-forming coccidia with several hosts, their indirect life cycle requires an intermediate host. The definitive host, that is the host in which sexual reproduction of parasite takes place, is dog and coyote. Cows, goats, sheep, horses, poultry and also dogs serve as intermediate hosts, which could be infected *via* food and water contaminated with sporulated *N. caninum* oocysts. However, the most frequent route of *N. caninum* infection is vertical transmission, from infected dam to her offspring (2).

The aim of this study was to determine the seroprevalence of neosporosis in aborting sheep and goats in the Eastern Slovakia.

MATERIAL AND METHODS

For the presence of antibodies to *N. caninum* 313 sera of sheep and 18 sera of goats coming from five districts of the Košice region and 69 sera of sheep from five districts of the Prešov region were examined. Serum samples were collected in 2007–2008 from the Slovak Veterinary and Food Administration (SVFA) in Košice and Prešov. Specific serum antibodies were detected using the indirect ELISA method according to the manufacturer's instructions (ID-VET, Innovative diagnostics, France). Optical density was measured at 450 nm (OD450) (ThermoLabsystems, Opsys MR, USA). Results were expressed as S/P percentage: S/P% = OD**450** sample/OD450 positive × 100. Serum samples with the S/P% < 50% were classified as negative and the samples with P/S% ≥ 50% were classified as positive. Results were evaluated statistically by Fisher exact test. In case of goats due to small number of examined animal's statistical analysis was not performed.

Table 1. Seroprevalence of *Neospora caninum* in aborting sheep in Košice and Prešov regions

Košice region	*Neospora caninum*			Prešov region	*Neospora caninum*		
	examined	positive	%		examined	positive	%
Košice – surround	63	3	4.8	Poprad	34	1	2.9
Rožňava	127	5	3.9	Prešov	4	0	0
Spišská Nová Ves	106	4	3.8	Stará Ľubovňa	18	1	5.6
Gelnica	13	0	0	Svidník	9	0	0
Trebišov	4	0	0	Bardejov	4	0	0
Total	**313**	**12**	**3.8**	**Total**	**69**	**2**	2.9

Table 2. Seroprevalence of *Neospora caninum* in aborting goats in Košice region

Košice region	*Neospora caninum*		
	examined	positive	%
Košice – surround	8	2	25
Rožňava	5	0	0
Michalovce	3	1	30.3
Trebišov	2	0	0
Total	18	3	14.3

RESULTS AND DISCUSSION

In the districts of Košice region, out of 313 sera of aborting sheep were 15 positive (3.8 %) (Tab.1). In the region of Prešov, 2.9 % mean seropositivity was non-significantly (p = 0.203) lower in comparison with former region. The overall seropositivity in sheep post abortion in the Eastern Slovakia was 3.7 %. Antibodies to *N. caninum* were found in 3 serum samples out of 18 aborting goats (14.3 %), in the districts of Košice (Tab. 2).

Mean seroprevalence 3.8 % in aborting sheep is more than four times lower in comparison with 16.4 % seropositivity of aborting cows from Eastern Slovakia (3). Our results are in accordance with other studies: 3.2 % seroprevalence of neosporosis was detected in 62 sheep in Brazil (4); 10.3 % positivity in 117 sheep from Switzerland (5) and 0.45 % in aborting sheep from Great Britain (6). In a large Italian study involving up to 1010 sheep without any reproduction disorders 2 % *N. caninum* seropositivity was reported (7).

In aborting goats in the Eastern Slovakia low occurrence of seropositivity was reported. Similar results are achieved (8) in 486 examined goats in Sri Lanka (0.7%) but higher 6.1 % of *N. caninum* seroprevalence was found in goats in Costa Rica (2) and 6.4 % in Brasil (9).

Due to the pasture breeding of Slovak sheep and goat flocks, we expected higher occurrence *N. caninum* in these animals. However, this first report of *N. caninum* seroprevalence in small ruminants in Eastern Slovakia indicates sporadic appearance when compared with aborting cows. We may suppose that this non-frequent occurrence of neosporosis in sheep and goats have minor impact on reproduction of these animals and have less subsequent effect on economic loss.

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EFFECT OF APPLICATION OF DIALYSABLE LEUKOCYTE EXTRACT ON EXPERIMENTAL SALMONELLOSIS IN CHICKENS

Kokinčáková, T., Herich, R., Levkutová, M.

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

kokincakova@uvm.sk

ABSTRACT

The presence and translocation of salmonellae were monitored in the chicken's caecum, liver and spleen using PCR. The preventive treatment with DLE reduced the presence of *S. enterica* subsp. *enteritidis* in the caecum but not in the examined internal organs.

Key words: chickens; dialysable leukocyte extract; PCR; prevention; salmonellosis

INTRODUCTION

Salmonellosis is defined as a clinical disease caused by infection with a member of the *Salmonella* group. It is known more than 2200 serotypes of *Salmonella*. Salmonellosis is usually intestinal infection of young poultry with or without clinical symptoms. Presence of salmonellae in caecum can lead to contamination of eggs and carcasses. Contaminated products are one of the most important causes of salmonellosis in human. Dialysable leukocyte extract (DLE) or transfer factor (TF) is defined like immunomodulator prepared from leukocytes, which can increase the antiinfection immunity. Liubchenko *et al.* (5) described application of transfer factor for treatment of fungal, viral and bacterial infections including *S. cholerae suis* and *S. dublin*. With regard to infection of salmonellae a body of information was obtained for calves (6) and pigs (1) indicating perspective utilization of specific DLE in prevention of *Salmonella* infections in these animals.

The aim of the work was to evaluate the protective effect of preventive parenteral application of DLE on colonization of caecum by *S. enteritidis* and its transmission to internal organs.

MATERIAL AND METHODS

Animals and treatment

Experimental infection was carried out using *S. enterica* subsp. *enteritidis* phage type 4. As immunomodulator was used specific and non-specific DLE prepared from chickens' and porcine leukocytes, respectively. A total of 120, one-day-old chickens hybrid Isa Brown were divided into 6 groups. The chickens of the group (SE) were infected with 1×10^8 CFU.ml^{-1} of *S. enteritidis* at age of 4 days. The chickens of the group with applied specific DLE and *S. enteritidis* (SE + CTF) were preventively treated with parenteral application of specific DLE at days 2 and 3 of life and infection with *S. enteritidis* was performed at day 4 in a dose 1×10^8 CFU.ml^{-1}. The same treatment except of application of the non-specific DLE was done in group (SE + PTF). The next two groups (CTF and PTF) were treated only with specific or non-specific DLE at age of 2 and 3 days at the same dose 0.2 ml parenterally. The C group was used as non-inoculated control group. Ten birds from each group were euthanized after 2 and 15 days *post infection*. The caecum, liver and spleen from each bird were aseptically removed and used for PCR detection.

DNA isolation and PCR

Isolation of bacterial DNA was performed using the Wizzard Genomic DNA purification kit (Promega, USA).

The sequences of the primer pairs used for DNA amplification of *invA* gene region of *Salmonella* spp. were as follows: 5˝-ACAGTGCTCGTTTACGACCTGAAT-3“ and 5“-AGACGACTGGTACTGATCGATAAT-3“ (Invitrogen, USA).

RESULTS

Two days after infection was found 60 % of positive samples in caecum, 100 % in liver and 30 % in spleen in birds with applied *S. enteritidis* alone (group SE). In group with application of chicken's DLE were salmonellae found in all samples from liver and in 30 % of samples from spleen. Presence of *Salmonella* spp. was not observed in content of caeca. Similar results were recorded in group treated with non-specific porcine DLE. 15 days after infection with *S. enteritidis* were salmonellae found in group SE in 100 % of samples from caeca and all the samples of liver and spleen were negative. In group with specific DLE (SE + CTF) were diagnosed 30 % of *Salmonella* spp. positive samples in caecal content. When the non-specific DLE was applied, birds showed 100 % of *Salmonella* spp. positivity of samples from caeca. The samples of liver and spleen were in both groups treated with DLE negative.

DISCUSSION

The rapid, cost effective and automated diagnosis of food borne pathogens throughout the food chain continues to be a major concern for the industry and public health. Poppe *et al.* (7) demonstrated that the course of infection with *S. enterica* depends on the bacterial strain, the host (age, breed, immune status), the environment and the experimental design (inoculum dose, housing). After crossing the intestinal mucosa through M cells salmonellae first encounters dendritic cells concentrated in the Peyer's patches (3). One of the alternatives to induce the immune system of the host during infectious disease is application of immunomodulatory agent.

Dialysable leukocyte extract (DLE) contains low-molecular substances of proteinous character with immunomodulative effect which are inevitable for proper functioning of the immune system. The sources of DLE include blood leukocytes or lymphatic organs (spleen, lymphatic nodes) or colostrum of immunised (specific DLE) or non-immunised animals (non-specific DLE) (2). Our results proved that application of specific and non-specific DLE reduced the presence of salmonellae in the content of caeca, but it does not have effect on translocation of pathogens to the liver and spleen.

The way of administration of immunodulatory agents is also very important, particularly in veterinary medicine, with regard to labour content. Studies of Kirkpatrick (4) require attention as they show that oral administration is just as good as the parenteral one which is very important particularly in chickens. Used parenteral application is suitable for experimental monitoring.

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Eimeria colchici – AMPLIFICATION AND SEQUENCING OF ITS-1 SECTIONS FOR PCR DIAGNOSIS

Major, P., Herich, R., Goldová, M., Levkut, M.

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

major_peter@uvm.sk

ABSTRACT

The study focused on analysis of ITS-1 sections of *Eimeria colchici* in pheasants that could serve in the future as suitable genetic markers for molecular diagnosis of *Eimeria* spp. in these birds. We used 2 isolates of *E. colchici* from blind sacs and a pure line of *E. tenella* (chicken) which served as a control. To amplify the ITS-1 regions, we used 3 sets of genus-specific primers. Samples of purified DNA fragments were subjected to sequencing. Analysis of results showed that coincident sequences ITS-1 were present in both isolates of *E. colchici.* With regard to *E. tenella,* the resultant sequence corresponded to the published data.

Key words: *Eimeria colchici;* ITS-1; PCR

INTRODUCTION

Coccidiosis in pheasants caused by parasitic protozoa of the genus *Eimeria* is a serious, worldwide spread disease affecting particularly young birds. Under stress situation, associated particularly with farmed pheasants, and after introduction of young birds to nature, coccidiosis can result in serious losses.

The accurate diagnosis of *Eimeria* species is based on morphological features of sporulated oocysts or on biological properties of eimerias (3). However, these conventional diagnostic methods are not reliable due to some morphological similarities among individual species (5). The modern molecular-genetic methods based on polymerase chain reaction (PCR) are considered rapid and reliable analytical tools. ITS-1 sections (Internal Transcribed Spacer) as parts of rDNA constitute genetic markers suitable for interspecies diagnosis.

MATERIAL AND METHODS

Two isolates of *Eimeria colchici* (C1, C2) were obtained from blind sacs of infected pheasants. Oocysts were concentrated by flotation on 1 mol saccharose and allowed to sporulate in 2.5 % solution of $K_2Cr_2O_7$ at laboratory temperature. After sporulation, they were treated with .....% solution of NaClO and washed several times in distilled water. A pure *E. tenella* (T) line, stored in a laboratory for one year, was used as a control. Walls of the oocysts were disintegrated by means of a teflon homogenizer and DNA was extracted using a commercial QIAmp® DNA mini kit (QIAGEN), following the enclosed procedure. The extract obtained was dissolved in 50 µl of deionized water.

Three sets of genus-specific primers common to *Eimeria* spp. were used to amplify the ITS-sections. They were BSEF (5-CTG TGA ATT CAT CGG A-3´) – BSER (5´-ATC GCA TTT CGC TGC GTC CT-3) (6,7), WW1 (5´-AAG TTG CGT AAA TAG AGC CC-3´) – WW3r (5´-CAA GAC ATC CAT TGC TGA AA-3´(2) and BSEF (5-CTG TGA ATT CAT CGG A-3´) – EEr (5´-GTC ACT TTG GTA GTC TAA AGA-3´). The PCR took place in a reaction volume of 50µl and consisted of 35 cycles (94 °C – 45 s, 56 °C – 30 s, 72 °C – 30 s). After the reaction, gel electrophoresis was carried out in 1 % agarose. The obtained fragments were extracted by means of a commercial QIAEX II Gel Extraction Kit (QIAGEN) and sent for subsequent sequencing.

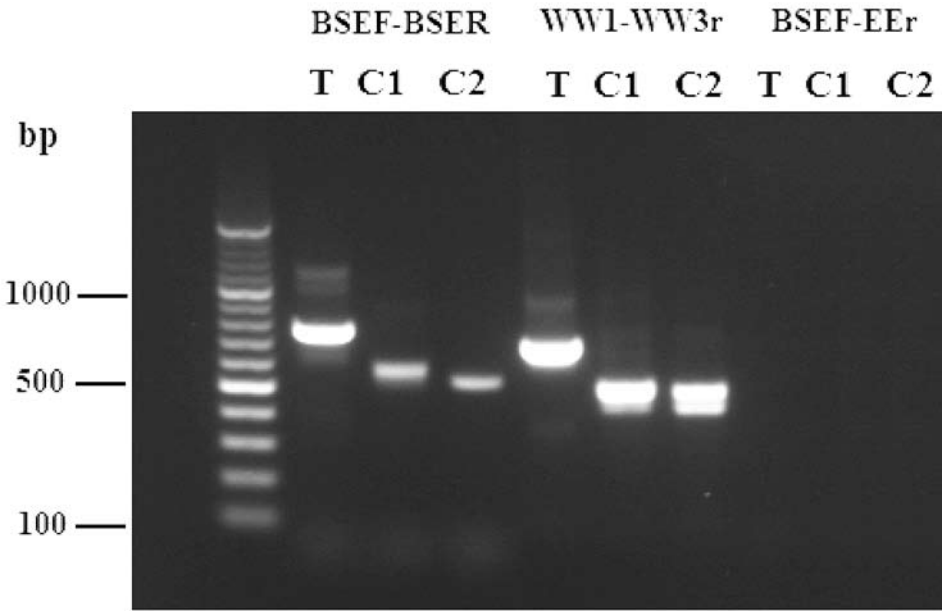

Fig. 1. Electrophoresis in 1% agarose. The PCR products of DNA samples of *Eimeria tenella* (T) and two isolates of *Eimeria colchici* (C1, C2) were obtained by means of 3 sets of genus-specific primers (BSEF-BSER, WW1-WW3r, BSEF-EEr)

RESULTS AND DISCUSSION

Three ITS-1 regions from two *Eimeria* species were amplified successfully using the first two sets of genus-specific primers, BSEF-BSER and WW1-WW3r. The last combination of primers (BSEF-EEr) provided negative result (Fig. 1). The size of amplicons in the first case (BSEF-BSER) was 753 bp for *Eimeria tenella* and 572 and 517 bp for the two isolates of *E. colchici*. In the second case (WW1-WW3r), fragments of size 655 bp were obtained for *E. tenella* and 482 and 469 bp for the two isolates of *E. colchici*. While only a single product was obtained with *E. tenella* in both cases, we observed a side fragment with both isolates of *E. colchici.* Duplication of fragments indicates the presence of two different types of sequences within the PCR product. This could result from contamination of the sample. After sequencing of the principal fragments, the resulting sequences were compared with one another. They were coincident in both isolates of *E. colchici* while in the case of *E. tenella* the resultant sequence corresponded to the gene bank data. Some authors point to the variability of ITS-1 regions even within one species (1, 2, 4, 7), however, such variability is less pronounced in comparison with the high variability among various species (2). It appears necessary to analyse ITS–1 sections also of other species of pheasant eimerias and after their mutual comparison to devise specific primers for reliable interspecies diagnosis.

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PREVALENCE AND SPECIES COMPOSITION OF FELINE ECTOPARASITOSES IN SLOVAKIA

Beracková, K., Kočišová, A.

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

ABSTRACT

In 2006–2007 we examined 79 cats with skin problems. The cats were kept either in apartments or in a combined way with access to a yard or were kept free in a rural area. Of the examined cats, 60 (75.9%) were positive for the presence of ectoparasites.The cats kept in apartments suffered from otodectosis the total prevalence of which reached 23.2%. Examination of cats with access to a yard showed that in the respective period 63.05% of patients were infested with ticks, notoedrosis was detected in one patient (6.25%) and otodectosis in 8 patients (27.85%). The majority of patients (73.8%) harboured fleas. Cats kept in the rural area were mostly infested with fleas (78.4%) and ticks (81.8%). With regard to species composition of individual ectoparasites, the diagnosed hard ticks included *Ixodes ricinus* (92%), *Dermacentor reticulatus* (4%) and *D. marginatus* (4%). Of the epidermis penetrating sarcoptic scab mites *Sarcoptes scabiei* var. *felis* was diagnosed in two cases (50%) and *Notoedres cati* (50%) in two cats, of the non-penetrating ones we diagnosed *Otodectes cynotis*. The most frequent ectoparasites found in cats were fleas, particularly the dog flea *Ctenocephalides canis* (72.5%), followed by (27.15%). In two rural cats we detected *Ceratophyllus gallinae* (0.2%) and in one *Chaetopsylla globiceps* (0.05%).

Key words: ***Ceratophyllus gallinae; Chaetopsylla globiceps; Ctenocephalides canis; C. felis; Dermacentor reticulates; D. marginatus; Ixodes ricinus; Notoedres cati; Otodectes cynotis***

INTRODUCTION

In the recent years the interest in keeping cats as household pets has been increasing considerably. One of the frequent problems the cat breeders have to face are parasites, particularly those which parasitise skin or subcutis. Ectoparasites harm their feline hosts by inducing hypersensitive skin reactions and acting as vectors of many bacterial, viral and parasitic disease agents. However, some can parasitise several animal species including the man (4). The aim of the present study was to present a review of prevalence of ectoparasites for the period of 2006–2007, in relation to various types of the environment in which the cats were kept.

MATERIAL AND METHODS

In the period of 2006–2007 we collected samples of ectoparasites from cats kept inside apartments without access to runs, those with access to a yard and from rural cats.

Ticks were removed from body surface by means of special tweezers and transferred to tubes (either dry or containing 70% alcohol). In case of suspected sarcoptic mites we performed a deep skin scraping, mixed the collected material with one drop of oil and examined microscopically. Fleas were collected from cats by combing them with a fine-tooth flea comb. They were transferred to tubes containing 70% alcohol. Individual ectoparasites were examined under a binocular magnifying glass or microscope at low magnification. Individual species of ectoparasites were diagnosed according to characteristic morphological attributes (3).

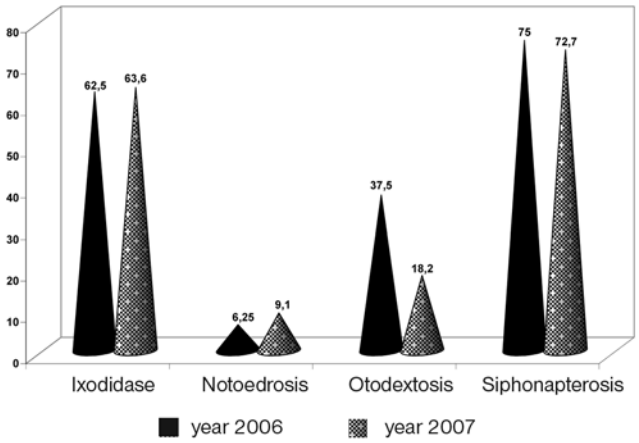

Fig. 1. Prevalence (%) of ectoparasitoses in cats kept in the combined way (apartment and access to a yard)

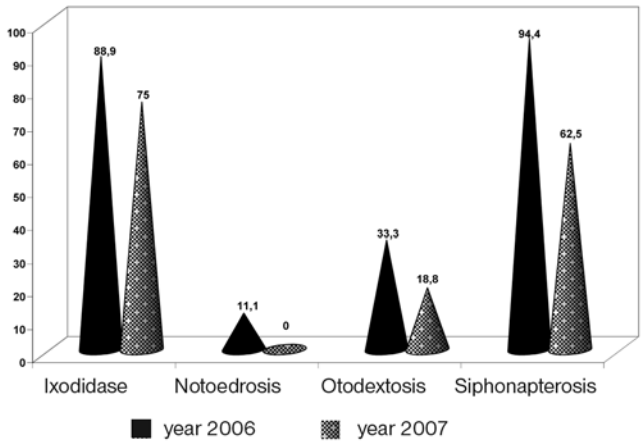

Fig. 2. Prevalence (%) of ectoparasitoses in rural cats

RESULTS AND DISCUSSION

In the observed period (2006–2007) we diagnosed high ectoparasite infestation in rural cats and in those with access to a yard while the cats kept exclusively in apartments were affected only by otodectosis (4 of 18 examined samples). Prevalence of ectoparasitoses in cats kept in combined way and in rural cats is presented in Figs. 1 and 2.

With regard to species composition of individual parasites, the examined cats harboured most frequently fleas *Ctenocephalides canis* (72.2 %) and *C. felis* (27.15 %). Our results are in contrast with those published in Germany, which reported prevalence of *C. canis* in the range of 0–12.45 % and that of *C. felis* between 81.5 and 83.5 % (5, 2). They resemble more the results obtained in England, showing higher prevalence of *C. felis* (96.4 %) than of *C. canis* (3.6 %) (2).

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INVESTIGATION INTO ROUTES OF SPREAD AND EPIDEMIOLOGICAL PATTERNS OF TRICHINELLOSIS BY GENETIC METHODS*

Ševcová, D., Hurníková, Z., Šnábel, V.

Parasitological Institute, Slovak Academy of Sciences
Hlinkova 3, 040 01 Košice
The Slovak Republic

ABSTRACT

Among 243 samples collected from wildlife of Slovakia (red foxes, wild boars, stone martens, brown bear, wolf, lynx, European polecat) in the period of 2005–2007, as much as 96.7% of animals harboured *Trichinella britovi*, which is of lesser infectivity and pathogenicity for humans. The predominance of *T. britovi* in wildlife follows the pattern obtained from neighbouring central-European countries. A more pathogenic *T. spiralis* was detected in Slovakia only sporadically, in two foxes (0.8%) and in mixed infection with *T. britovi* in 4 foxes (1.6%). Notably, non-encapsulated *T. pseudospiralis* was found in the two cases (in fox and wild boar) in eastern Slovakia that substantiated its limited presence in our territory following the outbreak in pig farm in 2003. DNA and allozyme data indicated the likely introduction of *T. pseudospiralis* to central Europe from Scandinavia through the raptorial birds that temporarily reside in eastern Slovakia during the wintertime.

Key words: allozymes; Australian region; DNA sequences; genotype; *Trichinella*,; Palearctic region

INTRODUCTION

Trichinellosis is globally distributed zoonotic disease caused by the ingestion of raw or undercooked meat containing *Trichinella* parasites. The genus *Trichinella* is currently partitioned into eight species and three additional, taxonomically yet unclassified genotypes, T6, T8 and T9. Four species (*T. spiralis, T. pseudospiralis*, *T. britovi*, *T. nativa*) are recognized in Europe, the two former species of which have a cosmopolitan distribution. The dissemination of *T. spiralis* was facilitated by the European colonization of other parts of world, whereas the spread of *T. pseudospiralis* (known to occur in at least three continents) is probably related to its infectivity to birds. The latter species is the first discovered non-encapsulated *Trichinella* species – Garkavi (2), and more than 30 isolates attributed to the species have been so far detected in mammals and birds. The growing reports of its presence in human outbreaks (Russia, France, Thailand) suggest that this genus is of higher public health concern than originally thought. The objectives of the present study were to evaluate the epidemiology of *Trichinella* species in sylvatic animals in Slovakia and to more thoroughly investigate genetic polymorphism in *T. pseudospiralis*.

MATERIAL AND METHODS

In 2005–2007, a total of 243 *Trichinella* isolates were collected from wild animals hunted in different regions of Slovakia, namely from 215 red foxes (*Vulpes vulpes*); 19 wild boars (*Sus scrofa*); 3 stone martens (*Martes foina*); 1 brown bear (*Ursus arctos*); 1 wolf (*Canis lupus*); 1 lynx (*Lynx lynx*); 1 European polecat (*Mustela putorius*). Samples were identified at species level by multiplex PCR analysis, targeting regions of expansion segment V (ESV) and internal transcribed spacer (ITS) rDNA according to Zarlenga *et al.* (4). Genetic structuring in 10 *T. pseudospiralis* isolates, 7 of which originated from the Palearc-

* **Running head:** Spread and transmission patterns of trichinellosis

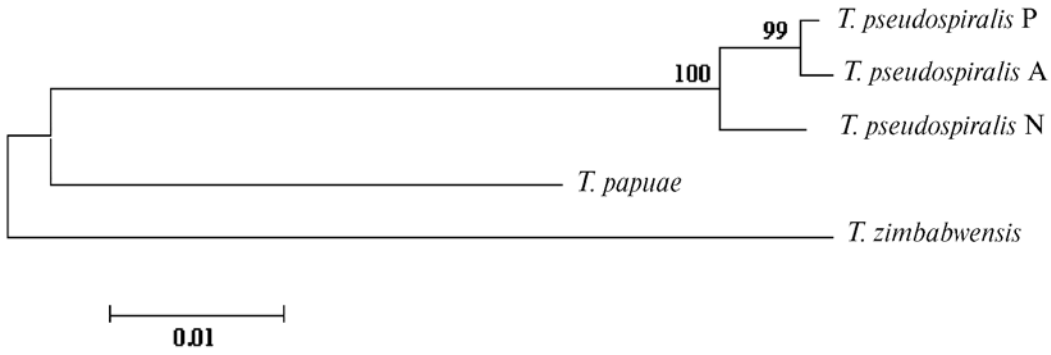

Fig. 1. Phylogenetic relationships in *T. pseudospiralis* isolates from the Palearctic (P), Nearctic (N), and Australian regions (A) and *T. papuae* and *T. zimbabwensis* isolates inferred by neighbor-joining analysis of the sequences of *cox1*, cytochrome P450 and cynate lysate. Bootstraps values are indicated at branching points

tic region (from Caucasus, Kamchatka, Kazakhstan, Finland, Slovakia, 2 isolates from Sweden), 2 from the Nearctic region (Alabama, Texas in the USA), and 1 from the Australian region (Tasmania), was assessed by DNA sequencing. As reference, the two isolates pertaining to the remaining non-encapsulated species (*T. papuae* and *T. zimbabwensis*) were simultaneously examined. Sequences at 4 selected gene loci were screened for three protein-coding genes of the cytochrome P450 gene, the cynate lyase gene, the mitochondrial gene *cox1*; followed by the 18S ribosomal RNA gene. A part of these isolates (origin from Slovakia, Finland, Caucasus, Tasmania, USA), being maintained by passages in laboratory mice, was studied for polymorphism also by allozyme analysis, employed *via* isoelelectrofocusing in thin layers of polyacrylamide gels. Following enzyme systems were tested using this approach: adenylate kinase (AK, 2.7.4.3), phosphoglucomutase (PGM, EC 5.4.2.2), malic enzyme (ME, EC 1.1.1.40), peptidase B, substrate leucyl-glycyl-glycine (PEP-B, EC 3.4.11.4).

RESULTS AND DISCUSSION

In total, 243 samples collected from wildlife of Slovakia were identified at species level using multiplex PCR approach. Our findings revealed *Trichinella britovi* as predominant species in our territory, with 96.7 % of animals infected with this species. These findings are consistent with observations that *T. britovi* is the prevailing species in the red fox population in Europe. The distribution of this species in the Slovak Republic in wildlife is comparable to those observed in neighboring Poland, Czech Republic, Hungary and Ukraine (Cabaj, 1). *T. spiralis* was detected in Slovakia only sporadically, in two foxes (0.8 %) and in mixed infection with *T. britovi* in 4 foxes (1.6 %). This species is perpetuating in our country in a low extent probably as a residuum of its former presence in domestic cycle. Of importance are records of the mixed infections of *T. britovi*/*T. pseudospiralis* detected in wild boar and fox from region of eastern Slovakia, where outbreak of trichinellosis in a pig farm has launched in 2003 due to the *T. pseudospiralis* causative agent (Hurníková *et al*., 3). These findings are suggestive for possible importation of the parasite into sylvatic cycle in this area and offer further support for upward trend for non-encapsulated *T. pseudospiralis* to be detected in Europe.

Gathered data demonstrated that genetic variation in *T. pseudospiralis* was mainly induced by the different continental origin (Fig. 1). In a well-defined cluster (bootstrap value 100) obtained by concatenated sequence data of three genes, the Palearctic and Australian isolates appear to be more similar group that could be related to a recent colonization of the Australian region with infected birds from the Palearctic region that migrated along Indo-Malaysian regions. In Palearctic group of isolates, a striking genetic similarity was recorded between Slovak isolate and Scandinavian isolates (unlike other Palearctic isolates exhibiting subtile genetic differences) by both DNA and allozyme methods (PGM polymorphism). *T. pseudospiralis* is widespread in the Scandinavian region owing to environmental humidity and cold temperature favoring its survival in the host carrion. Its introduction to central Europe has likely taken place *via* migration of raptorial birds from north of Europe (Black sea/Mediterranean flyway) wintering in Slovakia, rather than *via* another bird-migrating routes, e. g. East Atlantic flyway. The three non-encapsulated species were clearly separated that reinforce the classification of *T. pseudospiralis* along with recently recognized *T. papuae* and *T. zimbabwensis* as separate species.

ACKNOWLEDGEMENTS

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INTRASPECIES VARIABILITY OF INFRAPOPULATIONS OF *Fascioloides magna* BASED ON SELECTED VARIABLE SECTIONS *COX*1 AND *NAD*1 OF GENES OF MITOCHONDRIAL DNA

Bazsalovicsová, E., Kráľová-Hromadová, I. Špakulová, M., Turčeková, Ľ.

Parasitological Institute of SAS, Hlinkova 3, 040 01 Košice
The Slovak Republic

bazsal@saske.sk

ABSTRACT

The study investigated genetic variability of *Fascioloides magna* within three infrapopulations originating from Canada, Slovakia and Croatia, based on sequences of variable sections of two genes of mitochondrial DNA, subunit 1 cytochrome oxidase (*cox*1) and nicotinamide dehydrogenase (*nad*1), by means of sequencing and the SSCP (Single Strand Conformational Polymorphism) method. Comparison of genetic structure of parasite infrapopulations from distant territories allowed us to assess for the first time the extent of intraspecies variability of *F. magna*. Results of the research form a basis for more extensive phylogeographic study of the parasite and explanation of historic processes of its spreading.

Key words: *Fascioloides magna*; mitochondrial genes

INTRODUCTION

The giant liver fluke *Fascioloides magna* (Trematoda: Fasciolidae) is a serious, originally North American, liver parasite of a variety of wild and domestic ruminants. The first mention of the occurrence of *F. magna* in Europe was in the natural park La Mandria in Italy (1). At present it occurs besides Italy also in southwestern and central parts of Bohemia (2) and in the area of Danubian flooded forests covering regions of Slovakia, Hungary, Croatia and Austria (3).

Molecular methods are capable of revealing genetic variability of geographically distant populations using mitochondrial (mt) genes as the most suitable approach as their analysis may contribute to explanation of origin and ways of spreading of both the parasite and its host. On the basis of complete sequences of the subunit 1 cytochrome oxidase (*cox*1) and nicotinamide dehydrogenase (*nad*1), shorter sections were selected which showed characteristically higher frequency of mutations (4) and which could be used for more detailed population studies of *F. magna*.

The aim of the present study was to determine genetic variability of *F. magna* within three host infrapopulations originating from Canada, Slovakia and Croatia and to compare these geographically distant infrapopulations. The SSCP (Single Strand Conformational Polymorphism) method and sequencing were selected for determination of the structure of variable sections of *cox*1 and *nad*1 genes of mt DNA and frequency of occurrence of haplotypes. Comparison of results of direct sequencing and the SSCP method was used to evaluate the reproducibility and reliability of the SSCP method.

MATERIAL AND METHODS

Adult *Fascioloides magna* originated from liver of the following hosts from the respective locations: red deer (*Cervus elaphus*), Čičov, Slovakia (73 animals); red deer, Tikveš, Croatia (30 animals); elk (*Cervus elaphus canadensis*), Banff, Canada (38 animals). Isolation of genomic DNA, PCR amplification, purification of PCR products, sequencing and analysis of sequences was carried out according to (4).

The SSCP method was performed in a SSCP apparatus (The DCode™ Universal Mutation Detection system) follow-

ing the protocol supplied by the producer. Electrophoretic separation of denatured PCR products was carried out in 12 % polyacrylamide gel.

RESULTS

Sequence analysis of the *cox*1 gene (384 bp) showed presence of 2 different haplotypes in 73 *F. magna* isolated from liver of red deer originating from Slovakia. The first haplotype (CO1-Ha1/SR) was found in 68 samples (93.2 %) while the second one (CO1-Ha2/SR) was detected in 5 samples (6.8 %). Both haplotypes showed 97.9 % similarity (Tab. 1) and differed in 8 nucleotide substitutions (Tab. 2). The SSCP analysis confirmed the occurrence of two different haplotypes. The investigated *nad*1 mt gene (405 bp) also indicated presence of two haplotypes. Haplotype ND1-Ha1/SR occurred in the same 68 individuals (93.2 %) which showed presence of CO1-Ha1/SR haplotype. The remaining 5 individuals (6.8 %) harboured haplotype ND1-Ha2/SR. Both ND1 haplotypes showed 98.8% similarity (Tab. 3) and differed in five nucleotide substitutions (Tab. 4). The SSCP analysis again proved the occurrence of two haplotypes.

Sequence analysis of the *cox*1 gene (384 bp) in 30 *F. magna* isolated from the liver of red deer from Croatia detected only one haplotype CO1-Ha1/HR, identical with haplotype CO1-Ha1/SR (Tab. 1). SSCP profiles of the *cox*1 region were coincident in all 30 individuals. The analysed *nad* 1 mt gene (405 bp) showed identical structure in 29 (of 30 analysed) *F. magna* originating from Croatia. Their haplotype marked as ND1-Ha1/HR, was fully coincident with the Slovak ND1 haplotype ND1-Ha1/SR (Tab. 3, 4). One *F. magna* (3.3 %) harboured one nucleotide substitution (Tab. 4), however, the SSCP method failed to detect presence of this unique sequence. Haplotype of this individual parasite (ND1-Ha3/HR) showed 99.8 % similarity with haplotypes ND1-Ha1/HR and ND1-Ha1/SR (Tab. 3). Its similarity with haplotype ND1-Ha2/SR was a little lower (98.5 %) (Tab. 3).

Analysis of sequences of the *cox*1 gene (384 bp) in 38 individual *F. magna* isolated from the liver of elk from Canada revealed presence of 2 additional, structurally different haplotypes. The haplotype CO1-Ha3/CA occurred in 26 parasites (68.4 %) while the second haplotype CO1-Ha4/CA was observed in 12 samples (31.6 %). Both haplotypes showed 99.7 % similarity (Tab. 1) and differed only in one nucleotide substitution (Tab. 2). Both Canadian haplotypes showed considerable similarity with haplotype CO1-Ha1/SR/HR (98.7 and 99 %) (Tab. 1). The similarity of two Canadian haplotypes with the haplotype CO1-Ha2/SR was a little lower (96.6 and 96.9 %) (Tab. 1). The difference between both haplotypes represented by change in only one nucleotide was not detected on SSCP profiles. Analysis of sequences of the *nad*1 mt gene (405 bp) in all 38 individuals originating from Canada confirmed presence of two structurally different haplotypes. Haplotype ND1-Ha4/CA was observed in 34 individual parasites (89.5 %). The remaining 4 parasites (10.5 %) harboured haplotype ND1-Ha5/CA. Both ND1 haplotypes showed 99.8 % similarity (Tab. 3) and differed only in one substitution (Tab. 4) which, however, was not detected by the SSCP method. Both Canadian ND1 haplotypes showed 98.3–99.0 % similarity with Ha1-Ha3 haplotypes of *F. magna* originating from Slovakia and Croatia (Tab. 3).

DISCUSSION AND CONCLUSION

The genetic structure and variability of individual *Fascioloides magna* isolated from one host has not yet been revealed. The present study of infrapopulations of *F. magna* from hosts originating from three various territories indicates the presence of 2 genetic lines

Tab. 1. Similarity of haplotypes of the *cox*1 gene (CO1-Ha) in %

	Ha1/SR	Ha1/HR	Ha4/CA	Ha3/CA	Ha2/SR
Ha1/SR	-	-	-	-	-
Ha1/HR	100 %	-	-	-	-
Ha4/CA	98.7 %	98.7 %	-	-	-
Ha3/CA	99 %	99 %	99.7 %	-	-
Ha2/SR	97.9 %	97.9 %	96.6 %	99.7 %	-

Tab. 2. Nucleotide substitutions of haplotypes of the *cox*1 gene (CO1-Ha)

	21	27	86	90	105	120	181	224	230	249	264	273	348
Ha1/SR	T	G	C	A	T	T	G	T	T	G	T	C	C
Ha1/HR	T	G	C	A	T	T	G	T	T	G	T	C	C
Ha4/CA	C	G	T	A	A	T	G	T	T	A	T	C	T
Ha3/CA	C	G	C	A	A	T	G	T	T	A	T	C	T
Ha2/SR	T	A	C	G	T	C	A	C	C	G	C	T	C

Tab. 3. Similarit of haplotypes of the *nad*1 gene (ND1-Ha) in %

	Ha2/SR	Ha1/HR	Ha1/SR	Ha3/HR	Ha4/CA	Ha5/CA
Ha2/SR	-	-	-	-	-	-
Ha1/HR	98.8%	-	-	-	-	-
Ha1/SR	98.8%	100%	-	-	-	-
Ha3/HR	98.5%	99.8%	99.8%	-	-	-
Ha4/CA	98.5%	98.8%	98.8%	99%	-	-
Ha5/CA	98.3%	98.5%	98.5%	98.8%	998%	-

Tab. 4. Nucleotide substitutions of haplotypes of the *nad*1 gene (ND1-Ha)

	15	41	52	171	205	249	256	312	348
Ha2/SR	A	C	G	T	T	G	G	C	A
Ha1/HR	A	C	G	C	C	A	A	T	A
Ha1/SR	A	C	G	C	C	A	A	T	A
Ha3/HR	G	C	G	C	C	A	A	T	A
Ha4/CA	G	T	A	T	C	A	A	C	G
Ha5/CA	G	T	G	T	C	A	A	C	G

Explanatory note: Position of nucleotides of the amplified zone is expressed by numbers above the nucleotides

(haplotypes) as in red deer in Slovakia and Croatia as in elk in Canada. The presence of two haplotypes has been documented by sequence analysis of both mt genes. Accurate determination of the number and frequency of individual haplotypes and study of geographic distribution of *F. magna* requires molecular analysis of a greater number of individuals originating from a greater number of hosts and enzootic areas.

The two methods, SSCP and sequence analysis, used in parallel, failed to provide coincident results. When using SSCP to analyse individual *F. magna* from Croatia and Canada, with individual haplotypes differing only in one nucleotide substitution, we observed no difference at the level of SSCP profile. However, when analysing profiles CO1 and ND1 of haplotype of Slovak parasites in which individual haplotypes differed in eight or five nucleotide substitutions, we were able to record the difference. Because of that, when carrying extensive population studies of *F. magna,* it is recommended to substitute the screening SSCP method with another method with higher reproducibility (e.g. HRM – High Resolution Melting) which can disclose also one-nucleotide substitutions.

Results obtained in our study present initial information about intraspecies variability of individual *F. magna* within infrapopulations and indicate presence of several genetic lines of this parasite. Extending these results by additional information will contribute to explanation of the origin and ways of spreading of *F. magna* on both European and North American continent.

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CHARACTERISTICS AND EPIDEMIOLOGICAL GENOTYPING OF *S. aureus* ISOLATES FROM VARIOUS SOURCES

Mašlanková, J., Tkáčiková, Ľ.

ABSTRACT

The present study analysed isolates of *Staphylococcus aureus* (SA) originating from various sources (human n = 93, sheep milk n = 49, sheep cheese n = 24, bryndza n = 6) on the basis of polymorphism in the coagulase (*coa*) gene. Amplification of *coa* section of the gene allowed us to determine that the examined group of *S. aureus* consisted of 5 types differing in the number of 81 bp long tandem repetitions. By means of RFLP we were able to determine 16 subtypes in the isolates from sheep products and 25 subtypes in human isolates. The isolates from sheep milk and cheese contained most frequently the subtype D1 (36.7%) and the most frequent subtypes in human isolates were C1 (16.1%) and C3 (16.1%). Nine subtypes (A1, A2, B1, C1, C3, C4, D1, D3 and D4) occurred in both groups of SA isolates.

Key words: *coa* gene; RFLP-PCR; *S. aureus*

INTRODUCTION

The methods such as serotyping, biotyping and testing of antibiotic resistance were used until recently to determine epidemiological profiles of SA (4). At present many authors use molecular methods, such as ribotyping, polymorphism in the coagulase gene (*coa*) polymorphism encoding the X region of proteine A (*spa* gene). The 3´-end of *coa* gene contains highly polymorphic 81 bp long region suitable for differentiation of SA isolates (3).

The aim of the present study was to use *coa* gene polymorphism to identify subtypes of SA in human isolates and isolates from sheep milk and cheese.

MATERIAL AND METHODS

Bacterial strains. Samples of SA (n = 199) were isolated from raw sheep milk (n = 73), sheep cheese (n = 24) and bryndza (n = 6) and from clinical human samples (n = 93).

Isolation of DNA. DNA was isolated from broth culture of SA (1).

PCR amplification was carried out according to Hookey *et al.* (2). The PCR product was cleaved with *Hae*III. Electrophoresis of samples was carried out in 2% agarose.

RESULTS AND DISCUSSION

We detected 5 PCR products of the *coa* gene (A–500 bp; B–580 bp; C–660 bp; D–740 bp and E– 820 bp). The isolates from sheep milk and cheese harboured most frequently (40.8%) the *coa* type with 7 repetitions (type D) which was present in all isolates from bryndza and in 45.8% and 40.8% isolates from sheep cheese and milk, resp. The type D was found only in 20.4% human isolates of SA. Of all human isolates, 44.1% carried the *coa* type C. The *coa* type E was not detected in human isolates, this type was found only in isolates from sheep cheese.

The use of PCR-RFLP method for analysis of the *coa* gene enabled us to characterise SA isolates in more

Tab. 1. Polymorphism in the *coa* gene of isolates of *S.aureus*

Source	Coagulase				
	500 bp	580 bp	660 bp	740 bp	820 bp
	A	B	C	D	E
Human (n = 93)	10 (10.7 %)	24 (25.8 %)	40 (43.0 %)	19 (20.4 %)	-
Sheep milk (n = 49)	10 (20.4 %)	-	19 (38.8 %)	20 (40.8 %)	-
Sheep cheese (n = 24)	2 (8.3 %)	1 (4.2 %)	7 (29.2 %)	11 (45.8 %)	3 (12.5 %)
Bryndza (n = 6)	-	-	-	6 (100 %)	-

detail. In SA isolates from sheep milk and cheese we were able to detect 16 *coa* subtypes. The highest polymorphism in isolates from sheep milk and cheese was observed in the type C. The D1 subtype was found in the highest proportion (36.7 %) in isolates from sheep milk and cheese. The human isolates exhibited higher polymorphism in comparison with isolates from sheep milk and cheese. The RFLP method allowed us to determine altogether 25 *coa* subtypes. The highest polymorphism in human isolates was observed in subtypes B and D. The *coa* subtypes C1 and C3 were most frequent, both found in 16.1 % of human isolates of SA. The type E was not detected in human isolates. Comparison of *coa* subtypes in human isolates and isolates from sheep milk and cheese showed that 9 subtypes (A1, A2, B1, C1, C3, C4, D1, D3 and D4) occurred in both groups of SA isolates.

Some studies proved the possibility of transfer of SA between humans and animals, humans being the potential source of infection of animals (5).

The results on polymorphism of the coa gene obtained in this study can contribute to explanation of the occurrence of individual SA strains in humans and animals.

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EFFECT OF PROBIOTICS ON THE LEVEL OF NON-SPECIFIC IMMUNITY IN IMMUNOSUPPRESSED DOGS

Haladová, E., Mojžišová, J., Čížek, M., Smrčo, P.

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

haladova@uvm.sk

ABSTRACT

The strain for oral administration was isolated from 8-day old and typed by BBL Crystal identification system as *Lactobacillus jensenii*. The cultured and diluted lactobacilli culture was administered daily *per os* to 10 dogs. Rectum swabs were taken during six samplings to determine bacteriological status of the observed dogs. Blood samples were collected in parallel and were subjected to immunological analysis. After a 4-week gradual decrease, *Proteus* spp. was not recovered from any animal. Administration of the probiotic resulted in a significant increase in many immunological parameters (Tab. 1).

Key words: bacteriological status; *Lactobacillus jensenii;* immunological analysis

INTRODUCTION

The term probiotic originates from two Greek words "for life" and implies live microbial supplement which, through improvement of intestinal microbial balance, has a beneficial effect on the host (2). It also stimulates significantly its immune system and anti-tumour and anti-cholesterol activities.

MATERIALS AND METHODS

The *Lactobacillus jensenii* probiotic, intended for *per os* administration, was tested to determine the inhibitory activity against selected pathogens. Cultured and diluted lactobacilli were administered *per os* daily to 10 dogs of various breeds and both sexes, 4 weeks old, originating from a dog shelter. Rectal swabs were taken to determine bacteriological status of dogs and their blood was sampled in parallel on days 0, 7 and 28 by puncture into *v. cephalica* and subjected to immunological analysis. The sample taken on day 0 confirmed the assumed immunosuppression.

The blastogenic response of blood lymphocytes to mitogens was evaluated by ELISA BrdU (colorimetric) test using 20 $\mu g.ml^{-1}$ phytohaemaglutinine PHA – P (Sigma, USA).

The phagocytic ability of blood leukocytes was determined by ingestion of 2-hydroxyethyl metacrylate particles (MSHP, diameter 1.2 µm, ARTIM Prague, CR) (4). The **chemotactic activity** was determined by polymorphonuclear leukocyte (PMNL) chemotaxy under agarose according to Mareček and Procházková (1).

The metabolic activity of pghagocytes – iodonitro tetrazolium test was carried out according to Mareček and Procházková (1).

Statistical analysis – ANOVA-test (GraphPad InStat).

RESULTS AND DISSCUSION

Perdigon *et al.* (3) observed that feeding fermented milk containing *Lactobacillus acidophilus* and *L. casei* to mice increased phagocytic and lymphocytic activity in the respective animals.

Increased PHA was observed also in our study with the highest significance on day 21 after administration of the probiotic. The chemotactic activity was affected

Tab. Immunological parameters of dogs following administration of probiotics

Parameter	Sample 0	Sample 1	Sample 2 Day 7	Sample 3 Day 14	Sample 4 Day 21	Sample 5 Day 28
				Lactobacilli		
PHALe %	31.4 ± 8.7	38.5 ± 4.1	35.75 ± 8.76	32.88 ± 14.03	30.25 ± 9.17	37.9 ± 4.98
PHI Le	7.14 ± 1.34	10.06 ± 3.2	10.98 ± 2.09	11.75 ± 3.23*	14.49 ± 3.94**	10.54 ± 2.7
IMA	1.96 ± 0.76	2.85 ± 0.89	1.53 ± 0.48	2.07 ± 0.59	1.50 ± 0.43	1.56 ± 0.75
ChI	1.25 ± 0.11	1.14 ± 0.81	1.23 ± 0.81	1.40 ± 0.16	1.73 ± 0.27*	1.76 ± 0.32
SI	2,44±1,26	2.98 ± 0.43	3.22 ± 0.65*	2.86 ± 0.45	2.82 ± 0.34	2.78 ± 0.65

PHALe – phagocytic activity of leukocytes; PHLe – phagocytic index of leukocytes
IMA – index of metabolic activity of phagocytes; ChI – index of chemotactic activity
SI – stimulative index of lymphocytes; * – $P \leq 0.05$; ** – $P \leq 0.01$

after 3 weeks of probiotic treatment. The stimulative index (SI) of lymphocytes increased significantly after 7 days of probiotic treatment. Yumuto *et al.* (5) described a negative effect of *Lactobacillus arabinosus* on incidence of tumours in the large intestine of mice. In dogs we observed a positive effect of *L. jensenii,* particularly in terms of suppression of pathogenic microflora (*Ptoeus* spp.), which proved that the tested strain was not only able to adhere to intestinal mucosa but also persist there throughout the experiment.

After 4 weeks of the experiment we were unable to recover *Proteus* spp. from any of the dogs in which it was present at the beginning of the experiment.

CONCLUSION

The strain *Lactobacillus jensenii* used in our study protected dogs against pathogenic micro-organisms in their intestinal apparatus and participated in stimulation of non-specific immune response in immunosuppressed individuals.

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DETECTION OF MYCOTIC FLORA AND PARASITIC FAUNA IN RAPTORS OF SLOVAKIA

Smrčo, P.[1], Mojžišová, J.[1]
Čonková, E.[2], Letková, V.[1], Goldová, M.[1]

[1] Department of Epizootiology and Parasitology, University of Veterinary Medicine,
[2] Department of Pharmacology, University of Veterinary Medicine,
Komenského 73, 041 81, Košice
The Slovak Republic

ABSTRACT

In the Slovak Republic presence of mycotic and parasitic pathogens among 62 individuals of 9 species from order Falconiformes was analyzed during years 2006–2007. From group of mycotic pathogens *Candida albicans* (27.4%), *Rhodotorula mucilaginosa* (16.13%) and *Aspergillus flavus* (9.68%) were detected mixed infection of *Aspergillus flavus* and *Candida albicans* in two individuals and *Candida albicans* with *Rhodotorula mucilaginosa* in seven cases was observed. Parasitological examination by flotation methods: 15 from 62 samples of faeces (24.19%) were positive. Eggs of internal parasites were detected: fam. Trichostrongylidae (12.9%), *Capillaria* spp. (4.83%), *Porrocaecum* spp. (1.61%), and *Syngamus trachea* (1.61%) and eggs from classis Trematoda (1.61%). Oocysts of protozoa were also found: *Eimeria* spp. (1.61%).

Key words: aspergillosis; candidiosis; endoparasites; fungi; raptors

INRODUCTION

In this study we were concentrated to detection of pathogenic fungi and parasites in birds of prey. Parasites should be a cause of weakness of bird's immune system. Then an organism is more susceptible to infection induced by commensal and conditionally pathogenic microorganisms.

MATERIAL AND METHODS

Animals

Sixty two diurnal raptors representing 9 species from order Falconiformes were sampled.

Methods

Coprological analysis. Samples of faeces for parasitological investigation were collected in aviaries from under perch. Faeces were stored in plastic boxes with potassium dichromate solution. After flotation were samples investigated under microscope.

Mycological analysis. Samples were obtained by introducing a sterile dry cotton swab into the crop and cloaca and placed in transport media Fungi-Quick (Copan, Italy). The swabs were cultured on two plates of Sabouraud's dextrose agar (SDA) and one on HiCrome Candida agar (HiMedia, India). The one of SDA plate and HiCrome agar were incubated at 37 °C for up to 7 days. Another SDA plate was incubated at room temperature for two weeks. The identification of was based on macroscopic observation of colonies and by microscopic examination of a wet mount, using lactophenol cotton blue solution (Merck). For distinction of yeasts were used rapid HiCandida Identification Kit (HiMedia, India).

RESULTS

62 individuals of 9 species were examined to presence of parasites and pathogenic moulds and yeasts.

Out of 62 examined samples of faeces, fifteen (24, 19%) were positive. Most common eggs detected were

of Trichostrongylidae fam. (12.9 %), *Capillaria* spp. (4.83 %), *Porrocaecum* spp. (1.61 %), *Syngamus trachea* (1.61 %) and Trematoda (1.61 %). In one sample oocysts of *Eimeria* spp. (1.61 %) were found.

Pathogenic fungal flora were isolated from 34 (54.83 %) out of the 62 sampled birds. Highest prevalence was established in *Candida albicans* – 18 samples (27.4 %); 10 swabs from crop and 8 from cloaca were positive, than in *Rhodotorula mucilaginosa* 10 samples (16.13 %); 7 from crop and 10 from cloaca and *Aspergillus flavus* was isolated from 6 samples (9.68 %), 3 from crop and 3 from cloaca.

Mixed infection of *Aspergillus flavus* and *Candida albicans* in two individuals (*Falco peregrinus* and *Falco cherrug*) and *Candida albicans* with *Rhodotorula mucilaginosa* in seven cases was observed (*Buteo buteo*, *Aquila chrysaetos*, *Accipiter gentilis*, *Falco cherrug* (2×) and *Falco peregrinus* (2×)).

In seven cases (11. 29 %) parasites and fungi were diagnosted both together.

DISCUSSION

Aspergillus spp. causes the disease under immunocompromised situations of the host or when the bird is exposed to an overwhelming number of spores (4, 7, 10). Aspergillosis occurs frequently in birds of prey held in captivity (2, 7, 13), i.e. in zoos (for conservancy, research or education), falconry and those that are maintained in rehabilitation centers. This disease is responsible for 15 to 30 % of deaths in birds of prey exhibited in zoos, rarely occurs in free-living birds (6). In spite of aspergillosis is often considered the main fungal cause of losses in the wild bird population (6, 8).

Some species as goshawk *(Accipiter gentilis*), gyrfalcon (*Falco rusticolus*), rough-legged hawk (*Buteo lagopus*), immature red-tailed hawk (*Buteo jamaicensis*), golden eagle (*Aquila chrysaëtos*)) are more susceptible to aspergillosis while others are resistant: prairie falcon *(Falco mexicanus*) and Harris hawk (*Parabuteo unicinctus*) (1, 6, 7, 11, 12, 13, 14).

Wieliczko *et al.* (15) were evaluating the health status of goshawk chick nesting in Wrocław vicinity. From examined 33 chicks from 11 nests they did not isolate *Aspergillus* spp., which was isolated just from nest litter. Crop candidiasis was found in 63 % of the 35–40 day old chicks.

Garcia (5) examined presence of fungal flora in trachea of birds from a wildlife rehabilitation centre in Spain. They are presenting 34.93 % positivity of birds. From 146 sampled birds 24 (16.43 %) were positive for presence of moulds, and 24 samples (16.43 %) were positive to yeasts and from tracheas of three birds (2.05 %) both moulds and yeasts were cultivated.

In our study were discovered 54.83 % prevalence of pathogenic moulds and yeasts occurrence (*Aspergillus* sp. 9.68 %, *Candida* sp. 29.03 % and *Rhodothorulla* spp. 16.13 %) in crop and cloaca of sampled raptors. Typical clinical sings were not observed.

22. 58 % samples of faeces were positive to presence of parasites and six different species were described in our work. Ferrer *et al.* (3) present 79 % and Lierz *et al.* (9) 58.3 % of positive samples of faeces in investigated diurnal raptors. In nine cases (11.90 %), parasites, moulds or yeasts were detected together. We suppose that weaken of organism by parasites should have a influence to breaking through of mycotic disease.

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