FOLIA VETERINARIA

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

ISSN 0015-5748

2

LV • 2011

EDITORIAL BOARD

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

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

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

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

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

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

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

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

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

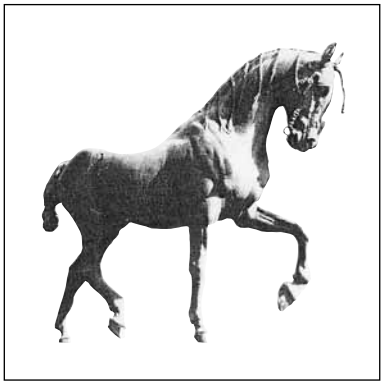
Sadzba: **Sapfo publishers**, Szakkayho 1, 040 01 Košice

EV 3485/09

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

Indexed and abstracted in AGRIS, CAB, EBSCO

FOLIA VETERINARIA, 55, 2, 2011



54th STUDENT SCIENTIFIC CONFERENCE

May 4th, 2011

The aim of the 54th Student scientific conference (ŠVOČ) organised in the academic year 2010/2011 was to present results fo scientific investigations carried out by undergraduate and PhD. students. The papers were presented in the following four sections:

1. Pharmaceutical and pre-clinical – 2. Clinical
3. Hygiene of food and the environment
4. Post-graduate students A and B

UNIVERSITY OF VETERINARY MEDICINE AND PHARMACY IN KOŠICE

FOLIA VETERINARIA, 55, 2, 2011

CONTENTS

FOLIA VETERINARIA, 55, 2: 5–6, 2011

250 YEARS OF VETERINARY EDUCATION

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ABSTRACT

The year 2011 is the World Veterinary Year. Based on the needs of society, Claude Bourgelat founded the first veterinary education institution in the world in Lyon, France in 1762. Owing to this event, veterinary medicine became an official science taught at the level of higher education. Since then, veterinary medicine progressed considerably and has become an important tool in the management of public health. The aim of veterinarians today is to watch over the health of both animals and humans. This paper is devoted to the history of the establishment of this institution and to conduct an overview of veterinary education in Slovakia.

Key words: education; history; university; school; veterinary medicine

INTRODUCTION

In 2011 we commemorate the 250th anniversary of the founding of the first veterinary school in the world in Lyon, France. Veterinary medicine belongs to the group of biological sciences. Its essential aim is prevention, diagnosis and treatment of animal diseases and in collaboration with human medicine to observe and investigate epidemiological and epizootological situations in the world. Veterinarians are responsible for the health of human populations by the constant control of food products of animal origin; and as the colourful slogan goes, *"from the stable to the table"*. Veterinary medicine plays an irreplaceable role in the management and protection of the environment and biodiversity.

Claude Bourgelat's contribution

On January 2nd, 1762, Claude Bourgelat, a colonel in the French army, founded the first veterinary school in the world in Lyon, France. The foundation of an educational institution of this kind was called for by the unfavourable health situations in animal herds throughout the world. C. Bourgelat was born in 1712. Already by the age of 28 years he became known as the *"Grand Equerry"* which means verbatim *"High steward of the king's stables"*. In July, 1761, Bourgelat, together with his friend Henri-Léonard Bertin, presented a project dealing with the establishment of a veterinary school. In August, 1761, a King's Board resolution was passed which authorised Bourgelat to establish such a school. The training started in February 1762. In the first year, 6 students enrolled in the study. The majority of them were horseshoers but there were also human doctors from abroad. The students originated from Denmark, England, Austria and Italy and after successful graduation they founded similar veterinary schools also in their countries (8).

Establishment of veterinary schools in central Europe

Many graduates of the school in Lyon founded similar schools in their home countries. Thus in a short time, veterinary schools were established in Vienna, Turin, Padova, Copenhagen, Swedish Skara, Dresden, Giessen, Hanover, Bologna, Milan, Merlin, Munich, London, Madrid and Naples. The third oldest school in the world was established in Vienna. It was founded in 1767 by Ludovico Scotti, but was abolished later. The second Veterinary school in Vienna was founded by J. G. Wolstein in 1777 and exists up to this day. In Budapest, the Veterinary department was established in 1787 as part of the Medical faculty. In 1851, Viliam Zlámal founded an independent Veterinary institute which was transformed later into the Veterinary College. Veterinary education in the Czech Republic

started in the form of lectures at the Medical faculty in Prague. The Veterinary department was established in 1795. The Veterinary College in Brno was established in 1918 and its present name is Veterinary and Pharmaceutical University in Brno (3).

University of Veterinary Medicine and Pharmacy in Košice

After founding the Veterinary College in Brno, many students from Slovakia came to study at this institution. After the World War II, the college in Brno was in a desolate state from both a material-technical and personal points of view. The task to build a similar educational institution in Slovakia was undertaken by Prof. Ján Hovorka and Samuel Adámať, DVM. In July of 1949 there was established a preparation committee for the founding the Veterinary College in Košice. The training started on October, 5, 1949. Prof. Ján Hovorka was appointed the rector of this institution (1, 4).

Present system of veterinary education in Slovakia

In the 1960's, two secondary schools (originally agricultural and technical schools) were established in Slovakia. Gradually they were transformed into specialised veterinary schools. One is located in Nitra and the other in Košice. A private secondary specialised veterinary school was founded in Bratislava in 1993. The specialised competences of the graduates of these secondary schools include; care of the health and productivity, rearing, reproduction and artificial insemination of animals. After completing the school, the graduates can work as: an assistant in veterinary facilities; reproductive and sanitation technician; food hygiene technician; laboratory technician; worker in Zoos; and other breeding establishments.

Veterinary education on the level of a higher education institution is provided by the University of Veterinary Medicine and Pharmacy (UVMP) in Košice as the only university of this kind in Slovakia. Today it provides university education in seven study programmes for undergraduate, graduate, and postgraduate study (7).

Continuing life-long education in the veterinary field has been institutionalised since 1958. The Institute for Education of Veterinarians is an education and congress centre of the Ministry of Education and Rural Development of the Slovak Republic primarily focused on the development of human resources. In the period of its existence, more than 60,000 persons involved in the veterinary field, participated in courses organised by this institution (2). Stress is put on continuous education of the staff of State Veterinary and Food Administration of the Slovak Republic and State veterinary and food institutes in all fields of veterinary care, food hygiene and food inspection, veterinary legislation, protection of state territory, common agricultural policy of EU and laboratory diagnostics (6).

The Slovak Veterinary Chamber (SVCh) is an autonomous professional organization uniting veterinary surgeons that are involved in private veterinary practice and provide veterinary service in the territory of the Slovak Republic. It also takes care of the professional development of its members who are required to improve and update their professional knowledge. The educational opportunities include: lectures, seminars, workshops, study stays and internships in Slovakia and abroad. The Statute of SVCh is also responsible for the educational programme stating the details of continuous education of private veterinarians as well (5).

Celebrations and associated events of the World Veterinary Year 2011 are organised worldwide under the auspices of prominent world veterinary, agricultural and medical institutions, such as the Organisation for Animal Health (OIE), Food and Agriculture Organization (FAO) of the United Nations, World Health Organization (WHO), associations of veterinary surgeons of Africa, Asia, Europe, America, Australia and others. Forty-seven countries from around the world have joined the campaign with the motto *"Vet for health, Vet for food, Vet for planet".* There has been an effort to increase the awareness of the importance and work of veterinary surgeons, veterinary education institutions, professional and specialised organisations and thus enhance enforcement of activities contributing to preservation and improvement of public health. The quality of education in veterinary medicine throughout the world is constantly improved based on the latest development in veterinary, medicinal and biological sciences and up-to-date technical opportunities and trends.

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Selected papers from the 54th STUDENT SCIENTIFIC CONFERENCE with international participation, held at the University of Veterinary Medicine and Pharmacy in Košice on May 4, 2011.

FOLIA VETERINARIA, 55, 2: 7–9, 2011

COMPARISON OF THE INFLUENCE OF SELECTED PLANT EXTRACTS ON MITOCHONDRIA OF LEUKEMIC CELLS *IN VITRO*

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ABSTRACT

Individual extracts from horse chestnut, arnica and hops were applied *in vitro* to leukemic Jurkat cells and the isolated mitochondria from these cells. The influence of plant extracts on mitochondria was observed by means of the determination of the proteins in individual samples and monitoring of the fluorescence of endogenous fluorofores in the control and experimental (exposed to extracts) mitochondria. Fluorofores in nanomolar concentrations were observed by means of the 3D images of the excitation-emission matrix. The resultant fluorescence was manifested graphically as two fluorescent excitation-emission zones. The content of proteins and fluorescence in the first excitation zone was decreased in all experimental mitochondria in comparison with the control. The cytotoxic effects of the tested extracts on mitochondria decreased the most following exposure to arnica and the least after exposure to horse chestnut.

Key words: cytotoxic effect; fluorescence; mitochondria; plant extracts

INTRODUCTION

The treatment of diseases based on herbs and their extracts is used today more extensively due to the lower incidence of undesirable side effects. The action of herbs depends on the presence of a mixture of several biologically active components. The principal active component of horse chestnut seeds (*Aesculus hippocastanum, Hippocastanaceae*) is aescin, a mixture of triterpene saponins, used for the treatment of chronic venous insufficiency (11, 12), haemorrhoids (1) and post-operative oedemas. Studies *in vitro* proved also an apoptotic action and an antiproliferative potential of β-aescin (13) against leukemic cells (4). The most important components of arnica montana flowers (*Arnica montana, Asteraceae*) are bitter resins and essential oils. The essential oils contain sesquiterpene lactons exhibiting toxic effects (10) which limits their peroral use. The anti-tumour action of hops flower cones (*Humulus lupulus, Cannabaceae*) have been ascribed predominantly to prenylated chalcones. Some of them, particularly xantohumol, induced apoptosis of leukemic cells *in vitro* and *in vivo* (8).

Our study investigated the potential cytotoxic action of plant extract on mitochondria of leukemic cells *in vitro* by monitoring fluorescence of their endogenous fluorofores.

MATERIAL AND METHODS

We used Sigma-Aldrich chemicals and Jurkat cells (T-cell lymphoma) provided by Dr. Hajduch (Olomouc, CR), which were divided to control (a) and three experimental groups. The individual groups of experimental cells (b, c, d) were exposed to plant extracts (Calendula a.s., Nová Ľubovňa) of horse chestnut (0.125 mg.ml^{-1}),

arnica (0.5 mg.ml^{-1}) and hops (2 mg.ml^{-1}), respectively dissolved in RPMI 1640 medium. The cells were cultivated for 72 h in the RPMI 1640 medium at t = 37 °C with access to air. The mitochondria of the control and experimental cells were isolated according to Mela and Seitz (7). The concentration of proteins was determined according to Bradford (3). Excitation-emission 3D fluorescent matrices (EEM) of samples were analysed in the respiration medium (pH = 7.4) in nanomolar concentration in wavelength range λ = 200 – 500 nm using a luminescence spectrophotometer Perkin Elmer LS 55 (USA) and quartz cuvettes at t = 25 °C. Scanning rates were 1 200 nm.s^{-1}, the size of excitation-emission slits was 10/15 nm. The resultant 3D spectrum, presented in graphic form by means of software WinLab, was produced from 20 scans (simple fluorescence spectres of mitochondria placed in a space with increment 10).

RESULTS AND DISCUSSION

The topographic presentations of EEM, which provided detailed information, were compared visually (Fig. 1) and mathematically and showed differences between the experimental groups of mitochondria in comparison with the control. The evaluation of the emission spectres obtained as horizontal sections of EEM at λ = 240 nm, revealed 2 fluorescence maxima pointing to the existence of 2 fluorescence zones (Fig. 1 and 2).

Fluorescence of mitochondria exposed to horse chestnut and hops was reduced but arnica increased the fluorescence in comparison with the control (Fig. 1 and 2).

Both extracts (horse chestnut and hops) reduced the content of proteins in mitochondria (0.338 mg.ml^{-1} and 0.26 mg.ml^{-1}, resp.) compared to the control (0.52 mg.ml^{-1}) which correlated with a reduced fluorescence of both fluorescent zones 1 and 2 (Fig. 2). The fluorescence zone 1 is characteristic of proteins while zone 2 reflects the presence of NADH+H+. The changes observed indicate the cytotoxic action of the investigated extracts (6, 9) which was observed *in vitro* and *in vivo*.

Arnica (Fig. 2) reduced the zone 1 fluorescence of mitochondria fluorofores the most and also the level of proteins in mitochondria (0.0039 mg.ml^{-1}) in comparison with the control mitochondria. In the zone 2, arnica induced the highest fluorescence (NADH+H+ fluorescence increases in a deficiency of oxygen) in comparison with all investigated groups which can be ascribed to the cytotoxic action of arnica as observed also by other authors *in vitro* (2, 5).

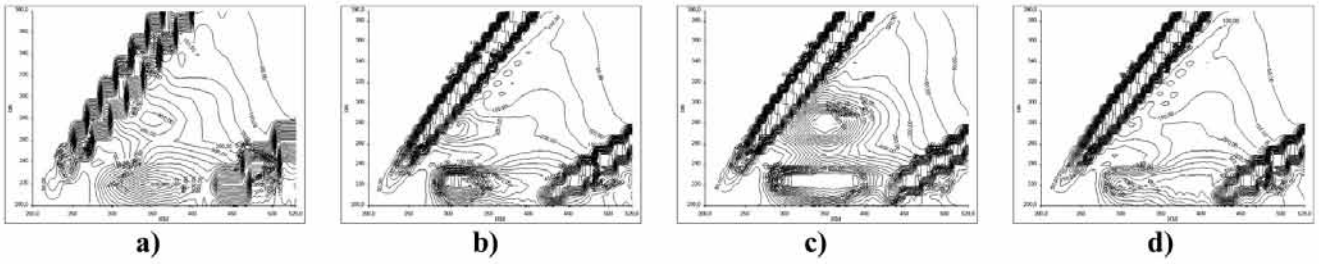

Fig. 1. Comparison of three-dimensional excitation-emission fluorescence topographic presentations (EEM) of control and experimental (extract-exposed) mitochondria: a) control, b) horse chestnut, c) arnica, d) hops

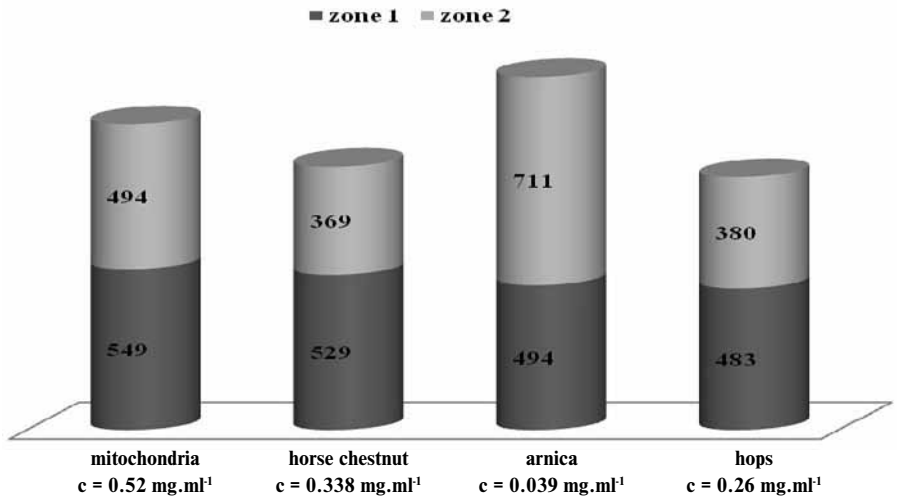

Fig. 2. Comparison of fluorescence of two fluorescence zones produced as a result of fluorescence of all fluorofores present in control and experimental mitochondria

CONCLUSION

Our results indicated the different cytotoxic actions of the tested plant extract not only on leukemic cells but also on the mitochondria themselves. The content of proteins in leukemic cells mitochondria decreased most after *in vitro* exposure to arnica extract which correlated with the results of 3D fluorescence excitation-emission analysis.

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Selected papers from the 54th STUDENT SCIENTIFIC CONFERENCE with international participation, held at the University of Veterinary Medicine and Pharmacy in Košice on May 4, 2011.

FOLIA VETERI NARIA, 55, 2: 10–11, 2011

EXPRESSION OF DOPAMINE RECEPTOR D4 TRANSCRIPT IN MOUSE EARLY EMBRYO

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ABSTRACT

Dopamine is a well-known neurotransmitter and a failure in the dopaminergic system is associated with many diseases. In addition, dopamine can play an important role in the basic developmental processes involving cell proliferation, differentiation, and migration. In this study, we examined the expression of one member of the dopamine receptor family in mouse preimplantation embryos. We used RT-PCR with oligonucleotide primers specific to dopamine D4-receptor, and showed that the D4-receptor mRNA is expressed in 4-cell embryos, 8-cell embryos as well as and in the morulas and blastocysts. Our results suggest that dopamine, present in the reproductive tract, could influence the embryo even in very early stages of development.

Key words: dopamine receptors; preimplantation embryo

INTRODUCTION

The development of the preimplantation embryo is regulated by its genetic program as well as by the factors of the surrounding environment. Preimplantation embryos are able to develop to the blastocyst stage *in vitro* in relative simple media but their developmental capacity is inferior when compared with their in-vivo counterparts, indicating the important role of factors from the maternal reproductive tract. *In vivo*, preimplantation embryos develop in the oviduct which provides an environment containing a variety of molecules which can influence the embryo. Biogenic monoamines, including dopamine, have been identified in the folicular and oviductal fluids of several mammalian species (3). Accumulating evidence indicates that these compounds can (besides their well-known neurotransmitter function) also play an important role in the basic developmental processes, such as embryogenesis and morphogenesis, controlling cell proliferation, differentiation, and migration (4). The aim of this study was to examine the expression of one member of dopamine receptor family – D4 receptor – in mouse preimplantation embryos.

MATERIAL AND METHODS

To obtain preimplantation embryos, female ICR mice (intact or mated with males) were killed, and unfertilized oocytes or embryos at various developmental stages were isolated by flushing them from the oviduct or uterus. The total RNA was extracted using TRIzol Reagent, and reverse transcribed with SuperscriptTM II Reverse Transcriptase. PCR amplification was carried out with Taq DNA polymerase (Invitrogen Life Technologies) using oligonucleotide primers specific to mouse dopamine D4 receptor and to mouse beta-actin. The initial denaturation step at 95 °C for 2 min was followed by 35 cycles of 94 °C for 30 sec, 62 °C for 45 sec and 72 °C for 45 sec. The PCR products were analyzed using electrophoresis on a 2 % agarose gel stained with SYBR Green I.

RESULTS AND DISCUSSION

We detected a PCR product corresponding to the dopamine D4 receptor (235 bp) in 4-cell embryos, 8- to 16-cell embryos, morulas and blastocysts. No PCR product was detected in oocytes, or in the reactions where reverse transcriptase (RT- reactions) or cDNA (Blank reaction) were omitted (Fig. 1A). Digestion of the 235 bp PCR product with restric-

tion enzyme BfaI produced DNA fragments of the expected sizes 142 bp and 93 bp, confirming the identity of the amplified sequence (Fig. 1B). The oocytes and embryos produced a PCR fragment corresponding to the beta-actin mRNA at all developmental stages, thus confirming the integrity of the RNA and the RT-PCR process (data not shown).

Agarose gels with separated PCR products are shown. Lanes in the panel A: Oc, oocytes; 4c, 4-cell embryos; 8c, 8- to16-cell embryos; Mo, morulas; Blast, blastocysts; PC, positive control tissue (brain); M, molecular weight markers. Lanes in the panel B: ND, non-digested PCR product amplified with D4 receptor-specific primers; D, the 235 bp PCR product digested with BfaI; M, molecular weight markers. The sizes of the markers and the predicted sizes of the PCR products (non-digested or digested) in base pairs (bp) are indicated to the left and the right of the panels respectively.

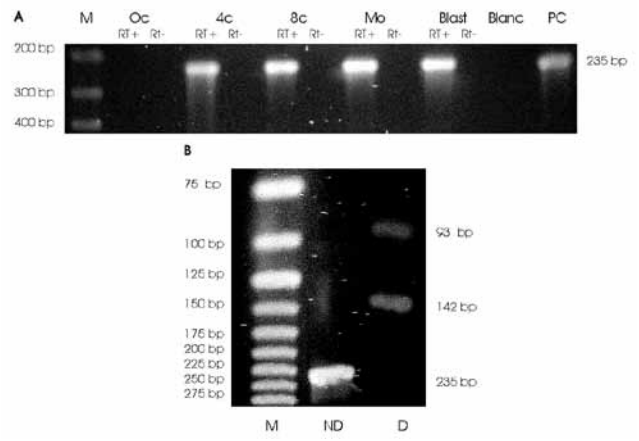

Fig. 1. RT-PCR analysis of dopamine D4 receptor in mouse oocytes and preimplantation embryos

Several adrenergic receptors have been shown to be expressed in mouse preimplantation embryos (1). The expression of D4 receptor mRNA, demonstrated in the present study, suggests that all main types of catecholamine receptors (alpha adrenergic-, beta adrenergic- and dopamine- receptors) can be expressed in preimplantation embryos. Moreover, receptors for other biogenic monoamines have been found in oocytes and preimplantation embryos (2, 5).

The potential role of receptors for biogenic monoamines in the regulation of preimplantation development has been examined in experiments where agonists/antagonists activating/inactivating the receptors were added to the embryos cultured *in vitro*. Administration of adrenergic agonists to mouse preimplantation embryos significantly inhibited cell proliferation (1). The effect of dopamine on preimplantation embryos has not yet been tested, but the similarity in G protein coupling between the D4 receptor and some adrenergic receptors suggest that these receptors can regulate similar cellular processes. Moreover, a ligand cross-binding between adrenergic and dopamine receptors is well-known. Other biogenic monoamines have also been demonstrated to be capable of influencing the development of preimplantation embryo. Administration of histamine receptor H2 agonist to mouse blastocysts incubated *in vitro* stimulated blastocyst hatching, which suggests a role of the receptor in blastocyst implantation into the uterus (5). Embryo exposure to 5-HT1D serotonin receptor agonists significantly reduced the cell number, and increased the number of embryos with apoptotic and secondary necrotic nuclei (2).

The exact physiological role of dopamine and other biogenic monoamines in the development of preimplantation embryo is unknown. The examination of the expression of dopamine receptors at the protein level as well as confirmation of receptor functionality in preimplantation embryos is necessary. Moreover, the effect of dopamine on preimplantation embryo development must be considered in the context with other biogenic monoamines present in the oviduct.

ACKNOWLEDGEMENT

This publication is the result of the project 26220120043 implementation supported by the Research & Development Operational Programme funded by the ERDF, and was also supported by the Slovak Academy of Sciences under contract VEGA 2/0041/09.

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Selected papers from the 54th STUDENT SCIENTIFIC CONFERENCE with international participation, held at the University of Veterinary Medicine and Pharmacy in Košice on May 4, 2011.

FOLIA VETERINARIA, 55, 2: 12–14, 2011

BENDIOCARB AND POTENTIAL OXIDATIVE INJURY OF AN ORGANISM

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ABSTRACT

This study investigated the potential influence of bendiocarb on the antioxidative enzyme system of the liver and kidneys in female rabbits following administration of 5 mg.kg^{-1} b. w. for 30 days. The specific activity of superoxide dismutase (SOD) and catalase (CAT) in the liver remained unchanged but the activity of glutathione peroxidase (GSHPx) was inhibited significantly on day 3. The kidneys showed significantly decreased specific activity of SOD from day 10 of the experiment. No significant changes in the activity of CAT and GSHPx were observed in any experimental group. The inhibition of activity of some enzymes could result from the accumulation of reactive oxygen species (ROS). The changes observed in the antioxidative enzyme system confirmed the development of ROS after exposure to bendiocarb. The oxidative damage to the investigated organs was confirmed by the increased levels of thiobarbituric acid reactive substances (TBARS), an indicator of lipid damage.

Key words: antioxidative enzymes; bendiocarb; kidneys; liver

INTRODUCTION

Bendiocarb is an insecticide from the group of carbamate pesticides used in public health and agriculture. It is resorbed by all common pathways, undergoes rapid metabolic changes and is eliminated by the kidneys. One of its pharmacodynamic properties is an inhibitory effect on acetylcholine esterase (2). The inhibition of acetylcholine esterase by carbamate pesticides may result in oxidative damage by means of the production of free radicals in the target tissues (4). These radicals are highly reactive components that may damage important biomacromolecules and alter their functions (6). The long-term increased level of free radicals leads to oxidative stress. The extent of organ damage depends on the effectiveness of its antioxidant system. An important part of this system are enzymes (superoxide dismutase SOD, catalase CAT, glutathione peroxidase GSHPx) which prevent the formation of free radicals or repair damage caused by their action.

The aim of this study was to observe the influence of bendiocarb on the production of reactive oxygen species (ROS) in the liver and kidneys of female domestic rabbits and thus prove its toxic effects independent of inhibition of acetylcholine esterase.

MATERIAL AND METHODS

The experiment was carried out on clinically healthy female domestic rabbits (*Oryctolagus cuniculus domesticus*) obtained from an accredited animal farm (Nitra, SR). Experimental animals (6 in each group) were administered bendiocarb (96 % Bendiocarb, Bayer) *per os* at a dose of 5 mg.kg^{-1} b. w. per day. Owing to the adverse side effects of bendiocarb (diarrhoea, dehydratation and alopecia) after 10 days of the experiment, the dose mentioned was administered every 48 h. The animals were sacrificed on days 3, 10, 21 and 30 of the experiment. The study was carried out in agreement with the requirements of institutional ethical authority (Decision 2647/07-221/5).

Samples of the liver and kidneys from the experimental animals were used to prepare 25 % (w/v) homogenates in 5 mmol.l^{-1} TRIS-HCl buffer, pH 7.8. After centrifugation (105 000 g, 1 h, 4 °C) the total proteins were determined in supernatants by the method of Bradford (1). The determination of SOD was based on the spec-

trophotometric measurement of the inhibition rate of cytochrome (c) reduction (550 nm). The activity of CAT was measured as a decrease in H_2O_2 in the reaction mixture at 240 nm. The glutathione peroxidase activity was determined by the kinetic measurements of the consumption of NADPH+H+ for the reduction of glutathione produced at the removal of peroxides (340 nm). The TBARS, products of lipid peroxidation, produces coloured substances with thiobarbituric acid with absorption maximum at 535 nm. The specific activity of enzymes was expressed in $U.mg^{-1}$ protein. The Student t-test was used for statistic evaluation of the results.

RESULTS AND DISCUSSION

The developing free radicals cause changes in the antioxidative system and depending on the state of the organism, can induce oxidative stress.

Superoxide dismutase provides primary antioxidative protection of cells against ROS.

The specific activity of SOD in the kidneys increased by day 3 of bendiocarb administration but by day 10 it decreased significantly (Fig. 1). The extent of the decrease of SOD activity depends on the degree of oxidative stress (5). The inhibition of the SOD activity may result from the increased production of superoxide anion radical (7). The decreased SOD activity in the kidneys caused a decreased production of H_2O_2. Our observations failed to detect significant changes in the activity of CAT and GSHPx-H_2O_2 enzymes participating in its degradation (Tab. 1).

The activity of SOD in the liver showed no significant changes. GSHPx-H_2O_2 activity was inhibited significantly on day 3 of the experiment but in the following period it increased gradually to the control level (Tab. 1).

The changes in the activities of the antioxidative enzymes in the liver and kidneys indicate oxidative attack on the investigated organs. The level of substances reacting with thiobarbituric acid (TBARS) is one of the factors that reflect the extent of oxidative damage to the organs (3). Significantly increased levels of TBARS detected in the kidneys on days 3 and 10 of the experiment indicate oxidative damage to the kidneys (Fig. 2). The antioxidative enzyme system of the kidneys could not compensate sufficiently for the increased production of oxidants. In the liver we recorded a significantly changed level of TBARS on day 21 of the experiment

Table 1. Specific activities of catalase and glutathione peroxidase in organs of domestic rabbits

Specific activity $U.mg^{-1}$	Kidneys					Liver				
	Control	Day 3	Day 10	Day 21	Day 30	Control	Day 3	Day 10	Day 21	Day 30
CAT	**136** ± 56	**180** ± 18	**167** ± 27	**217** ± 15	**185** ± 22	**72** ± 15	**87** ± 19	**86** ± 18	**66** ± 7	**81** ± 14
GSHPx-H_2O_2	**0.13** ± 0.03	**0.17** ± 0.01	**0.14** ± 0.03	**0.18** ± 0.03	**0.17** ± 0.03	**0.12** ± 0.02	**0.070*** ± 0.007	**0.09** ± 0.01	**0.093** ± 0.006	**0.11** ± 0.01

The values are means ± SD (n = 6). Student *t*-test was used for evaluation; * – $P < 0.05$

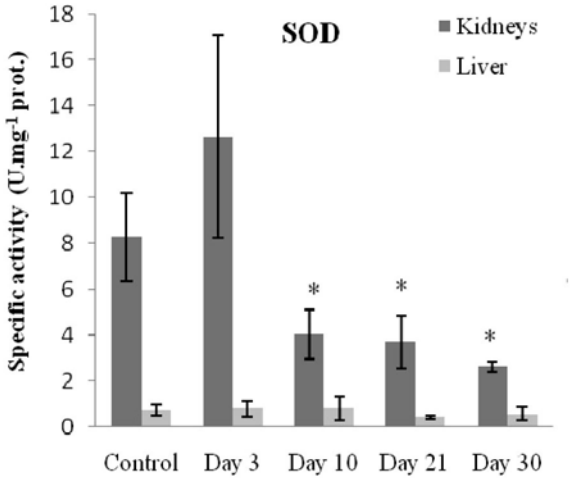

Fig. 1. Specific activity of SOD in homogenates of rabbit kidneys and liver after administration of bendiocarb

The values are means ± SD (n = 6). Student *t*-test was used for evaluation; * – $P < 0.05$

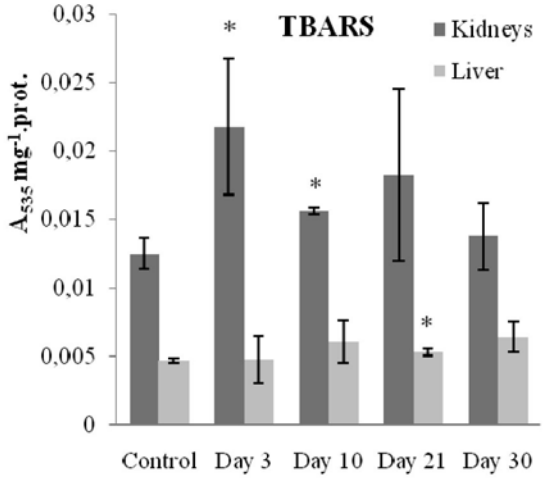

Fig. 2. Content of TBARS in homogenates of rabbit kidneys and liver after administration of bendiocarb

The values are means ± SD (n = 6). Student *t*-test was used for evaluation; * – $P < 0.05$

(Fig. 2). The insufficient antioxidative capacity of liver resulted in a disturbed balance between the oxidants and antioxidants with an increased exposure to bendiocarb.

The results obtained indicated that the animals were exposed to the action of free radicals. The toxicity of bendiocarb is not related only to the inhibition of acetylcholine esterase but also to the production of ROS which can enhance its total toxicity. Free radicals can develop due to the cyclic biotransformation of bendiocarb and its metabolites (8).

ACKNOWLEDGEMENT

The study is part of the project VEGA 2/0021/09 of the Institute of Medical Chemistry of UVMP in Košice in collaboration with ÚFHZ SAV in Košice.

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Selected papers from the 54th STUDENT SCIENTIFIC CONFERENCE with international participation, held at the University of Veterinary Medicine and Pharmacy in Košice on May 4, 2011.

FOLIA VETERINARIA, 55, 2: 15–17, 2011

HYPERTROPHIC CARDIOMYOPATHY IN CATS

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ABSTRACT

Hypertrophic cardiomyopathy (HCM) of cats is an autosomal dominant (AD) disease affecting all breeds of cats. In Slovakia HCM has been diagnosed most frequently in Maine Coon (MCO) and Ragdoll (RAG) cats. This study investigated the prevalence of HCM in 15 MCO cats (9 females, 6 males) from breeding cattery Redi Cats, SR, between 2009 and 2011. After taking detailed anamnesis, each cat was examined clinically, focusing on the cardiovascular system. No clinical signs of HCM were observed in the experimental group. Samples of blood (n = 6) and cheek swabs (n = 11) were obtained from the experimental animals and sent for DNA testing for HCM. The results showed that 8 out of 15 examined cats carried heterozygous mutations (HCM/N) in the MYBPC3 gene. In 7 cats, the DNA test was supplemented with echocardiographic examination (ECHO) which failed to prove HCM. We prepared pedigree analysis of the examined animals. Subsequently, a questionnaire dealing with HCM was prepared and handed out at a cat exhibition held in Bratislava-Ružinov in March 2011. At the exhibition we examined clinically one cat with evident signs of HCM. Previous DNA testing of this cat indicated that the animal carried the HCM/HCM genotype.

Key words: cat; heart; HCM; hypertrophic cardiomyopathy

INTRODUCTION

Hypertrophic cardiomyopathy (HCM) is the most common heart disease in cats. It is a condition characterised by an abnormal inward thickening of the left ventricle, papillary muscles of the left ventricle and interventricular septum (5). Because of this increased thickness, the myocardium becomes too stiff and does not extend sufficiently which leads to incomplete filling of the heart. This also reduces the volume of blood that the heart can pump with each contraction (2). Haemodynamically, HCM involves diastolic dysfunction, i.e. the heart muscle cannot fully relax during diastole (3). Diastolic dysfunction results in increased pressure in the left ventricle and subsequent dilatation of the left atrium. Blood stasis inside the dilated left atrium may lead to development of thrombi and thromboembolic disease (10). A common example is the paralysis of back limbs due to a blood thrombus stuck in the terminal aorta. This condition is commonly referred to as a saddle thromboembolus (4). HCM occurs in two stages: 1. Asymptomatic (compensatory) – an affected cat does not show visible signs. HCM has been detected by preventive echocardiographic examination (ECHO) or the presence of cardiac murmurs and arrhythmias. 2. Symptomatic stage (congestive heart failure) – accompanied by pulmonary oedema or pleural exudates (3), less frequently by ascites (2), while a cough is uncommon (9). An attentive owner will notice that the cat becomes withdrawn, slightly apathic and apeptic. Sometimes he may remember a brief episodes of painfulness and limping, collapse or dyspnoea after play or exposure to stress (3).

With the increasing number of cats with breed predisposition to HCM in Slovakia, the number of patients has also increased. The increasing prevalence may be ascribed to the possible awareness of breeders. A higher detection rate is supported by a better instrumentation level of veterinary outpatient units. Advanced USG machines can detect even subclinical forms of HCM which may have been overlooked in the past. The use of DNA tests, particularly in some cat breeds, can become an important contribution.

The aim of our study was to evaluate the health of cats at the breeding cattery Redi Cats, SR, on the basis of individual exami-

nation and to prepare a pedigree analysis. We also tried to assess the prevalence or detection of HCM in cats in SR by means of a questionnaire which was also intended to inform breeders about the risk of HCM in cats and potential ways of detecting of this disease.

MATERIAL AND METHODS

We analysed the health of 15 cats from the breeding cattery Redi Cats, SR, owned by Ing. Zuzana Drevová. We took the anamnesis of all 15 animals (9 females, 6 males) and examined them clinically, focusing on the cardiovascular system, particularly on HCM screening examinations. DNA tests for HCM were carried out and the results served for pedigree analysis of the tested animals. We proceeded as follows: 7 animals were selected for detailed monitoring between 2009–2011 based on the following criteria: they had to be kept at the station from 1 to 5 years of age; had to be clinically healthy and fed with the same high-quality feed. Besides the clinical examination, they were subjected to ECHO and DNA testing for HCM. The remaining 8 experimental animals were only DNA tested by withdrawing 1 ml of blood from *v. cephalica antebrachii* into a tube containing EDTA and transferring the samples in a thermos bottle for DNA testing for HCM to Genomia laboratory (CR). DNA testing was carried out also on cheek swabs taken from 11 animals by a special sterile swabbing brush (Cytobrush). After swabbing and drying, the swabs were placed back into respective envelopes and sent for DNA testing for HCM to the Animal DNA laboratory (Austria). Blood of two of these animals was examined at Genomia (CR).

Of the 7 animals that were subjected to ECHO one was examined with Siemens Sono Line Adara 3.5–5 MHz convex probe and six with Chison 600 VET, 3.5–6 MHz microconvex probe. The animals were auscultated with a phonendoscope 3M™ Littmann® Electronic Stethoscope 3200.

In conclusion, on the basis of DNA tests and respective calculations, the pedigree analysis of the examined 15 cats was made.

We tried to assess the prevalence of HCM in cats in Slovakia by means of a questionnaire, "Hypertrophic cardiomyopathy in cats", handed out at a cat exhibition held in Bratislava-Ružinov on March 12, 2011. One cat carrying HCM/HCM genotype according to previous DNA testing was examined clinically at the exhibition.

RESULTS AND DISCUSSION

None of the 7 animals observed in detail between 2009 and 2011 showed any signs of HCM during the clinical examinations. One anamnesis revealed that, two years ago, the owner observed in one male cat, weakness and painfulness of back limbs, which culminated by a transient 1-day paralysis of the pelvic limbs. The owner could not say whether the temperature of the limbs was changed. Within one week, the problems disappeared but the animal tired easily or was unwilling to play. It started to be aggressive and became emaciated. However this could be related to its participation in many exhibitions. After neutering, its condition improved. It did not suffer from any disease in the past. The clinical examination detected a slight heart murmur. According to Tilley (10), a heart murmur is present in the majority of HCM affected cats. However, heart murmur is not characteristic of HCM. Up to 30 % of heart murmurs in cats are of unknown origin and some of them never acquire HCM (8). Only Doppler ECHO can accurately identify their origin (1).

The results of the DNA testing for HCM: Blood (Genomia, CR): A31P (HCM/N), and A74T (N/N). The ECHO values of all cats were in the standard range. Total evaluation of DNA tests for HCM: of 15 examined animals, 8 (53.33 %) had genotype HCM/N and 7 (46.67 %) genotype N/N. The incidence of HCM according to gender: in the group of 9 females, 53.33 % had genotype HCM/N and 46.67 % genotype N/N. In the group of 6 males, 50.00 % had genotype HCM/N and 50.00 % genotype N/N. Genotype HCM/HCM was not detected as we were able to test only 15 animals. According to Tilley (11), mutations (A31P, A74T) in the MYBPC3 gene are inherited by the AD mode. The influence of genotype on phenotype manifestation of HCM in MCO has not been clearly explained.

HCM is a disease common in all feline breeds. In some breeds, such as Abyssinians and Siamese cats, the risk of HCM is low (7). It has been estimated that the disease is heritable in at least 60 % of the cases. Hereditary HCM is characterised as a disease with AD mode of inheritance and is genetically and clinically heterogenous (6).

The questionnaires handed out at the cat exhibition in Bratislava-Ružinov in March, 2011, were filled out by 43 breeders. Of the 42 breeders interested in hereditary diseases of cats 39 had heard about feline HCM, 30 were acquainted with the clinical symptoms of HCM and 11 were interested to find out more about the disease. Twelve of 19 breeders of MCO and RAG cats have their cats tested for HCM by means of the DNA test, 5 breeders showed interest in subjecting their cats to DNA testing for HCM and 2 refused DNA testing of their cats. The most frequent way of DNA testing involved taking cheek swabs and sending them to laboratories in the following succession: Animal DNA laboratory (Austria); Genomia (CR); and Laboklin (Germany). The preventive testing was based primarily on DNA tests; less frequently on echocardiography. Five breeders confessed that they had or presently have animals with clinical signs of HCM. At the exhibition we were given an opportunity to examine a cat with clinical signs of HCM and four times confirmed the HCM/HCM genotype.

CONCLUSION

DNA tests showed that 53.33 % of the tested cats from the evaluated breeding cattery were positive for HCM. Testing for HCM is not compulsory for any feline breed in Slovakia. No study has been presented on the incidence of HCM in catteries in Slovakia. The incidence of HCM in MCO and RAG breeds in Slovakia has been estimated to 75 % but the clinical signs are observed only in 25 %. The majority of cat owners do not wish to give publicity to the data on incidence of feline HCM in Slovakia. Obligatory examination of breeding cats for HCM by means of DNA tests could improve the

existing situation. HCM positive dominant homozygotes should be eliminated from breeding. It is also necessary to prevent crossbreeding of the heterozygotes. Gradual elimination of HCM positive animals from breeding programmes could eliminate this feline disease completely from the Slovak catteries.

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Selected papers from the 54th STUDENT SCIENTIFIC CONFERENCE with international participation, held at the University of Veterinary Medicine and Pharmacy in Košice on May 4, 2011.

FOLIA VETERINARIA, 55, 2: 18–19•, 2011

THE INFLUENCE OF HOOF-PASTERN ANGLE ON DEVELOPMENT OF EQUINE ORTHOPAEDIC DISEASES

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ABSTRACT

This study focused on the most frequent diseases of horses related to over angulated (soft) pastern and upright pastern stance. We examined 8 horses with either of these irregularities. The animals were examined clinically and radiographically. Seven horses showed signs of lameness. The most common findings associated with upright pastern was podotrochlosis (4 cases) and subluxation of the pastern joint (1 case). In the horses with over angulated (soft) pastern we recorded the calcification of the superficial digital flexor tendon (1 case) on both hind legs and habitual luxation of the patella in the proximal direction (1 case). No pathological changes were detected in one horse.

Key words: joint; lameness; pastern; angle

INTRODUCTION

Diseases of joints and tendons are the most frequent causes of lameness and permanent disability of sport horses. The predisposition to the development of these diseases is higher in horses with irregular stance. A characteristic feature of horses with upright (angled more toward the vertical) pastern is a broken hoof-pastern axis, i. e. the angle of the hoof wall in relation to the ground is smaller and the angle of the pastern is bigger than in horses with normal angulation of the hoof and pastern (4). Such conformation produces a predisposition to damage to: the distal sesamoid bone; bursa podotrochlearis; deep digital flexor tendon; and the digital extensor tendon. (6). On the other hand, an over angulated (soft) pastern, the angle of the hoof in relation to the ground is bigger and the angle of the pastern is smaller than with the normal alignment (4). This affects mostly the interosseous muscle and superficial and deep digital flexor tendons (6). The aim of our study was to investigate the damage to the locomotor apparatus of the horses with over angulated (soft) and upright pastern.

MATERIAL AND METHODS

In our study we examined 8 horses with over angulated (soft) pastern or upright pastern. The horses were of the following breeds: 4 Slovak warm-blooded, 2 Old Kladruber, 1 English thoroughbred and 1 Appaloosa. The age of horses was as follows: 3 years – 1 horse; 7 years – 1 horse; 8 years – 3 horses; 12 years – 1 horse, 17 years – 1 horse; 25 years 1 – 1 horse. Their use was as follows: 3 hobby horses, 2 event horses, 2 city police horses and 1 Western horse. The animals were examined clinically and radiographically. Seven horses showed signs of lameness.

RESULTS

The upright pastern stance was observed in 5 horses. In 4 of them, X-ray examination revealed changes in the distal sesamoid bone indicative of podotrochlosis and in one (mare) also a chip fracture at the dorso-medial edge of the sesamoid bone. In one horse we diagnosed a subluxation of the pastern joint. All horses from this group showed signs of lameness.

The over angulated (soft) hind pastern stance was observed in 3 horses. In the 25-year old Slovak warm-blooded mare, we diagnosed calcification of the superficial digital flexor tendon located proximal to the pastern joint on one

leg and distal on the other. On one leg we also found calcification of the distal angled sesamoidean ligaments. In a 3-year Appaloosa mare, we observed bilateral luxation of the patella in a proximal direction. Only one horse from this group showed no signs of lameness.

DISCUSSION

Despite frequently presented assertion that the hoof-pastern axis should not be broken, i.e. that the angle of the hoof wall in relation to the ground should be the same as the angle of the pastern, this assertion has been supported by few proofs. However, this idea is supported by the fact that the straight angle ensures optimum connexion of the coffin pastern and distal sesamoid bones and minimizes the impact with the horse movements (5).

Contrary to claims that an over angulated (soft) pastern results in damage to the interosseous muscle (1), Marks (3) cited several authors who stated that long upright pastern in competition horses is associated with a high incidence of the suspensory apparatus problems. In jumping and dressage horses, the upright pastern, in particular, predisposes the horses to diseases of the proximal interphalangeal joint (3). In our study, we found a subluxation of the pastern joint in one horse with the upright pastern. Beeman (1) described, that the short upright pastern increases the pressure forces acting on: the pastern joint; digital joints; and on the hoof.

Our observations revealed podotrochlosis in 4 horses and damage to the superficial digital flexor tendon in 1 horse which is in agreement with the report by Curtis (2) that the broken hoof-pastern axis can result in various orthopaedic disorders, such as: mechanical laminitis; palmar pain syndrome; arthrosis of the coffin and pastern joints; damage to the flexor tendons; and lesions on the dorsal side of the hoof wall.

CONCLUSION

This study focused on 5 patients with upright pastern and 3 with over angulated (soft) pastern. The breed, age and use of horses was very diverse. Seven patients had marked changes in their locomotor apparatus which prevented their full use.

Irregular hoof-pastern angulation is relatively frequent in the horse population and is associated with disorders of the relevant structures. Therefore, one should pay attention to the first signals of problems, such as decreased performance and reduced willingness to work, that may herald potential changes, and ensure early and effective diagnostics and therapy.

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Selected papers from the 54th STUDENT SCIENTIFIC CONFERENCE with international participation, held at the University of Veterinary Medicine and Pharmacy in Košice on May 4, 2011.

FOLIA VETERINARIA, 55, 2: 20–22, 2011

TEMPORAL SUCCESSION OF THE OESTROUS CYCLE AND OVULATION AFTER ADMINISTRATION TO MARES OF $PGF_{2\alpha}$ IN PREPARATION OESTROPHAN

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ABSTRACT

This study investigated the occurrence of oestrus and ovulation in horses after the administration of Oestrophan, a $F_{2\alpha}$ prostaglandin preparation containing the active ingredient of cloprostenol. We examined 18 mares of three breeds; Slovak warm-blooded Horse, American Quarter Horse and Hutsul. They were dividend according to housing conditions and stress factors present at insemination. Oestrus detection was based on the external and internal symptoms and a gynaecological examination. Ovulation was detected by repeated ultrasonographic rectal examinations of the ovaries in 6 h intervals. In five mares, the ovulation appeared 39–87 h after administration of Oestrophan, in twelve 105–177 h and in three 207–231 h after administration of the preparation. When expressed in days, oestrus appeared within 4 days after the administration of Oestrophan and the subsequent onset of ovulation was detected within a maximum of 5 days after observation of oestrus signs. In mares that were in oestrus at our first examination, we detected the onset of ovulation within 5 days.

Key words: mare; oestrus; ovulation; prostaglandin

INTRODUCTION

Mares are seasonally polyestrous breeders; meaning that they come into heat every 21–22 days, mostly from March to November, depending upon the climate, i.e. undergo 15 to 17 cycles during this portion of the year. The onset, duration and termination of the mating season is affected by the external and internal environments and is highly individual with respect to breed and individual horses (1, 4). The usual length of oestrus is 4–7 days but this can change in the course of a year (2). The factors affecting the time from the onset of oestrus up to ovulation is, the diameter of pre-ovulation follicle during luteolysis; the bigger the follicle, the shorter the oestrus (6, 7). Ovulation is affected by many factors: hormonal control; daylight period; keeping mare in the stable or on pasture; and last but not least, nutrition and stress. The uterine $PGF_{2\alpha}$ controls viability of the Corpus Luteum (CL) in the cycling mare (3, 8). Prostaglandin, the pulsating secretion $PGF_{2\alpha}$ of the uterine lining, appears on day 14 post-ovulation if the mare is not pregnant and causes lysis of the CL. This results in an almost immediate decrease in the circulating progesterone and the mare begins to show signs of oestrus (5).

MATERIAL AND METHODS

Our research project was carried out on 18 mares of three breeds, namely 8 American Quarter Horse mares, 2 Hutsul mares and 8 Slovak warm-blooded Horse mares. The mares were in active breeding age, ranging between 5 to 18 years old and their body weight was between 280–560 kg. The way of keeping the mares was not the same. We used the $F_{2\alpha}$ prostaglandin preparation Oestrophan inj. a. u. v. (Bioveta a.s., SR) to produce luteolysis and induce oestrus. The active ingredient of the preparation is Cloprostenolum (250 μg of sodium salt in 1 ml). One ml of the preparation was administered twice, with intervals between administrations of 12 h, namely at 18 : 00 and 06 : 00 hours. It was administered within days 6–15 of the luteal phase of the cycle. In Hutsul mares, with regard to their body size, we used 1 ml dose and repeated the treatment after 5 days. At the onset of oestrus, we considered the time when the

mare exhibited signs of heat and was willing to accept the stallion's advances. Ovulation was detected by repeated ultrasonographic rectal examination of ovaries in 6 h intervals using an instrument ALOKA SSD–500 (Tokyo MURE HITAKA – SH Co., Ltd. Japan) equipped with the probe UST-588-U, 5,0 MHz.

RESULTS AND DISCUSSION

For use of the preparation Dinolytic inj. a.u.v., the manufacturer reports that ovulation should occur between days 8 and 10 when administered during diestrus. For Oestrophan, the manufacturer reports the onset of ovulation on days 4 to 6 from the administration during diestrus. Our results are presented in Table 1, showing the results in hours. Because the mares were examined every 6 hours our error can be only in this range which is in fact related to the time of ovulation in mares in different time intervals during the day.

When Oestrophan was administered in the luteal phase of the oestrous cycle, the mares came to oestrus within 3 to 4 days and ovulation occurred within 6 to 9 days following administration of the preparation. In mares in heat, the onset of

Table 1. Occurrence of oestrus and ovulation after administration of $PGF_{2\alpha}$ (year 2010)

Mare	Administration of Oestrophan		Ovulation			
	Date	Time	Date	Range (hrs)	Onset (hrs)	Days
Slovak warm-blooded horse						
Lady	May 14 and 15	18:00 and 6:00	May 18	78–84	81	3.37
Salima	Sept 9 and 10	18:00 and 6:00	Sept 14	120–126	123	5.13
Sidória1	April 6 and 7	18:00 and 6:00	April 13	156–162	159	6.62
Sidória2	May 21 and 22	18:00 and 6:00	May 24	66–72	69	2.88
Candila	May 20 and 21	18:00 and 6:00	May 29	228–234	231	9.62
Sunny	Sept 10 and 11	18:00 and 6:00	Sept 17	174–180	177	7.37
Lolita	July 16 and 17	18:00 and 6:00	July 20	84–90	87	3.62
La Jolie	Aug 20 and 21	18:00 and 6:00	Aug 26	150–156	153	6.37
Lady1	April 9 and 10	18:00 and 6:00	April 15	120–126	123	5.13
Lady2	April 27	6:00 and 18:00	April 29	42–48	45	1.87
Hutsul						
Divá	June 28, July 4	18:00	July 7	204–210	207	8.6
Taja	June 30, July 4	18:00	July 9	204–210	207	8.6
American Quarter Horse						
Tasalena	April 29 and 30	18:00 and 6:00	May 1	36–42	39	1.62
Joany	May 26 and 27	18:00 and 6:00	June 2	154–162	157	6.54
Royal	May 26 and 27	18:00 and 6:00	June 1	132–138	135	5.6
Melody	May 29 and 30	18:00 and 6:00	June 3	132–138	135	5.6
Running	June 7 and 8	18:00 and 6:00	June 12	102–108	105	4.3
Kety	June 7 and 8	18:00 and 6:00	June 12	102–108	105	4.3
Holly	June 7 and 8	18:00 and 6:00	June 12	102–108	105	4.3
Cadilac	June 7 and 8	18:00 and 6:00	June 14	156–62	157	6.54

ovulation was recorded on days 2 to 5 post-administration of Oestrophan. When reporting this time in hours, the onset of ovulation was recorded in five mares within 39 to 87 hrs, in twelve mares within 105 to 177 hrs and in three mares within 207 to 231 hrs following the administration of Oestrophan.

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Selected papers from the 54th STUDENT SCIENTIFIC CONFERENCE with international participation, held at the University of Veterinary Medicine and Pharmacy in Košice on May 4, 2011.

FOLIA VETERINARIA, 55, 2: 23–24, 2011

ONSET OF THE FIRST POST-PARTUM OESTRUS IN HUTSUL MARES KEPT AT THE NATIONAL STUD FARM IN TOPOĽČIANKY

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ABSTRACT

The onset of the first post-partum oestrus occurs in mares on days 5 to 20 post-partum. It is affected by the long-lasting suppression of the function of the hypothalamic-hypophyseal-ovarian axis during gravidity. This first post-partum oestrus is advantageous due to its reliability, early onset, good conception rate and regularity. Its disadvantages are the high incidence of embryonic mortality in mares suckling their foals and the subsequent anoestrus. An opinion prevails that this first post-partum oestrus should not be used or used only to a limited extent.

The onset of the first post-partum oestrus was affected by the date of parturition and the day from parturition when this first oestrus began. Observation of the herd of 21 Hutsul mares kept at National Stud Farm (NSF) in Topoľčianky revealed the following information about 17 gravid mares:

- **Onset of the first post-partum oestrus was recorded no earlier than on day 8 and no later than on day12 post-partum,**
- **The length of the first post-partum oestrus was a minimum of 3 days with two mating attempts and no longer than 11 days, with the number of mating attempts equal to 6,**
- **The successfulness of mating attempts during this first post-partum oestrus was low and reached only 35. 3%.**

Key words: mare; post-partum oestrus

INTRODUCTION

In almost 90 % of mares, the first post-partum oestrus starts in a relatively short time after parturition; usually on days 5 to 20 (1, 3, 4). This is caused by the previous long-term suppression of the function of the hypothalamic-hypophyseal-ovarian axis during pregnancy with the eventual release of gonadotrophic hormones (2). This first post-partum oestrus was recommended for mating because of its reliable and early onset, good conception rate and regularity. The disadvantages include high incidence of embryonic mortality in mares suckling their foals (losses up to 30 %) and the subsequent anoestrus. Today an opinion prevails that this first post-partum oestrus should not be used or used only to a limited extent (5).

If no post-partum or other complications occur, with respect to ovarian activity the following four situations may take place:

I – the mare enters the first post-partum oestrus and continues with normal cyclical ovarian activity,

II – the mare enters the oestrus and a) is mated and becomes pregnant; b) is not mated, Corpus Luteum (CL) does not undergo regression and a state of prolonged luteal phase (pseudogravidity) is induced,

III – the mare is in heat, ovulation occurs, CL is subject to regression, but no ovarian activity (anoestrus caused by acyclicity),

IV – the mare does not enter post-partum oestrus, absence of ovarian activity, and thus develops acyclicity.

The aim of our study was to observe the onset and characteristics of the first post-partum oestrus in mares kept at NSF in Topoľčianky.

MATERIAL AND METHODS

We observed a closed herd of 21 pure bred Hutsul mares between 2008 and 2009 kept at NSF Topoľčianky (farm Hostianske). Their ages ranged was 5 to 16 years of age and they appeared to be

clinically healthy and in good condition. They were supplied hay supplemented with concentrates from a common manger and were housed all year long in a free stable situation with unlimited access to pasture and a consistent appropriate light regimen. Of these 21 mares, 17 were pregnant from previous mating season and 4 were nongravid.

Ultrasonographic (USG) rectal examinations were carried out with an instrument ALOKA SSD-500, Tokyo MURE HITAKA-SH Co., Ltd. Japan, with a probe UST-588-U, 5 MHz. The rectal USG examinations of mares at NSF Topoľčianky were performed in order to prove ovulation and gravidity or were carried out twice a month to identify problem mares. More frequent regular examinations were prevented by the considerable distance of the farm Hostianske and the veterinary surgeon's schedule.

RESULTS AND DISCUSSION

The onset of the first post-partum oestrus in 17 gravid mares at NSF Topoľčianky was affected by the date of parturition and the day from parturition when this first oestrus began. The first post-partum oestrus was recommended for mating due to its reliable onset, regularity and good conception rate, although it resulted in high embryonic mortality in suckling mares and risk of subsequent anoestrus. Its characteristics are summarised in Table 1.

Table 1. The first post-partum oestrus in 17 mares at the NSF Topoľčianky in 2009

Onset of first post-partum oestrus			Oestrus length (days)/ number of mating attempts			Successfulness (%)
Earliest (day)	Latest (day)	Mean ± SD (day)	Shortest	Longest	Mean	
8	12	10.12 ± 4.3	3/2	11/6	6.2 ± 2.2 days 3.6 ± 1.3 attempts	35.3

The onset of the first post-partum oestrus ranged between days 8 and 12 post-partum (mean 10.12 ± 4.3 days). The length of the oestrus varied between 3 and 11 days with 2 to 6 mating attempts observed respectively (mean oestrus length was 6.2 ± 2.2 days; mean number of mating attempts 3.6 ± 1.3. Successfulness of mating reached only 35. 3 %).

The onset of the first post-partum oestrus in mares at the NSF Topoľčianky was affected by the parturition date. Low successfulness of this oestrus could be explained by the risk of embryonic mortality. The fact that 2 to 6 mating attempts per oestrus were needed may also play some role as it is recommended to minimise the number of attempts per oestrus. The optimum is one mating attempt per oestrus.

CONCLUSION

The first oestrus occurred on days 8 to 12 post-partum (mean 10.12 ± 4.3 days), lasted for 3 to 11 days and the number of mating attempts was 2 to 6 (mean 6.2 ± 2.2 days with 3.6 ± 1.3 mating attempts, successfulness 35.3 %). It is recommended to decrease the number of mating attempts per oestrus to the optimum number of 1 attempt at a suitable time to decrease the incidence of embryonic mortality.

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Selected papers from the 54th STUDENT SCIENTIFIC CONFERENCE with international participation, held at the University of Veterinary Medicine and Pharmacy in Košice on May 4, 2011.

FOLIA VETERINARIA, 55, 2: 25–26, 2011

PREVALENCE OF NEOSPOROSIS IN DOGS IN EASTERN SLOVAKIA

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ABSTRACT

We investigated the occurrence of neosporosis in dogs, caused by the protozoan *Neospora caninum*, in selected districts of the Košice region of Eastern Slovakia. The small number of dogs examined prevented us from in depth statistical evaluations, so the results of the seroprevalence are only basically orientational. The mean seroprevalence of *N. caninum* antibodies in Eastern Slovakia, determined by cELISA, reached 25.46%. The seroprevalence in rural dogs reached 56%, while none of the examined urban dogs were positive. Seropositive dogs were analysed on the basis of age, gender, breed, clinical signs, alimentary habits, lifestyle, place of keeping and use. This was the first survey that confirmed the occurrence of neosporosis in dogs in Slovakia.

Key words: dogs; Eastern Slovakia; *Neospora caninum;* neosporosis

INTRODUCTION

The causative agent of neosporosis is *Neospora caninum* (4), a multihost, worldwide distributed protozoan of the phylum *Apicomplexa* (3). *N. caninum* has been detected in Slovakia in free living animals (10) and farm animals (8, 11). Several species of domestic and free living terrestrial (4) and marine mammals (2) and birds can serve as the intermediate hosts (5). Dogs (*Canis familiaris*) are both the intermediate and final hosts of this parasite. A characteristic feature of the parasite is its heteroxenous developmental cycle consisting of three infectious stages, i. e., tachyzoits, bradyzoits and oocysts. Neosporosis is transmitted horizontally and vertically (4) and can be either localised or generalised (6). The clinical signs appear mostly in young, congenitally infected dogs as ascendant paralysis of the pelvic limbs (3). In adult dogs, the infection is frequently latent or the clinical signs are not pronounced (9). Anti – *N. caninum* antibodies have been detected also in humans (7), but the presence of the parasite in human tissues has not been demonstrated. The zoonotic character and potential of this disease remains unclear (4).

The aim of our study was to determine the occurrence and seroprevalence of *N. caninum* antibodies in dogs in Eastern Slovakia and to identify individual groups of dogs at risk.

MATERIAL AND METHODS

Serological examination was carried out on 55 dogs from the Košice region by the indirect enzyme immunoanalysis (cELISA) using a commercial kit (*Neospora caninum* antibody test kit, cELISA (VMRD, Inc.)). We prepared a questionnaire for dog owners and used this questionnaire to divide dogs into several groups. The results of seroprevalence in these groups served as a basis for the identification of risk factors related to the development of the disease.

RESULTS AND DISCUSSION

The seroprevalence (P) of *N. caninum* antibodies in Eastern Slovakia reached 25.46 % (Table 1). In rural dogs, the seroprevalence was as high as 56 % (Table 2). Similar results in rural dogs were recorded in Argentina (P = 54.2 %) (1). The majority of seropositive rural dogs originated from farms on

which *N. caninum* was confirmed also in the farm animals (8, 11). This indicates that this parasite circulates between the diverse animals on the farm. A high risk group are dogs kept on farms or out of towns (93 % seropositive) which consume thermally untreated meat or viscera (P = 44.83 %) as has been reported by some authors (1, 3). With regard to gender-related prevalence (P = 32 %), bitches are at a higher risk (57 % seropositive). The highest seroprevalence (P = 50 %), was recorded in dogs in the age groups 3–5 and 5–7 years of age. Neosporosis was more frequently reported in dogs older than one year (3). As far as the use of dogs is concerned, the highest prevalence (71.4 % seropositive) was detected in watchdogs (Table 2). The prevalence of neosporosis was higher (P = 15.39 %) in dogs that were allowed to move freely in nature once a week (14 % seropositive) and the highest (P = 52.17 %) in dogs with unlimited access to free nature (86 % seropositive). The dogs that were not allowed to go to free nature, or only sporadically, showed negative results. The majority of seropositive dogs (79 %) appeared healthy at the time of their clinical examination (P = 21.15 %), just as reported by other authors (9, 3). Health changes were observed only in three seropositive dogs. The breed-related seroprevalence was higher in mongrels (P = 45 %) compared to pedigreed dogs (P = 14.29 %).

N. caninum, therefore causes problems in dogs in Slovakia. Dogs kept on farms pose a risk to other farm animals and permits the circulation of the parasite. It is therefore necessary to deal with this problem with regard to both the risk to animal health and potentially, the risk of *N. caninum* to the human population.

Table 1. Prevalence of neosporosis in districts of the Košice region

District	Number of examined dogs	Number of seropositive dogs	Prevalence (P) (%)
KE	30	0	0.00
KS	8	8	100
TV	5	4	80.00
RV	12	2	16.67
Total	**55**	**14**	**25.46**

KE – Košice; KS – Košice-surroundings; TV – Trebišov; RV – Rožňava

Table 2. Prevalence of neosporosis according to origin and use of dogs

Dogs	Number examined	Number seropositive	Prevalence (P) (%)
Urban	30	0	**0.00**
Rural	25	14	**56.00**
Companion	25	2	**8.00**
Watchdogs	23	10	**43.48**
Hunting	7	2	**28.57**

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Selected papers from the 54th STUDENT SCIENTIFIC CONFERENCE with international participation, held at the University of Veterinary Medicine and Pharmacy in Košice on May 4, 2011.

FOLIA VETERINARIA, 55, 2: 27–29, 2011

PREVALENCE OF TRICHINELLOSIS IN DOMESTIC AND FREE-LIVING ANIMALS

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ABSTRACT

Trichinellosis is a serious zoonotic helminth disease of humans, free-living carnivores, omnivores and farm animals. The transfer of the disease is associated with the food chain. Its characteristic features are the existence of natural foci and cosmopolitan occurrence, with the exception of Antarctica. The epizootology of the agent includes two cycles of transfer, i. e. sylvatic and domestic. The dominant species in the sylvatic cycle is *Trichinella britovi*, while *T. spiralis* predominates in the domestic cycle. In Europe, in addition to these species, *T. nativa* was found in northern territories and *T. pseudospiralis* has been transferred by birds.

The monitoring of the incidence of trichinellosis between 2007 and 2010 was carried out in collaboration with the State Veterinary Institute (SVI) in Zvolen. This project involved the examination of 216,270 animals: 51,370 wild boars, 164,713 pigs, 41 bears, 144 foxes and 2 badgers, from different regions of Slovakia. The presence of larvae in muscle tissues was determined by the artificial digestion of group samples in accordance with EU legislation (1). The species identification was carried out by multiplex PCR using specific primers from the zone of expansion segment V (ESV). Of the examined animals, positivity was detected in 13 wild boars (0.02%) and 7 foxes (4.86%) from different Slovak districts. Molecular analysis confirmed the presence of *Trichinella britovi* in all positive samples.

Key words: epizootology; monitoring; prevalence; *Trichinella* spp.

INTRODUCTION

Diseases transmissible from animals to humans, referred to as zoonoses, are currently in the limelight for both physicians and veterinarians. Trichinellosis is one of the more important parasitic infections because its transfer is related to the food chain. The host becomes infected by consumption of meat infested with the larvae. The parasite exhibits low host specificity and can infect practically all mammals and even birds and reptiles. Its natural reservoirs are carnivores and omnivores.

The nematode, *Trichinella spiralis,* was first reported in 1835. An important keystone in the systematics was the finding of non-capsule forming species, capable of infecting also birds; *T. pseudospiralis,* in 1972 and subsequently there was recognized two separate capsule forming species, *T. nelsoni* and *T. nativa*. The development of evolutionary and systematic biology and the introduction of molecular methods for the identification of species enabled the ability to distinguish the 8 known species of today; *T. spiralis, T. nativa*, *T. britovi, T. pseudospiralis, T. murrelli, T. nelsoni, T. papuae* and *T. zimbabwensis,* and three genotypes, *Trichinella* T6, T8 and T9.

Epizootological and epidemiological complexes associated with trichinella infection involves two cycles of transfer, i. e., sylvatic and domestic. The sylvatic cycle has been maintained in the long term predominantly by cannibalism and carrion feeding. The principal reservoir in Europe is the red fox (*Vulpes vulpes*) and wild boar (*Sus scrofa*) (8). Other carnivores and omnivores play only a secondary role in the circulation of trichinellosis in natural foci, due to their

low population density. In Europe, four *trichinella species* have been identified: *T. spiralis, T. britovi*, *T. nativa* and *T. pseudospirali.* The dominant species in the sylvatic cycle is *T. Britovi*, while *T. spiralis* predominates mostly in the domestic cycle or in sylvatic cycle in the areas where these two cycles overlap. In Finland, and some parts of Sweden, *T. nativa* has been frequently detected and the species *T. pseudospiralis*, transmissible also by birds, has been increasingly detected in various European countries (8).

In Slovakia, trichinellosis circulates in the sylvatic cycle and outbreaks of various intensity occur sporadically. From 1962 until 2009, 12 trichinellosis outbreaks were recorded, most of them in Eastern Slovakia, in the districts of; Rožňava, Prešov, Michalovce, Vranov nad Topľou, Bardejov and Spišská Nová Ves (2). In the case of the largest outbreak in 1998 in the village of Valaská, the disease was confirmed serologically in 336 patients who were infected by consumption of sausages containing meat from dogs that were fed meat from infected wild boars. The causative agent was *T. britovi*. In another large outbreak, affecting 11 people, was recorded in 2001 in the district of Komárno which had been, up to that time, free of this disease. The source of infection was infected meat of a domestic pig which was most likely fed with wastes from a wild boar. The causative agent was *T. spiralis,* the species highly pathogenic to humans (2). In 2008 there was a trichinellosis outbreak in the village of Dlhá Ves, district Rožňava, affecting 13 people who consumed meat and meat products from a pig which subsequently tested positive for *Trichinella*. The agent was *T. britovi*. The district of Rožňava has been considered an endemic location for some time with a high proportion of infected foxes and repeated positive findings in wild boars (3).

MATERIAL AND METHODS

The monitoring of trichinellosis was carried out between 2007 and 2010 at the State Veterinary Institute (SVI) in Zvolen. Examined were; 51,370 wild boars, 164,713 pigs, 41 bears, 144 foxes and 21 badgers from various regions of Slovakia. Muscle samples weighing 5 g were taken from predilection sites (diaphragm muscles in wild boars, foreleg muscles in carnivores) and examined by the method of artificial digestion in accordance with Commission Regulation (EC) No. 2075/2005 (1).

Identification of *Trichinella species* was carried out by multiplex PCR. Extraction and PCR amplification of DNA was conducted according to Pozio *et al.* (7) using species specific primers from the zone of expansion segment V (ESV). The obtained PCR products were separated by electrophoresis on agarose gel and visualised under UV light.

Table 1. Number and species of examined animals and animals positive for *Trichinella* between 2007 and 2010

Year	Animal species	Examined animals	Positive animals	District of origin of positive animals*	Prevalence	*Trichinella species*
2007	Wild boar	10,260	4	BS, IL, PD, RV	0.04 %	*T. britovi*
	Pig	35,799	0			
	Bear	12	0			
2008	Wild boar	10,846	2	RV, MT	0.02 %	*T. britovi*
	Pig	54,275	0			
	Bear	6	0			
2009	Wild boar	10,081	3	LV, BB, ZV	0.03 %	*T. britovi*
	Pig	36,879	0			
	Bear	11	0			
	Fox	78	3	PU, LC, PT	3.84 %	*T. britovi*
2010	Wild boar	20,183	4	RV, ZV, PP, KK	0.02 %	*T. britovi*
	Pig	37,760	0			
	Bear	12	0			
	Fox	66	4	RA, BR, VK, RV	6.06 %	*T. britovi*

* BB – Banská Bystrica; BS – Banská Štiavnica; BR – Brezno; IL – Ilava; KK – Kežmarok; LC – Lučenec; LV – Levice; MT – Martin; PD - Prievidza; PP – Poprad; PT – Poltár; PU – Púchov; RA – Revúca; RV – Rožňava; VK – Veľký Krtíš; ZV – Zvolen

RESULTS

The monitoring conducted between 2007 and 2010 included 216,270 animals of which 20 were found positive (13 wild boars, 0.025 % and 7 foxes, 4.861 %). The origins of the positive animals are shown in Table 1. The prevalence of trichinellosis among wild boars differed in individual years (0.04 % in 2007, 0.018 % in 2008, 0.03 % in 2009 and 0.02 in 2010). Foxes were monitored for the first time in 2009 when the prevalence was 3.84 % and in 2010 it increased to 6.06 %. Species identification showed that all positive animals were infected with *T. britovi*.

DISCUSSION

In Slovakia trichinellosis has circulated for about 50 years exclusively within the sylvatic cycle. In nature, the parasite is maintained mostly by means of infected red foxes in the population of which the prevalence persists at the level of 13 % (5). The SVI in Zvolen started with the examination of foxes for trichinellosis in 2009 and focused mostly on the southern districts of Central Slovakia. The prevalence among foxes determined by SVI reached 3.84 % in 2009 and 6.06 % in 2010 which is lower compared to the observation of the previous authors. Positive foxes were found in all examined districts which indicates that trichinellosis occurs in foxes throughout Slovakia.

The monitoring of wild boars throughout Slovakia showed that the prevalence of this disease was low for a long time (0.06 %). Between 2000 and 2004, all positive animals were hunted down in the long-term endemic regions of Central and Eastern Slovakia. Since 2005, Hurníková *et al.* (6) has repeatedly recorded infected animals also outside these regions. The role of wild boars in spreading the parasite was confirmed also by our results, because in 2007 we recorded infected boars also in the districts of Prievidza and Ilava of the Nitra region, where positivity in boars was detected for the first time in 2006. Additionally, two infected wild boars were hunted down in the districts of Banská Štiavnica and Rožňava. In 2008, the parasite was detected in one wild boar from the district of Martin and in one from the endemic region in the district of Rožňava. In the following year, besides two positive animals from Central Slovakia (districts Banská Bystrica and Zvolen), one positive wild boar was found in the district of Levice where trichinellosis in wild boars was confirmed as late as in 2006. In 2010, all positive animals originated from the endemic regions (districts of Rožňava, Zvolen, Poprad and Kežmarok).

Trichinella spiralis and *T. britovi* are the two most frequent species which circulate in agricultural, wooded and semi-natural European countries (8). Our results showed exclusive presence of the species *T. britovi* in all infected animals in Slovakia which is in agreement with the long-term observations of Hurníková and Dubinský (4) who stated a high predominance of this species in Slovakia (98.9 %). *T. spiralis* and *T. pseudospiralis* occurred in Slovakia only sporadically, mostly in the form of mixed infection with *T. britovi*. The species *T. spiralis* was the causative agent of an outbreak in 2001 in Komárno and was detected in several foxes in Eastern Slovakia. The species *T. pseudospiralis* was identified in swine and synanthropic mammals in Eastern Slovakia already in 2003 and in the following years was repeatedly detected in foxes and wild boars in Eastern Slovakia (4).

Regular monitoring of domestic pigs, wild boars, horses, foxes and other indicator animals, as stipulated by the Directive No. 2003/99/ES of the European Parliament and the EU Council on monitoring of zoonoses and their causative agents, of November 17, 2003, is an important tool for the evaluation of changes in the prevalence of zoonoses and prevention of infections.

ACKNOWLEDGEMENT

The study was supported by the project "INFEKTZOON – centre of excellence for animal infections and zoonoses" and the operation programme Research and development financed by the European fund for regional development.

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Selected papers from the 54th STUDENT SCIENTIFIC CONFERENCE with international participation, held at the University of Veterinary Medicine and Pharmacy in Košice on May 4, 2011.

FOLIA VETERINARIA, 55, 2: 30–32, 2011

OCCURRENCE OF ENDO- AND ECTOPARASITES IN LLAMAS AND ALPACAS IN THE CZECH REPUBLIC

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ABSTRACT

In recent years, the number of breeders of llamas and alpacas have been increasing in the Czech Republic as well as in neighbouring countries. For this reason, the Institute of Pathological Morphology and Parasitology, VFU Brno, made an effort to investigate parasitosis in these animals and recommend suitable prevention and therapy. The present study investigated the occurrence of endo- and ectoparasites in animals of the genus Lama, particularly in the Czech Republic. The prevalence of endoparasites, based upon the examination of the faeces of 101 animals by the flotation method, was as follows: representatives of the family Trichostrongylidae (with the exception of the genus *Nematodirus*) 38%; *Nematodirus* spp. 16.7%; *Trichuris* spp. 21.3%; *Capillaria* spp. 16.7%; *Eimeria* spp. 63% (of that *E. macusaniensis* 1.9%); *Giardia intestinalis* 5.6%; and *Monieza* spp. 1.9%. The occurrence of ectoparasites was evaluated during the clinical examinations of animals which was supplemented, in some cases, by the microscopic examination of the skin scrapings. This allowed us to diagnose chorioptic scabies in two alpacas.

Key words: chorioptes; eimeria; Lama; trichuris

INTRODUCTION

The rearing of llamas and alpacas has spread throughout the world and in recent years the number of animals of the genus Lama have increased in the Czech Republic as well as in the neighbouring countries. Parasitosis significantly affects the health of these animals (1, 8).

The ectoparasites of the families Sarcoptidae and Psoroptidae have infected llamas (1, 3, 8, 10). Of the endoparasites, these animals are most frequently affected by gastrointestinal parasites: nematodes of the genera *Nematodirus, Trichostrongylus, Cooperia* and others in the small intestine and stomach, *Trichuris* spp. in the large intestine (1, 2, 8). *Lamanema chavezi,* the most pathogenic helminth of llamas and alpacas, has been reported in South America and New Zealand (4). Coccidiosis of the young was caused by invasion of representatives of the genus *Eimeria* spp. Six species were described in animals of the Lama genus: *E. alpacae, E. lamae, E. punoensis, E. peruviana, E. invitaensis* and *E. macusaniensis* (1, 5, 8,).

The majority of parasites of llamas and alpacas also parasitize other ruminants with which they share pastures (1, 8). Thus the spectrum of Lama parasites in Europe can differ from parasites of llamas and alpacas in the Americas or New Zealand. It is desirable therefore, to map the prevalence of parasites of llamas and alpacas kept in our territory.

MATERIAL AND METHODS

Between April 2010 and March 2011 samples of faeces were collected from 101 animals, 49 llamas and 52 alpacas, from herds in the Czech Republic and one herd in Germany. Repeated samples were taken from 7 animals. Altogether 108 samples of faeces were examined by the flotation method using a solution of specific weight 1.3 (7). The findings were evaluated semi-quantitatively (Table 1)

If the findings were ++ or higher we determined the number of oocysts or eggs per 1 g of faeces (OPG, EPG) by the FLOTAC method (6).

Table 1. Number of various parasite forms in one microscopic viewing field used for semiquantitative determination of invasion intensity

	Number of oocysts, cysts or eggs in the viewing field	
	***Eimeria* spp., *Giardia intestinalis* Magn. 200 ×**	**Family Trichostrongylidae, *Trichuris* spp., *Capillaria* spp., *Moniezia* spp.; Magn. 100 ×**
Negative	0	0
Sporadic	1–2	1–2
Mild invasion +	6–10	3–5
Medium invasion ++	11–15	6–9
High invasion ++	16–20	10–12
Very high invasion ++++	> 21	> 13

Examinations for ectoparasites were carried out when indicated. In case of skin changes (e. g. crusts), we took skin scrapings and examined them microscopically.

RESULTS AND DISCUSSION

Of 108 examined samples of faeces, 87 were positive (80.6 %). Trichostrongylidae (except for *Nematodirus*) eggs were detected in 41 samples (38 %), the genus *Nematodirus* in 18 samples (16.7 %), *Trichuris* in 23 (21.2 %) and *Capillaria* in 18 samples (16.7 %). The tapeworm *Moniezia* spp. was found in 2 alpacas. *Eimeria* spp. oocysts were found in 68 samples (63 %), two of them containing oocysts of *E. macusaniensis*. *Giardia intestinalis* cysts were present in 6 animals (5.6 %) (Fig. 1).

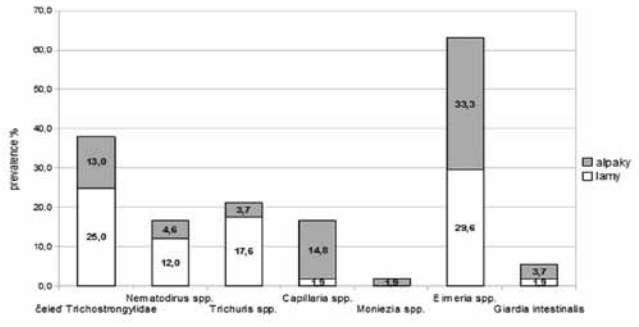

Fig. 1. Prevalence of individual parasites in llamas (white) and alpacas (shaded)

Two samples with Trichostrongylidae eggs (304 EPG and 143 EPG) and three with *Eimeria* spp. oocysts (402 OPG, 560 OPG) were evaluated as ++ and +++, respectively (a small amount of a third sample prevented us from determining the OPG). One llama had *Trichuris* spp. (+++) in its faeces, i. e. the diagnosis was clinical trichuriosis which was manifested by a worsened nutritional status. *Chorioptes* spp. were found in skin scrapings taken from the affected changed skin areas (ventral abdomen, interdigital spaces) of two alpacas.

The most widespread types of scabies in llamas and alpacas have been reported as chorioptic and sarcoptic (1, 8). The type caused by *Chorioptes bovis,* diagnosed in two alpacas, may be more problematic diagnostically. Contrary to the disease caused by Sarcoptes sp., it is usually not manifested by pruritus and thus skin changes may escape the attention for some time (1, 3).

Of the endoparasites, the highest prevalence was observed with *Eimeria* spp., which corresponded to the studies conducted in America and Europe (5, 9). Oocysts of *E. macusaniensis*, described as the most pathogenic, were present in 2 samples. *Trichuris* spp. invasion, which induces haemorrhagic enterocolitis, is associated with total apathy, diarrhoea and weight loss which was observed in the llama with clinical trichuriosis (8). In positive samples, we found a low total number of parasites (OPG and EPG).

CONCLUSION

We determined the prevalence of some endoparasites in llamas and alpacas. Of 108 samples of the faeces examined, 80.6 % were positive. We detected mostly low intensity infestations, not associated with altered health. Clinically, trichuriosis was diagnosed in one llama and chorioptic scabies in two alpacas which were repeatedly treated. Because parasitic invasions may affect significantly the health of animals, it is important to know the prevalence of individual parasites in llamas and alpacas kept in our territory, and to assess the intensity of invasion and consider the targeted therapy needed.

ACKNOWLEDGEMENT

We wish to express our thanks to Prof. MVDr. Vlasta Svobodová, CSc., for supervision of this specialised study. Our thanks belong also to MVDr. Taťána Husáková and MVDr. Eva Vernerová.

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FOLIA VETERINARIA, 55, 2: 33–36, 2011

EVALUATION OF BASIC CHEMICAL AND MICROBIOLOGICAL PARAMETERS IN LAKE SEDIMENTS IN THE PROTECTED AREA OF TAJBA

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ABSTRACT

***Emys orbicularis* (Linnaeus, 1758), is a semi-aquatic, fresh-water turtle. Tajba National Nature Reserve (NNR) was declared by the Slovak Parliament in 1966 as an *E. orbicularis* sanctuary. This study is the first one to perform habitat quality evaluation in Tajba. We collected throughout 2009 in monthly intervals, sediment samples which were analysed for biochemical and microbiological parameters. In addition, we collected surface water samples which were analysed to determine the heavy metal content of five selected elements. Our aims were to provide knowledge regarding the quality of the Tajba oxbow as a *E. orbicularis* habitat by establishing acceptable ranges for each environmental parameter. Also, we wanted to extend the knowledge regarding each analysed parameter's interaction and interface with the other parameters as a part of a holistic complex which is influenced by the surroundings and natural processes. Our results showed that during 2009, there were three main events which led to the elevation in all tested contaminants, resulting in a decrease of water quality and elevated exposure of *E. orbicularis* to adverse factors which have the potential to harm their survival. The first event in February (human origin indicated), the second event between May to July (agricultural origin indicated); and the third event in September (both urban and agricultural origins indicated).**

Key words: biochemical and microbiological parameters; *Emys orbicularis;* habitat; heavy metals; oxbow; sediment; surface water; water quality

INTRODUCTION

Emys orbicularis (Linnaeus, 1758), is a semi-aquatic fresh-water long living turtle. *E. orbicularis* is included in the IUCN Red List (5) and is listed among the species covered by the directive 92/43/EEC as a species of community interest. Tajba-Streda nad Bodrogom in Slovakia (48° 23' N, 21° 47' E) is listed as both a Special Protected Area (SPA) and a Site of Community Importance (SCI). Tajba's aquatic part, is an oxbow of the river Bodrog, and is situated about 1 km northeast of the village of Streda nad Bodrogom. Habitat degradation, fragmentation and loss, introduction of competitors or predators, road-kill, pollution, reduced clutch size and reduced hatching success are all potent catalysts in the decline of biodiversity (1, 7). The main underlying causes are the intensification of agriculture and the development of infrastructure, which are closely connected to increased fertilizer input in the floodplain which is a significant factor in the reduced water quality of the habitat (4). Furthermore, a significant positive correlation was found between the contaminant's sediment concentration to chromosomal damage, indicating that genotoxic habitats have genotoxic effects on *E. orbicularis* (6). Owing to the turtles' longevity and their relative high trophic level in the food chain, they have the potential to accumulate significant levels of some toxic pollutants, which are known to be immunosuppressants, and can be vertically transmitted. The pollution damage to eggshells allows for secondary invasion of bone-destroying microbes (3).

MATERIAL AND METHODS

We collected sediment and surface water samples in monthly intervals during 2009. Each sample was processed in order to acquire a biochemical analysis, microbiological profile and heavy metal element's content, for the purpose of constructing a water quality profile in the Tajba oxbow. The methods which were applied in processing the sediment samples included; filtration and evaporation for further biochemical and microbiological analysis. The surface water samples were analysed for their heavy metal content in a specialized lab.

Biochemical analysis

All the results were obtained on the basis of dry matter, which was determined by evaporation and combustion of volatile solids to a constant weight from which organic matter was calculated by subtracting the evaporative value. The pH was determined by a HACH EC30 pH meter. The total-nitrogen (Nt) was determined by the mineralisation method using a HACH 'Digesdahl', model 23130-21. Ammonia-nitrogen (N-NH4) was determined by Parnas-Wagner steam distillation, followed by a volumetric titration. Chemical oxygen demand (CODcr) was determined by a HACH DR-4000 model.

Microbial analysis

Prior to the cultivation, the sediment samples were diluted with a saline solution at 1 : 10 initial ratio, and then were cultivated on solid nutrient media – psychrophilic bacteria at 22 °C, mesophilic, coliforms and faecal steptococci at 36–37 °C, and faecal coliforms at 43–44 °C.

RESULTS

Results of analysis are presented in Tables 1 and 2, and Fig. 1

Table 1. Sediment biochemistry analysis results

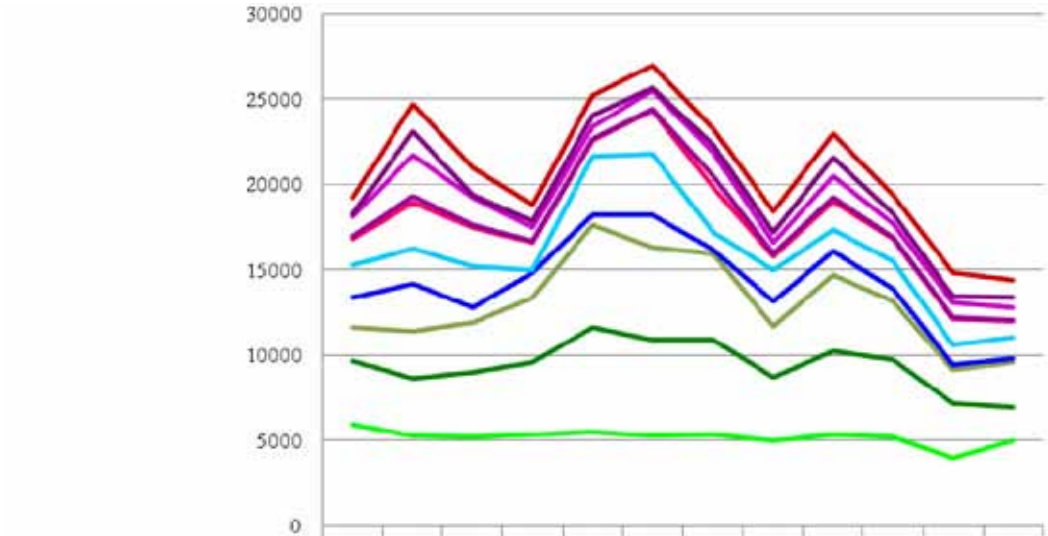
	Jan'	Feb'	Mar'	Apr'	May	June	July	Aug'	Sep'	Oct'	Nov'	Dec'
pH	6.8	5.96	7.81	7.2	7.7	7.89	6.89	6.94	7.05	7.1	7.75	6.76
DM %	15.8	10.99	25.26	50.76	3.47	12.77	8.02	21.29	7.68	6.94	6.21	4.59
Organic matter %	9.78	6.63	80.37	59.14	99.54	91.34	96.71	83.75	96.27	97.33	97.64	98.98
Inorganic matter %	90.22	93.37	19.63	40.86	0.46	8.66	3.29	16.25	3.73	2.67	2.36	1.02
Nt ($mg.kg^{-1}$)	1889	2055	2402	162.48	3395	3586	1022	1782	1194	1647	1194	1240
NH4 ($mg.kg^{-1}$)	>1	11.21	11.2	61.63	11.2	11.2	11.2	44.82	22.41	11.2	33.6	22.4
CODcr ($mg.kg^{-1}$)	1750	2750	840	1479.5	578	1893	210	1513	1435	675	250	229

Table 2. Sediment microbiology analysis results

$Log_{10}CFU.mg$	Jan'	Feb'	Mar'	Apr'	May	June	July	Aug'	Sep'	Oct'	Nov'	Dec'
Mesophilic	5954	5255	5204	5341	5477	5301	5380	4982	5322	5230	3996	4954
Coliforms	3699	3398	3771	4255	6114	5602	5531	3699	4892	4505	3204	2000
Faecal coliforms	2000	2778	2954	3763	6041	5415	5041	3000	4462	3447	1978	2602
Psychrophilic	N/A	N/A	N/A	N/A	N/A	N/A	5041	4518	5176	4477	4477	5079
Faecal Streptococci	0	1	0	0	0	2301	0	3518	0	2000	1114	0

N/A – Not Attempted

Over time changes in Heavy metals levels, COD,
total nitrogen & Microbiological parameters



	Jan'	Feb'	Mar'	Apr'	May	June	July	Aug'	Sep'	Oct'	Nov'	Dec'
Cr (ng/L)	950	1600	1600	960	1100	1300	940	1300	1400	1100	1400	1000
As (ng/L)	110	1400	110	350	720	180	410	570	1100	590	390	550
Ni (ng/L)	1200	2400	1600	820	690	1100	1400	610	1200	830	850	730
Pb (ng/L)	130	330	150	60	90	90	660	160	240	70	110	80
Cu (ng/L)	1500	2700	2300	1600	960	2500	2600	820	1700	1300	1500	980
Nt mg/kg	1889	2055	2402	162,48	3395	3586	1022	1782	1194	1647	1194	1240
CODcr sediment (mg/kg)	1750	2750	840	1479,5	578	1893	210	1513	1435	675	250	229
Faecal coliforms (log10CFU/mg)	2000	2778	2954	3763	6041	5415	5041	3000	4462	3447	1978	2602
Coliform bacteria (log10CFU/mg)	3699	3398	3771	4255	6114	5602	5531	3699	4892	4505	3204	2000
Mesophilic bacteria (log10CFU/mg)	5954	5255	5204	5341	5477	5301	5380	4982	5322	5230	3996	4954

Fig. 1. Changes in heavy metals levels, COD, total nitrogen and microbiological parameters with time

DISCUSSION

Our results showed that during 2009, there were three main events which led to the elevation in all tested contaminants and subsequently to a decrease in water quality and the elevated exposure of the *E. orbicularis* to adverse factors which have the potential to harm their survival. The first event (in February), in which all metals and faecal-coliforms/ faecal streptococci ratio increased indicated human origin contamination (urban – sewage runoff); the second event (May to July), in which the biochemical parameters in general and ammonia levels specifically, increased indicated an agricultural origin (fertilizer runoff) pollution; and the third event (September), in which the increase was most likely due to pollution from both urban (according to faecal-coliforms, As and Cr levels) and agricultural sources.

This study also showed the need for a broader landscape management scheme as a part of a more holistic view for the sustainability of *E. orbicularis* and Tajba as their habitat. There is a likely probability of adverse effects being derived from the agricultural land and the nearby village of Streda nad Bodrogom. This implies the need for better safeguards and protective zones in the Tajba NNR as noted by the EEA in 2010 (2). Monitoring the water quality of oxbow lakes is an important first step in conservation; accelerated eutrophication rates and toxic pesticide effects due to run-off effluent should be detected as soon as possible. Also, aerial pesticides applications should be avoided adjacent to the oxbow area. There is a need to develop tools to support the diagnosis and prediction of impacts of pollution upon biodiversity in general and upon *E. orbicularis* specifically, for the purpose of successful conservation of this species. We suggest conducting toxicities testing of egg-shells and the blood of *E. orbicularis*, and also of the E. orbicularis dietary components in order to have better knowledge of the direct impact of the pollutants found in Tajba on *E. orbicularis* and on it food web.

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Selected papers from the 54th STUDENT SCIENTIFIC CONFERENCE with international participation, held at the University of Veterinary Medicine and Pharmacy in Košice on May 4, 2011.

FOLIA VETERINARIA, 55, 2: 37–38, 2011

PROFILE FLUORESCENT ANALYSIS – NEW METHOD OF BIOLOGICAL MATERIAL DISTINCTION OF ANIMALS

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ABSTRACT

Metabolic fingerprinting refers to a measurement of a subclass of metabolites without sufficient analytical resolution to determine the metabolite levels of identities individually. Fingerprints can provide enough resolving power to distinguish between individual signals that can then be related to sample classifications. This method is based on the detection of differences and unexpected changes. Fluorescent spectroscopy is advantageous for fingerprinting methods because of its high sensitivity. Native fluorophores are natural components of biological fluids. Urine is a multi-component mixture consisting mainly of various compounds including fluorescent metabolites. Their composition, concentration and interactions are related to the metabolism of the organism. In this work, we detected differences between the urine of selected animal species *via* fluorescent analysis without any chemical derivation and we proved that our method can be also very simple and useful for the identification of biological fluids of different animal species. The differences between the fluorescent fingerprints of 9 selected mammals illustrated the variant composition of urine fluorophores which strongly suggests metabolic differences between the different animal species.

Key words: definition of material; fluorescence; profile analysis

INTRODUCTION

The metabolome represents the collection of all metabolites in a biological cell, tissue, organ or organism, which are the end products of cellular processes. Analysis of metabolome is the systematic study of the unique chemical fingerprints that specific cellular processes leave behind; the study of their small-molecule metabolite profiles (3).

Fluorescent techniques are suitable for fingerprint approaches because of theirs simplicity and high sensitivity. Among instrumental techniques, fluorescence spectroscopy is recognized as one of the most sensitive, therefore can reveal differences between complicated mixtures very quickly. It has proven to be a versatile tool for studying the molecular interactions in analytical sciences (4).

Urine contains a variety of organic and inorganic compounds including a number of natural fluorescent metabolites (1). The fluorophore composition of urine is related to different metabolic pathways. The analysis of fluorescence from a sample of urine without any added reagents provide useful information, but there is a need for critical evaluations of extremely concentrated (dense) or diluted urines. For elimination of this undesirable effect on spectral shape Kušnír *et al.* (2) introduced a new method of fluorescence evaluation – concentration matrices (CM). CM are created after syn-

chronous spectral scanning (ΔΛ – 30 nm) of a broad concentration scale of individual urines and a mathematical alignment of all scans.

The aim of this study was to demonstrate a rich potential for fluorescence fingerprinting of urine for detection of metabolic changes by simple comparative analysis. We analyzed urine samples from 9 different animal species.

MATERIALS AND METHODS

The urine samples used for examination were obtained from clinically healthy cows, dogs, guinea pigs, cats, horses and swine by mid stream free flow collection. Rabbits, rats and sheep samples were obtained by cystocentesis post mortem. All samples were kept frozen until the analysis.

The fluorescence was measured at room temperature, using a Luminescence Spectrophotometer Perkin Elmer LS 55 (USA), in 10 mm quartz cuvettes, except for rat and guinea pig samples which were measured in 5 mm microcuvettes. We created fluorescent concentration matrices from 12 synchronous spectra with ΔΛ 30 nm in a wide concentration range as described in the method by Kušnír *et al.* (2) for human urine.

RESULTS AND DISCUSSION

Profile fluorescent analysis allowed us to differentiate among urine specimens of cows, dogs, rabbits, guinea pigs, rats, cats, sheep, horses and swine with a high degree of significance. The CM is the global view on the total urine fluorescence (2). Figure 1 shows the example of swine urine concentration matrix. Every animal species fluorescent profile has its characteristic shape, wavelength of peaks centre and range of dominant peaks (Table 1).

Table 1. Specific location of most dominant peaks in concentration matrices of individual animal species

Animal species	Peak 1 [nm]	Peak 2 [nm]
Cow	347	380
Dog	350	
Rabbit	380	430
Guinea pig	385	430
Rat	345	
Cat	290	360
Sheep	278	
Horse	278	363
Swine	355	

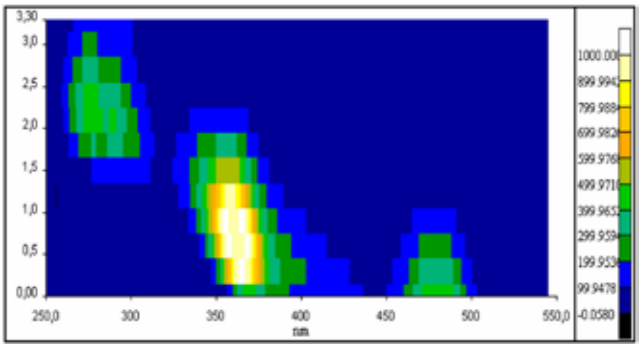

Fig. 1. The urine concentration matrix of swine

The conclusion is that the combination of the listed fluorescence properties helps to identify individual species. Our results also confirmed the metabolic differences between selected animal species which can improve laboratory analysis of body fluids of various animal species and reveal unknown metabolic differences.

ACKNOWLEDGEMENT

The study was supported by the project VEGA 1/0402/10.

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Selected papers from the 54th STUDENT SCIENTIFIC CONFERENCE with international participation, held at the University of Veterinary Medicine and Pharmacy in Košice on May 4, 2011.

FOLIA VETERINARIA, 55, 2: 39–41, 2011

POTENTIAL NEW USE OF X-RAY EXAMINATION IN DIAGNOSIS OF CANINE AND FELINE TRACHEAL PATHOLOGIES

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ABSTRACT

The aim of this study was to discuss some conventional X-ray methods of tracheal examination and compare them with the novel method of measurement of the tracheal diameter developed at our clinic. It was carried out at the University of Veterinary Medicine and Pharmacy (UVMP), Small Animals Clinic, on 188 animals in 2009. In dogs and cats it is not possible to determine the phases of the respiratory cycle according to the diameter of the trachea as it does not change with inhalation and exhalation as had been assumed by some authors. The diameter of the trachea in dogs can be estimated on the basis a 3-fold width of the dorsal third of the 3rd thoracic vertebra. The method more suitable in cats compared the diameter of the trachea with the height of 5th thoracic vertebra. Our observations did not reveal the calcification of tracheal rings in cats, while in dogs, it was observed at the age of 11.6 years on the average.

Key words: cat; diagnostics; dog; mean; trachea; X-ray

INTRODUCTION

Diseases of the trachea are most frequently diagnosed by x-ray examination, ultrasonography and endoscopy. The trachea is a semi-rigid, air filled tube that connects the larynx to the bronchial system. It runs ventrally to the cervical vertebrae and enters the thoracic cavity through the *apretura thoracis cranialis* where it diverges from the thoracic spines. Dorsal to the heart it divides into the *bifurcatio tracheae* and thence to the *bronchus principalis dexter et sinister*. The trachea is made up of cartilage rings that are dorsally opened and overlapped by means of the *musculus trachealis*. Dogs have 42 to 46 and cats 38 to 43 tracheal rings. X-ray examinations employ latero-lateral, dorso-ventral, ventro-dorsal or orthogonal projection. They are used to evaluate the position, diameter, opacity or lucence of the trachea. In diameter, the trachea is slightly wider than higher. The determination of the diameter of the trachea is an important tool in the diagnostics of tracheal collapse or stenosis (Fig. 1A, 1B) which are relatively frequent diagnoses in dogs and cats (7).

MATERIAL AND METHODS

This study was based on X-ray records of the animals examined at the University of Veterinary Medicine and Pharmacy (UVMP),

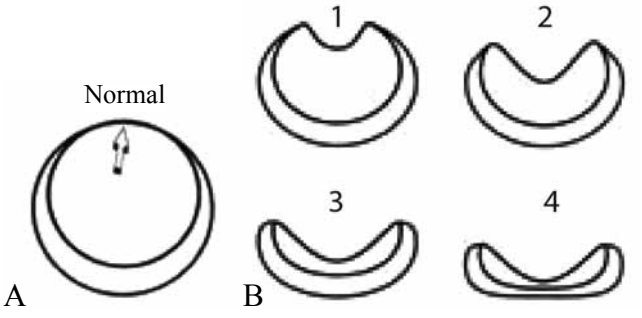

Fig. 1. A – normal tracheal diameter; B – individual stages of tracheal collapse 1–4: Stage 1 is reduction of tracheal lumen by 25%, stage 2 by 50%, stage 3 by 75% and stage 4 is a total collapse of the trachea (4)

Table 1. Mean tracheal diameters (cm)

	Cervical section			Mean	Thoracic section			Mean	Breath phase
	cranial	medial	caudal		SIC2	SIC4	SIC bifurc.		
Cats	0.6	0.5	0.5	**0.5**	0.5	0.5	0.5	**0.5**	inspiration 18x
	0.6	0.5	0.5	**0.5**	0.5	0.5	0.5	**0.5**	expiration 4x
Dogs	1.3	1.2	1.1	**1.2**	1.1	1.1	1.2	**1.1**	expiration 71x
	1.3	1.2	1.1	**1.2**	1.1	1.1	1.1	**1.1**	inspiration 95x

Small Animal Clinic. Available were right or left lateral X-ray records of 188 animals of which 166 were dogs (♂ n = 82 49.4 %; ♀ n = 84, 50.6 %) with a mean age of 8.5 years and 22 cats (♀ n = 16, 72.7 %; ♂ n = 6, 27.3 %) with a mean age of 4.4 years, which showed or failed to show signs of respiratory or cardiovascular diseases. Of this set of animals, we eliminated 12 dogs due to unreadability of the tracheal radiographs.

1. Topography of *bifurcatio tracheae* was determined on lateral x-ray records according to the number of right or left spatium intercostale (SIC) and made the respective comparisons.

2. Tracheal diameters were determined by three methods. According to Keally and McAllister (5), the trachea with should equal minimally 3-fold of the proximal third of rib width. We compared it with the height of Thoracic 5 and the widest part of the processus spinosus Thoracic 2.

3. Calcification of tracheal rings (CTR) is known as an age related process. We determined the mean age at which the calcification of the tracheal rings was observed in dogs and cats and its character of spreading throughout the trachea.

RESULTS

1. Determination of the most frequent positioning of *bifurcatio tracheae*

In cats the carina was located either in the 5th or 6th intercostal space (SIC) with a diameter of 5.4 mm. Identical values were obtained for both right and left SIC. In dogs it was located mostly in the 5th SIC with a diameter of 4.9 SIC. In four cases, we observed different locations of the *bifurcatio tracheae* shifted by 1 SIC (right and left SIC). In all these cases the shift was caused by pathology.

2. Determination of tracheal diameter

Table 1 summarises the results of the determination of the mean tracheal diameters of cats and dogs measured at three sites of the cervical section (cranial, medial, caudal) together with the respective total mean values and of those measured at three sites of the thoracic section (SIC2, SIC4, SIC *bifurcatio*) together with the respective total mean values.

Table 2 presents in the first column, the mean values of tracheal diameter, in the second column 3-fold width of the proximal rib end, in the third column the Th5 height and in the fourth column the width of processus spinosus of evaluated dogs and cats.

Table 2: Means of measurements based on X-ray records (cm)

	Mean tracheal diameter	Rib thickness	Th5 height	Width of processus spinosus
Dogs	1.16	1.2	1.1	1
Cats	0.5	0.75	0.5	0.6

3. Calcification of tracheal rings

No calcification of tracheal rings was observed in the examined cats. The age of examined cats (n = 22) was as follows: min = 0.5 y; max = 16 y; modus = 1 y; median = 3 y; mean = 4.4 y. In the examined dogs (n = 166) calcification was observed in 26 animals. In 10 dogs the calcification was evident throughout the trachea and in 16 dogs only in the cervical section was the calcification evident. The affected animals were 4 to 16 years old, their mean age was 11.6 years. Sixteen of the affected dogs were females and 10 males.

DISCUSSION

Thoracic radiography is one of the best available methods for the assessment of the heart, lungs and trachea. However, there are still problems with its interpretation (2, 3).

In cats, the position of *bifurcatio tracheae* was in the 5th or 6th SIC with a mean value of 5.4 and in dogs, it was in the 4th or 5th SIC, with a mean value of 4.9, which is in agreement with Schwartz, Johnson *et al.* (8) who reported that *bifurcatio tracheae* is commonly located in the 5th intercostal space.

The detailed analysis of individual measurements at six tracheal sites (3 cervical, 3 thoracic) showed that inspiration and expiration has no significant influence on tracheal diameter as stated by Schwartz, Johnson *et al.* (8) in contrast

with Ledecký *et al.* (6) who reported that at inspiration the tracheal diameter of the thoracic section is bigger and that of cervical section is smaller and the opposite applies at expiration. In the zone of apertura thoracis cranialis the tracheal diameter is by 6–7 % smaller than in the cervical or thoracic sections (1). The tracheal diameter decreases slightly in the caudal direction (9) which was confirmed also in our study.

The results obtained showed no calcification of tracheal rings in cats. However, to draw final conclusions, we would have to examine more than 22 cats. Also the cats examined in our study were relatively young (median age = 3 years). This could become the subject of our future studies. In dogs, CTR occurred in 16 % of the examined animals of which 61.5 % were females and 38.5 % males. It was observed most frequently in mongrels, Cocker Spaniels, Poodles, Dachshunds and Schnauzers. Calcification started in the cervical section and progressed in the caudal direction. It was most common in older dogs.

Radiographic diagnosis is an important part of the diagnostic process and should be evaluated in association with clinical signs and other special examinations.

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FOLIA VETERINARIA, 55, 2: 42–44, 2011

THE USE OF SKIN FLAPS IN CLINICAL PRACTICE DEALING WITH BIRDS OF PRAY INJURIES

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ABSTRACT

Skin defects are common problems in birds. They occur most frequently in wild birds kept in captivity. The predominant sites of skin damage in these birds are the heads, wings and legs. Rapid and effective therapy is essential due to the absence of freely available skin, damages at inaccessible sites and many other complications. In our clinical study we used a vascularized skin flap technique in the surgical therapy of skin defects in the Imperial Eagle (*Aquila heliaca*). In all of our patients, we observed the successful take of the skin flap and the eventual healing of the damaged site. All of the patients treated, began to use the affected limbs within two months of the surgery. Surgical therapy of skin defects in free living and exotic birds appears to be a suitable alternative of the therapeutical management of skin defects. Its use prevents the development of complications associated with open wound management.

Key words: skin defect; surgical therapy; vascularized skin flap

INTRODUCTION

Skin defects are common problems encountered in an avian clinical practice. They develop due to trauma, impacts, fighting, bites, insect stings or incorrect manipulation and breeding management (1). Other potential causes of skin defects are: inflammatory changes of the bodies and legs of birds (*pododermatitis*); frostbites; burns; and open fractures.

Early diagnosis of skin defects in avian patients plays an important role in the prevention of the spreading of the skin damages. It is important to consider the potential development of subsequent complications (5). The successful therapy of skin defects is a great challenge for veterinarians due to the anatomical specificities of avian skin, extent of damage and lack of excess skin at the defective site (6).

The integument of birds shares many similarities with that of mammals. However, there are also many skin modifications and differences (4). Its thinness, lack of subcutaneous tissue and poor blood supply reduce considerably the prospects of using methods that are successfully used for the therapy of skin defects in mammals (1). A frequent limiting factor in avian patients is the absence of excess skin at or near the skin defect that could be used to close the wound, or absence of papillomatous tissues in foot pads capable of withstanding the pressure of the avian body concentrated on the small area of its feet. These limitations call for new therapeutic methods in the management of skin defects.

A vascularised skin flap is defined as a partially separated segment of skin and subcutaneous tissue retaining its own blood supply during transplantation to the recipient site (1). By using skin flaps we prevent complications frequently associated with the management of healing of open wounds, involving excessive scarring, production of granular tissue unable to withstand pressure, resulting in skin contracture or prolonged healing of wounds (6).

MATERIAL AND METHODS

The clinical study was conducted at the Clinic for exotic and free living animals of the University of Veterinary Medicine and Pharmacy in Košice from October 2009 until March 2011. We treated eight Imperial Eagles (*Aquila heliaca*). They were examined clinically in order to judge the extent and seriousness of their skin

damage. When deciding about the treatment, we considered alternative methods of skin damage therapy and the use of vascularized skin flaps. When selecting the method, we focused on the aspects of overall health and social and working position of the avian patient.

Clinical cases

The group of patients comprised individuals with damaged skin on legs either due to injury by electrical current, insect sting or inflammatory disease of feet (*pododermatitis*). Before onset of the therapy, we evaluated thoroughly the health of the patient and extent of the damage. The first step involved removal of the necrotic part of the skin and initiation of the treatment of inflammation and the present infection. In the first period we treated the damaged skin by application of local packs with 1 % tannin-alcohol in combination with oxytetracyclin and polymyxin (Terramycin, PFIZER) in the form of an unguent for 2 weeks (Fig. 1). After this time, we evaluated the skin damage again and carried out the cleaning of the wound with removal of necrotic tissue (Fig. 2). To protect the wound against secondary contamination and drying, we applied a hydrocolloidal dressing in the form of a plaster (Hydrocoll, HARTMANN).

Fig. 1. Local pack with bandage

Fig. 2. Removal of necrotic tissue

The absence of large areas of skin and loss of papillomatous tissue from the feet required replacement tissue of an identical structure in the form of a vascularized skin flap. The skin flap was attached with suture material PROLENE 3/0 using simple U stitches. To produce a protective barrier against infection and to support granulation and epithelisation we applied 20 % Solcoseryl gel (VALEANT) and Betadineunguent (EGIS). The wound was again covered with hydrocolloidal plaster, dressing and an adhesive bandage (CoPoly).

RESULTS AND DISCUSSION

After surgery, the redressing and checking of the wound healing was done according to the seriousness of the situation in 1–2-week intervals for 1v2 months. The healing wound was checked for swelling, presence of wound secretion, purulence and drying out of the wound edges. The skin flaps were attached successfully to the recipient sites in all surgically treated avian patients. The healing of the skin defects was completed by 2 months after the surgery. All birds were able to apply a load with full use of the affected extremities.

Relatively few studies have dealt with the surgical therapy of skin defects by means of vascularised skin flap. Gentz and Linn (2) reported on use of simple skin flap in 3 birds with skin damage on their heads, The wounds of two birds healed without complications while necrotization occurred in the third one. Hannonon and McGehee (3) applied a vascularized skin flap to manage a large skin defect on an owl's wing. After 17 days, it becomes necrotic. However, after removal of the necrotic material, they observed the production of new granular tissue and the wound healed completely within one month.

By using the vascularized skin flap technique for therapy of skin defects, we avoided the complications frequently associated with the healing of open wounds. This method is suitable also for the therapy of extensive skin damages, even at sites with limited accessibility. The successful reports on this new method justify its use in avian clinical practice.

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Selected papers from the 54th STUDENT SCIENTIFIC CONFERENCE with international participation, held at the University of Veterinary Medicine and Pharmacy in Košice on May 4, 2011.

FOLIA VETERINARIA, 55, 2: 45–46, 2011

SPECIES COMPOSITION OF MOSQUITOES (*CULICIDAE*) IN BODROG AND HORNÁD RIVER-BASINS

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ABSTRACT

We present quantitative and qualitative evaluations of the species spectrum of larval stages of mosquitoes collected at six locations of Eastern Slovakia in the spring and summer of 2010. Altogether we collected 9,923 larvae of ten mosquito species with predominance of the so-called calamitous species, namely *Culex pipiens* (47.9%) (or *Culex torrentium*), *Aedes vexans* (36.8%) and *Ochlerotatus sticticus* (6.06%). Other collected species were early spring mosquitoes *Ochlerotatus punctor* (1.31%) and *Ochlerotatus cataphylla* (0.08%); late spring species *Ochlerotatus cantans* (4.57%), *Aedes cinereus* (1.47%) and *Culiseta annulata* (0.09%); summer mosquito *Anopheles maculipennis* (2.75%) and the relatively rare arboricolous species *Aedes geniculatus* (0.06%).

Key words: Eastern Slovakia; floods; larvae; mosquitoes

INTRODUCTION

Mosquitoes are one of the most important nuisance insects, vectors of diseases and temporary ectoparasites of humans and animals. Their numbers are so high in some countries that they pose serious health, economic and social problems (5). Presently, about 49 species of 6 genera have been found in the Slovak territory (8). The species composition of mosquitoes in Slovakia has been investigated intensively, particularly in the southern and western parts (9, 7, 6, 4) while more intensive research in Eastern Slovakia was conducted only in the fifties and sixties of the past century (12, 10, 11). The present knowledge of the fauna and ecology of mosquitoes in Eastern Slovakia is incomplete (2).

The aim of our study was to carry out entomologic surveillance of the species composition of mosquitoes in Hornád and Bodrog river-basins and observe and evaluate the influence of environmental factors on their life cycle, distribution and seasonal dynamics.

MATERIAL AND METHODS

From April until July 2010, we collected mosquito larvae at 6 previously flooded areas located in Eastern Slovakia: Košice – Ťahanovce, Šebastovce, Rozhanovce, Paňovce and Perín-Chým. The larvae were collected with a small-mesh strained which was swept over water surface or dipped a little below the water surface. Using a plastic pipette the larvae were transferred to tubes containing 96% alcohol to kill and fix the larvae (7). They were identified in a laboratory by means of a stereomicroscope and determination key (1, 5).

RESULTS

We made a total of 16 trips and collected 9,923 larvae of 10 mosquito species belonging to 5 genera (*Aedes, Culex, Culiseta, Ochlerotatus, Anophles*). The dominant groups were the so-called calamitous species, namely *Culex pipiens* (47.9%) (or *Culex torrentium*), *Aedes vexans* (36.8%) and *Ochlerotatus sticticus* (6.06%). An important characteristic of the flood species is that under suitable temperatures (25–30°C), their development is very rapid (7–14 days) and results in huge numbers of adults (imagoes). These species are, as a rule polycyclic with a tendency to over-infest at each subsequent

flooding provided that their ecological demands are met. Other identified species were: early spring species *Ochlerotatus punctor* (1.31 %) and *O. cataphylla* (0.08 %); late spring species *O. cantans* (4.57 %), *Aedes cinereus* (1.47 %) and *Culiseta annulata* (0.09 %); summer species *Anopheles maculipennis* (2.75 %) and arboricolous species *Aedes geniculatus* (0.06 %), collected in Rozhanovce. The Paňovce location was most abundant in mosquito species (7 species) while the highest number of larvae (almost 6,000 larvae of 5 species) was collected in the Šebastovce location. In this location we observed the highest increase in *Cx. pipiens* larvae between June 14 and 25, i. e. after flooding. Up to this time, the larvae of this species occurred in this location only sporadically. In the same time interval, together with this species, we diagnosed for the first time *Anopheles maculipennis* in a similar type of water biotopes.

DISCUSSION AND CONCLUSION

Territories with an abundance of mosquitoes, particularly of the calamitous species, include the southern part of Slovakia where the building of Waterworks Gabčíkovo disturbed the original structures of biotopes, with mosquito hatching sites and contributed to optimum mosquito larval development (6). Research conducted from 1999 to 2008 (3, 4) in inundated areas of the rivers Dunaj, Váh, Nitra, Žitava, Ipeľ and Latorica, confirmed the occurrence of 31 mosquito species of 6 genera. Of all the species found, the most important were the so-called calamitous species, namely *Aedes vexans, Aedes cinereus, Aedes rossicus, Ochlerotatus sticticus* and *O. cantans.* The larvae collected during our study in 2010 at the 6 targeted locations in Eastern Slovakia confirmed the presence of almost all calamitous species found in southern Slovakia with the exception of species *Aedes rossicus.* The species which dominated in our study was *Culex pipiens* or *Cx. torrentium* (4,757 larvae).

The changes in species composition of mosquitoes are affected by climate changes, particularly by climate warming in central Europe. The species composition in the observed locations was less diverse most likely because of repeated flooding in some places which could result in washing away of both mosquito eggs and larvae. In addition, some locations were inaccessible for a long time and, because of that, we probably could not collect larvae of some species as the days after flooding were hot, even tropical, and the developmental cycle of mosquitoes was shortened considerably.

ACKNOWLEDGEMENT

The study was conducted within the Project VEGA No. 1/0106/10 and basic research carried out by the National Reference Laboratory for Pesticides of the UVMP in Košice.

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Selected papers from the 54th STUDENT SCIENTIFIC CONFERENCE with international participation, held at the University of Veterinary Medicine and Pharmacy in Košice on May 4, 2011.

FOLIA VETERINARIA, 55, 2: 47–49, 2011

FFECTIVENESS OF SELECTED ESSENTIAL OILS AGAINST YEASTS OF THE GENERA *CANDIDA* AND *MALASSEZIA*

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ABSTRACT

The disc diffusion method (DDM) and microdilution method (MD) (CLSI M27-A2) were used to determine the susceptibility of yeasts of the genera *Candida* (11 samples) and *Malassezia* (3 samples) to 8 essential oils (EO) in concentrations of 30 % and 50 %. With DDM, the cinnamon, oregano, and clove EO affected most all of the tested isolates. The minimum inhibitory concentration (MIC) of EO with MD ranged from 40 to 320 µg.ml^{-1}.

Key words: *Candida*; disc diffusion method; essential oils; *Malassezia;* microdilution method

INTRODUCTION

Yeasts of the genera *Candida* and *Malassezia* are natural commensals of the skin and the mucosa of humans and animals. Under some conditions they act as opportunistic pathogens (9, 11). *Candida albicans* is the most frequent causative agent of animal candidoses (6). Various species of the genus *Malassezia* (*M. pachydermatis, M. equina, M. caprae, M. nana*) induce otitis and dermatitis in animals (7, 3). The therapy of mycoses is based on local or systemic active treatment. Ancillary therapy of fungal diseases also utilizes essential oils (EO) (2).

MATERIAL AND METHODS

The effectiveness of essential oils (EO) [bergamot, juniper, eucalyptus, clove, nutmeg, oregano, sage, cinnamon (Calendula, SR), in concentrations of 30 % and 50 %] and itraconazol against clinical isolates of yeasts (*C. albicans* – 6, *C. glabrata* – 5) and reference strains: *M. pachydermatis* CBS 1879; *M. sympodialis* CBS 8334; *M. equina* CBS 6999 (CBS-KNAW Fungal Biodiversity Centre, the Netherlands) was tested by the disc diffusion method (DDM) according to Ajvazjan (1) and by the standard CLSI microdilution method (MD) (9). For the control we used the reference strain *C. albicans* CCM 8215 (Czech Collection of Micro-organisms, Brno, CR) the susceptibility of which was evaluated against itraconazol (Hi-Media Laboratories Pvt. Ltd., Mumbai, India). The effectiveness of EO was verified by cultivation, i. e. by application of the content of wells of microtitration plates (50 µl) to plates containing nutrient media. The growth of yeasts was checked after 48 and 72 hours.

RESULTS AND DISCUSSION

Table 1 shows the size of the inhibition zones produced by EO at concentrations of 30 % and 50 %, affecting the yeasts of the genera *Candida* and *Malassezia* when tested by DDM. Cinnamon EO in 30 % concentration had no effect on the yeast of *M. sympodialis*. No susceptibility of yeasts was found to the other tested EO. These results agree with those of Rusenova and Parvanova (10), who reported that the size of the inhibition zones with cinnamon and oregano oil was 30 and 47 mm, respectively for *C. albicans* and 37 and 34 mm, respectively for *M. pachydermatis*. The antimycotic effects of itraconasol was sufficient and the size of inhibition zones exceeded 20 mm. Table 2 presents MICs of EO which inhibited the growth of *Candida* yeasts. It also shows

the number (n) and per cent of susceptible strains (S). At MIC 160 μg.ml^{-1}, oregano and cinnamon oils showed 100 % effectiveness against all isolates of *C. albicans* and *C. glabrata.* The effectiveness of clove oil reached 100 % against *C. albicans* and 80 % against *C. glabrata* 80 %. Manohar *et al.* (8) investigated the antifungal effectiveness of oregano oil against yeasts (*C. albicans*) *in vitro* and *in vivo* and reported higher MIC (0.25 mg.ml^{-1}). The MIC determined in our study ranged between 40 and 160 μg.ml^{-1} against isolates of *C. albicans.* The MICs of itraconazol in MD testing were > 0.25 μg.ml^{-1} for both the reference strains and clinical isolates.

Table 3 shows the MICs of reference strains. All strains were susceptible at MIC 160 μg.ml^{-1}.

Table 1. Size of inhibition zones (mm) produced by 30 % and 50 % EO

EO	Conc.	Isolates		Reference strains			
		CA	CG	CA	MP	MS	ME
Cinnamon	30 %	32–>40	22–>40	30	>40	16	30
	50 %	>40	38–>40	38	>40	20	33
Oregano	30 %	34–>40	32–>40	30	>40	28	35
	50 %	36–>40	40–>40	32	>40	30	40
Clove	30 %	35–>40	35–>40	34	40	>40	>40
	50 %	38–>40	31–>40	36	36	>40	>40

CA – *Candida albicans,* CG – *C. glabrata,* MP – *Malassezia pachydermatis,* MS – *Malassezia sympodialis,* ME – *Malassezia equina*

Table 2. Levels of MIC and effectiveness of oils against isolates of *Candida* yeasts

EO	*C. albicans*			*C. glabrata*		
	MIC (μg.ml^{-1})	n	S (%)	MIC (μg.ml^{-1})	n	S (%)
Oregano	160	1	16.7	80	2	40
	80	4	66.7	160	3	60
	40	1	16.7			
Cinnamon	160	6	100	80	4	80
				160	1	20
Clove	160	5	83.3	160	3	60
	40	1	16.7	320	1	20
				80	1	20

n – number of susceptible strains; S – susceptibility

Table 3. MIC (μg.ml^{-1}) of reference strains

EO	*M. pachydermatis*	*M. equina*	*M. sympodialis*	*C. albicans*
Oregano	80	80	80	80
Cinnamon	80	160	160	160
Clove	40	80	80	160

Cultivation checking of optically pure media confirmed the 100 % effectiveness. No growth of yeasts was observed on plates.

CONCLUSION

Fungal and bacterial pathogens may exhibit high levels of antimicrobial resistance in veterinary practice and this necessitates the need for alternative therapy (4). The results provided by the tested EO indicated their additional antifungal effect is significant. However, the yeasts were more susceptible to itraconazol. The effectiveness of essential oils has been ascribed to their active components but it appears necessary to test also the activity of these components that are credited with antimycotic effects.

ACKNOWLEDGEMENT

The study was supported by the projects APVV-0357-07 and VEGA No. 1/0736/08.

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Selected papers from the 54th STUDENT SCIENTIFIC CONFERENCE with international participation, held at the University of Veterinary Medicine and Pharmacy in Košice on May 4, 2011.

FOLIA VETERINARIA, 55, 2: 50–52, 2011

DETERMINATION OF PARAMETERS OF NON-SPECIFIC IMMUNITY IN THE COURSE OF PARVOVIRUS DISEASE IN DOGS

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ABSTRACT

Parvovirus disease of dogs caused by canine parvovirus type 2 (CPV2) is an acute, highly contagious disease manifested by gastroenteritis, myocarditis and immunosuppression. This investigation was carried on 6 anonymous puppies, 6–8 weeks old with clinical signs of parvovirosis and the confirmed presence of CPV2 in their faeces (rapid CPV Ag test Kit, Bionote, Korea). The parameters of non-specific immunity were determined and evaluated in these animals during their treatment and convalescence. Puppies with confirmed parvovirosis showed; a decreased index of metabolic activity (IMA) stimulation index (SI) of lymphocytes, chemotactic activity (CHA), increased phagocytic index of leukocytes, and phagocytic activity of neutrophils compared to the control dogs which were negative for CPV2.

Key words: canine parvovirus; enteritis; immunological examination; immunosupression

INTRODUCTION

The causative agent of parvovirus disease of dogs is canine parvovirus type 2 (CPV2), a member of the family Parvoviridae, genus *Parvovirus*. Parvovirosis is one of the most common viral diseases causing acute haemorrhagic enteritis in dogs (1). CPV2 occurs most frequently in 6–12 weeks old puppies because the younger animals are protected passively by maternal antibodies (4). These antibodies provide protection against CPV infection but can be blocked by viruses contained in the vaccines against CPV (5, 1). CPV2 infections spread by the oro-faecal route. Because the virus is eliminated 8–12 days after infection, its levels in the faeces of infected animals are high (2). After entering the body, the virus replicates locally in the lymphoid tissue of the oropharynx causing rapid development of viraemia accompanied with increased temperature and lymphopenia (8). Viruses cause varying degrees of immunosuppression as well as immunodeficiency. Prognosis in non-vaccinated animals is uncertain. The best preventive measure is vaccination.

MATERIAL AND METHODS

Animals

The experimental group (E) consisted of 6 sick dogs of different age and gender, kept under identical conditions, all with canine parvovirus diagnosis (rapid CPV Ag test Kit, Bionote, Korea). The control group (C), comprised of 6 clinically healthy dogs of different breeds, mean age 12 months, kept under the same conditions as the experimental dogs. Samples of blood were collected by puncture of *v. jugularis* or *v. cephalica antebrachii* four times in weakly intervals.

Immunological analysis

The phagocytic activity of leukocytes is expressed as the percentage of leukocytes phagocytizing 3 or more MicroSpheric Hydrophilic Particles (MSHP), and the phagocytic index is the ratio of the number of phagocytised particles and the number of all potential phagocytes present. The phagocytic activity of leukocytes was determined by the ingestion of 2-hydroxyethylmetacrylate particles (MSHP, diameter 1.2 µm, ARTIM Prague) (10).

The metabolic activity of leukocytes was determined by means of an iodine-nitrotetrazolium test adjusted by Mareček and Procházková (7). We used 2-(4-iodophenyl)-5-phenyltetrazolium chloride (INT) (Lachema Brno) as an indicator and Zymozan

(Sigma, USA) as a stimulant. The index of metabolic activity of leukocytes (IMA) was expressed as the ratio of activities of stimulated and non-stimulated cells.

The blastic transformation of lymphocytes was evaluated by ELISA BrdU (colorimetric) test using phytohaemagglutinin PHA-P (Sigma, USA) with a concentration of 20 µl.ml^{-1}. The level of blastogenic response of lymphocytes is expressed as the stimulation index (SI).

The chemotactic activity (CHA) was determined by the method of the migration of the polymorphonuclear leukocytes on agarose and evaluated as the chemotactic index, i.e., the ratio of the length of chemotaxis and spontaneous migration paths.

The results obtained were presented as means (x) with respective standard deviations (SD). Differences between groups were evaluated using the Student t-test.

RESULTS AND DISCUSSION

Examinations were carried out on 6 dogs. Three of them showed the typical clinical signs: apathy, somnolence, nausea, vomiting, watery and later bloody diarrhoea and dehydration. Six vaccinated dogs kept in the same environmental and hygiene conditions, free of parvovirosis, were used as the control. The results of the examinations are presented in Tables 1 to 4.

A marked increase in IMA ($P<0.001$) indicated a decreased capacity of the cells to process enzymatically the phagocytised material. We recorded a significant decrease in SI ($P<0.05$) in comparison with the control dogs. Specific protection mediated by Tc lymphocytes begins at the end of the viraemic stage of infection and plays a role in the process of recovery and elimination of the virus (9). A marked increase in CHA ($P<0.001$) in comparison with the controls indicates a decreased ability of phagocytes to migrate to the inflammation site. This may be caused by insufficient production of cytokines by antigen presenting cells, subpopulations of cytotoxic (Tc) and helper (Th) lymphocytes, fat cells controlling proliferation and maturation of haemopoietic myeloid line cells as well as their functional activity and chemotaxis. A marked reduction in IMA, SI and CH indicates immunosuppression, an important factor in the pathogenesis of canine parvoviral disease (6). After first sampling, we detected an increased levels of phagocytic activity of neutrophils which, however, declined in the course of the following samplings, indicating a decreased phagocytising ability of neutrophils with time.

The level of leukocytes (1st sampling) was reduced, but showed an increasing tendency during the following samplings. Toman *et al.* (9) mentioned leukocytopenia, lymphopenia and decreased function of lymphocytes in canine parvovirus disease. The comparison of dogs with clinical signs, with dogs with diagnosed parvovirosis but free of clinical signs, showed a marked decrease in leukocytes, CHA, SI and IMA. These parameters indicate serious immunosuppression.

Table 1. Comparison of non-specific immunity of groups C and E – 1st sampling

1st sampling	Le (×10^3)	IMA	SI	CH	PhANe (%)	PhINe
Sick dogs - group E						
x	6.4	1.53	4.19	1.28	69.89	11.1
SD	1.54	0.33	1.66	0.11	16.1	6.3
	**	**	Ns	***	Ns	Ns
Healthy dogs – group C						
x	9.45	2.13	2.95	1.92	58.97	7.23
SD	0.484	0.17	0.41	0.14	2.61	0.34

Le – leukocytes; IMA – index of metabolic activity; SI – stimulation index of lymphocytes; CH – chemotaxis; PhANe – phagocytic activity of neutrophils; PhINe – phagocytic index of neutrophils; Ns – non-significant; ** – P < 0.01; *** – P < 0.001

Table 2. Comparison of non-specific immunity of groups C and E – 2nd sampling

2nd sampling	Le (×10^3)	IMA	SI	CH	PhANe (%)	PhINe
Sick dogs - group E						
x	11.5	1.63	1.97	1.4	50.23	6.47
SD	1.82	0.19	0.88	0.18	14.89	2.96
	**	***	*	***	Ns	Ns
Healthy dogs – group C						
x	10.18	2.47	3.14	2.26	60.35	8.17
SD	1.82	0.19	0.88	0.18	14.8	2.96

Ns – non-significant; * – P < 0.05; ** – P < 0.01; *** – P < 0.001

Table 3. Comparison of non-specific immunity of groups C and E – 3rd sampling

3rd sampling	Le (×10^3)	IMA	SI	CH	PhANe (%)	PhINe
Sick dogs – group E						
X	10.68	1.16	1.59	1.09	55.13	7.45
SD	2.8	0.16	1.48	0.13	10.09	0.76
	Ns	***	Ns	***	Ns	Ns
Healthy dogs – group C						
X	10.38	2.37	3.052	2.16	60.48	8.3
SD	2.8	0.16	1.48	0.13	10.09	0.76

Ns – non-significant; *** – P < 0.001

Table 4. Comparison of non-specific immunity of groups C and E – 4th sampling

1st sampling	Le ($\times 10^3$)	IMA	SI	CH	PhANe (%)	PhINe
Sick dogs – group E						
X	7.233	1.37	1.58	1.17	67.33	8.06
SD	1.38	0.15	1.1	0.11	9.456	1.48
	**	***	*	***	Ns	Ns
Healthy dogs – group C						
X	10.2	2.36	3.13	2.14	60.97	8.52
SD	1.38	0.15	1.1	0.11	9.45	1.48

Ns – non-significant; * – $P < 0.05$; ** – $P < 0.01$; *** – $P < 0.001$

CONCLUSION

Canine parvovirus disease is an acute, progressive, highly contagious disease with frequently fatal consequences. Our study showed the disturbed reactivity of the immune system during parvovirus infection. The immunological analysis revealed significant decrease in IMA ($P<0.001$), CHA ($P<0.001$) and SI of lymphocytes ($P<0.05$) in the group of sick dogs. The marked suppression of the proliferation activity of lymphocytes, chemotactic activity and index of metabolic activity indicated immunosuppression. These immune system component play an important role in the protection of the body against the development of infection. Vaccination of puppies which no longer receive protection from maternal antibodies is one of the most important preventive measures.

ACKNOWLEDGEMENT

The study was supported by project VEGA 1/0615/09.

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Selected papers from the 54th STUDENT SCIENTIFIC CONFERENCE with international participation. held at the University of Veterinary Medicine and Pharmacy in Košice on May 4, 2011.

FOLIA VETERINARIA, 55, 2: 53–54, 2011

SEROLOGICAL MONITORING OF *FEBRIS CATARRHALIS OVIUM* IN THE SLOVAK REPUBLIC

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ABSTRACT

The goal of this study was to detect antibodies against bluetongue virus and the nucleic acids of bluetongue virus between 2008 and 2010 in the susceptible animal species in the Slovak Republic. The examination of antibodies against bluetongue disease was carried out by the ELISA method. Bluetongue virus (BTV) detection was performed using the method of real time PCR. Sentinel animals came from selected farms throughout the territory of the Slovak Republic (100 farms). Apart from these animals, other animals after moving between farms, after import and after abortion were examined. A total of 61,832 samples from animals (60,729 cattle, 1,103 sheep) were tested for antibodies against the BT virus infection and 3,903 samples from animals (3,868 cattle, 35 sheep) were tested for the presence of nucleic acids of BT virus. Of the total number of samples examined from animals during monitoring of the disease, 249 samples were BT seropositive (241 cattle, 8 sheep) and from cattle, 52 of the samples were positive for the virus.

Key words: antibodies; bluetongue; cattle; *febris catarrhalis ovium*; monitoring; sheep; virus

INTRODUCTION

Febris catarrhalis ovium (bluetongue, BT) is caused by bluetongue virus (BTV) which is included in the family *Reoviridae* and the genus *Orbivirus*. It is antigenically related to other *Orbiviruses*, for example, the virus of haemorrhagic disease of deer (3, 7). Twenty-four BTV serotypes have been identified (1). The etiological agent is one of the segmented double-stranded ds – RNA viruses (6). The viral genome is composed of 10 double-twisted segments of RNA that encode four non-structural proteins (NS1, NS2, NS3, NS3A) and 7 structural (VP1–VP7) proteins (4, 6). For identification and serotyping of BTV serotypes, polypeptides VP2 and VP7 have played an important role. Polypeptide VP7 has been species specific and polypeptide VP2 has been serotype specific (2, 3).

MATERIAL AND METODS

A total of 61,832 samples from animals (60,729 cattle, 1,103 sheep) were examined for antibodies against bluetongue virus disease, and 3,903 samples from animals (3868 cattle, 35 sheep) were examined for the presence of the nucleic acids of BT virus. Sentinel animals came from selected farms throughout Slovakia (100 farms). A second group of animals consisted of animals investigated during their movements of between farms, import of animals, females after abortion or after the birth of dead offspring. Serological and virological tests were performed in the reference laboratory for bluetongue disease in the State Veterinary Institute in Zvolen. The examination of antibodies against bluetongue disease virus (VP7 protein) was carried out by the ELISA method (ID VET Kit for detection of anti-VP7 antibodies by competitive ELISA). For the evaluation of samples a Microplates Reader MRX II. (Dynex Technologies) was used. The detection of the nucleic acids of bluetongue virus was performed using real time PCR method. Real time PCR iCycler IQ5 BIO-RAD with software for image evaluation samples was used.

RESULTS AND DISCUSSION

During serological monitoring of a total of 36,390 sentinel animals, we found 64 positive cases. The peak of positive cases was recorded in 2010 (63). Of 25,442 animals that were investigated in terms of movements of animals between farms, import of animals, as well as females after abortion, there were 185 positive samples (177 cattle, 8 sheep). The virological examination was carried out in 3,903 animals after their movements, imports or after abortion. Of this number, 52 animals showed the presence of nucleic acids of BT (Table 1).

The surveillance of bluetongue disease has been carried out according to Commission Regulation 1266/2007 (5). According to this regulation, all member countries are obliged to carry out surveillance of bluetongue consisting of entomological surveillance, clinical and serological monitoring of animals. Serological and virological surveillance should be implemented in the period of maximum vector activity, i.e. during the spring and summer months. Particular attention should be paid to newly introduced breeding animals, especially when imported from infected areas or animals on pasture.

Domestic and wild ruminants are susceptible to infection with BT. The global distribution of this disease has been localized in the regions with the occurrence of the vector – *Culicoides* midges. The most intensive testing was performed during the period of maximum vector activity, i.e. during the spring and summer months. Most sero-positive samples were found in the areas with the dominance of *Culicoides obsoletus* complex, i.e. in 2008 in the Nitra region (169 positive samples), and in 2010 in Trnava region (59 positive samples). The virological examinations of the animals were performed only in the case of positive serological findings. In our case 3903 samples were examined for the virus. Of this number 52 samples were positive for the virus. These were the animals investigated due to transfers, imports or animals after abortion or after the birth of dead offspring.

Our study covered all of Slovakia. Within regions, we tried to include a group of sentinel animals, which made a representative group of animals at risk of being infected with BT. The entire monitoring process was in accordance with the legislation for the control of BT. Those results are used to judge Slovakia as a possible BT free country.

ACKNOWLEDGEMENTS

The work was carried out within projects AV4/2041/08 and VEGA 1/0940/11.

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Selected papers from the 54th STUDENT SCIENTIFIC CONFERENCE with international participation, held at the University of Veterinary Medicine and Pharmacy in Košice on May 4, 2011.

Table 1. Number of examined and positive animals between 2008 and 2010

Test type	Investigated animals	Number of samples examined/ Number of positive samples that were recorded			Total
		Year 2008	Year 2009	Year 2010	
Serological testing	Sentinel animals	8,801/0	1,173/1	15,858/63	36,390/64
	Cattle – non-sentinel	9,200/175	15,139/2	-	24,339/177
	Sheep – non-sentinel	458/2	335/0	310/6	1,103/8
Virological testing	Cattle – non-sentinel	916/50	2,837/2	115/0	3,868/52
	Sheep – non-sentinel	4/0	11/0	20/0	35/0

FOLIA VETERINARIA, 55, 2: 55–57, 2011

STANDARDIZATION OF qRT-PCR IN ANALYSIS OF GENE EXPRESSION OF TLR RECEPTORS IN SWINE

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ABSTRACT

The aim of this study was to optimise qRT-PCR that was used to analyse the influence of β-glucans originating from *Pleurotus ostreatus* (pleuran, Natures) and *Saccharomyces cerevisiae* (Sigma) in three different concentrations (1 μg.ml^{-1}, 5 μg.ml^{-1}, 10 μg.ml^{-1}) on the changes of the gene expression of receptors TLR4 and TLR6 in the porcine intestinal epithelial line (IPEC-J2). Lipopolysaccharide (LPS) from *E. coli* (10 μg.ml^{-1}) was used as a control. The standardization of qRT-PCR included the selection of suitable primers and their optimal concentrations. The results of qRT-PCR revealed that lower concentrations of the tested glucans (1 μg.ml^{-1} and 5 μg.ml^{-1}) failed to significantly affect the gene expression of the investigated receptors in IPEC-J2 cells. On the other hand, the stimulation of IPEC-J2 cells with pleuran at the highest concentration (10 μg.ml^{-1}), as well as stimulation of cells with LPS, doubled the expression of mRNA receptors TLR4 and TLR6.

Key words: β-glucans; qRT-PCR; IPEC-J2; TLR

INTRODUCTION

TLR (Toll-Like Receptors) produce a large family of evolutionary-conserved receptors, serving as an important component of protection against invading micro-organisms. These receptors are expressed on cells of the immune system and also on cells that are constantly in contact with the outer environment (e.g. epithelial cells of the gastrointestinal and respiratory tracts). Therefore, their expression is not constitutive and changes rapidly in response to: pathogens; various cytokine; or environmental stress (1). Despite many *in vitro* and *in vivo* studies, the influence of immunomodulatory substances of the type of β-glucans on the expression of TLR receptors in swine, had been observed only at the level of blood mononuclear cells (2).

The aim of this study was to optimise conditions of the qRT-PCR method in relation to the analysis of the influence of β-glucans on changes in the gene expression of TLR receptors in the IPEC-J2 cell line.

MATERIAL AND METHODS

Cell line and cultivation conditions

Cells of IPEC-J2 (donated by VUVL, v. v. i., Brno, CR) were cultivated in complete medium in three 12-well plates at a concentration of 6×10^4 cells.ml^{-1} at 37 °C in an atmosphere of 5 % CO_2. After incubation for 72 h the following substances were added to the cells: glucan pleuran (Natures); glucan originating from *Saccharomyces cerevisiae* (Sigma); lipopolysaccharide (LPS) from *Escherichia coli* (positive control); and pure medium (negative control). The tests were carried out using three different concentrations of both types of glucans (1 μg.ml^{-1}, 5 μg.ml^{-1}, 10 μg.ml^{-1}) and one concentration of LPS (10 μg.ml^{-1}). After 24 h of stimulation the cells were stored at −20 °C in a conservation solution "RNAlater".

Isolation of RNA

Total RNA was isolated using a kit "Aurum total RNA" (Bio-Rad) and its concentration and purity was determined at 260 nm.

Transcription of RNA into complementary DNA (cDNA)

One μg of each RNA sample was transcribed by means of reverse transcriptase and primers oligo dT and Random (Fermentas).

qRT-PCR

The reaction mixture consisted of 1× SYBR Green Supermix (BioRad), 6 µl H2O, 1 µl forward a reverse primers (10 µM or 33 µM) and 5 µl of cDNA sample, which was prepared in duplicate. Thermal conditions were as follows: 95 °C for 3 min; 40 cycles (94 °C, 15 s; 58 °C, 30 s; 72 °C, 30.), 72 °C for 15 min with subsequent analysis of the melting curve.

RESULTS AND DISCUSSION

The first condition for the successful standardization of qRT-PCR was the control of the quality of individual cDNA samples which was confirmed by PCR with primers for β-actin and subsequent electrophoresis of amplicons in agarose gel. The second important step was the selection of suitable primers and their optimum concentration (10µM or 30 µM). According to our observations the lower concentration of primers (10 µM) appeared optimal for amplification of TLR4 and TLR6 (Fig. 1).

The relative expression of RNA receptors TLR4 and TLR6 was evaluated by the ΔΔCT method. It is based on the comparison of the quantity of individual observed genes with the so-called reference gene, the expression of which remains unchanged during the experiment (β-actin in our case) and also with the so-called calibrator (control sample of non-stimulated cells). These calculations give the so-called fold difference, or in our case, fold change in gene expression of the receptors TLR4 and TLR6 compared to non-stimulated control cells. Our results showed that the lower concentrations of the glucans used (1 µg.ml^{-1} and 5 µg.ml^{-1}) had no significant effect on the gene expression of the investigated receptors in the cell line IPEC-J2. On the other hand, the highest concentration of pleuran used in our study (10 µg.ml^{-1}), as well as LPS, resulted in a 2-fold expression of RNA receptors TLR4 and TLR6 (Table 1).

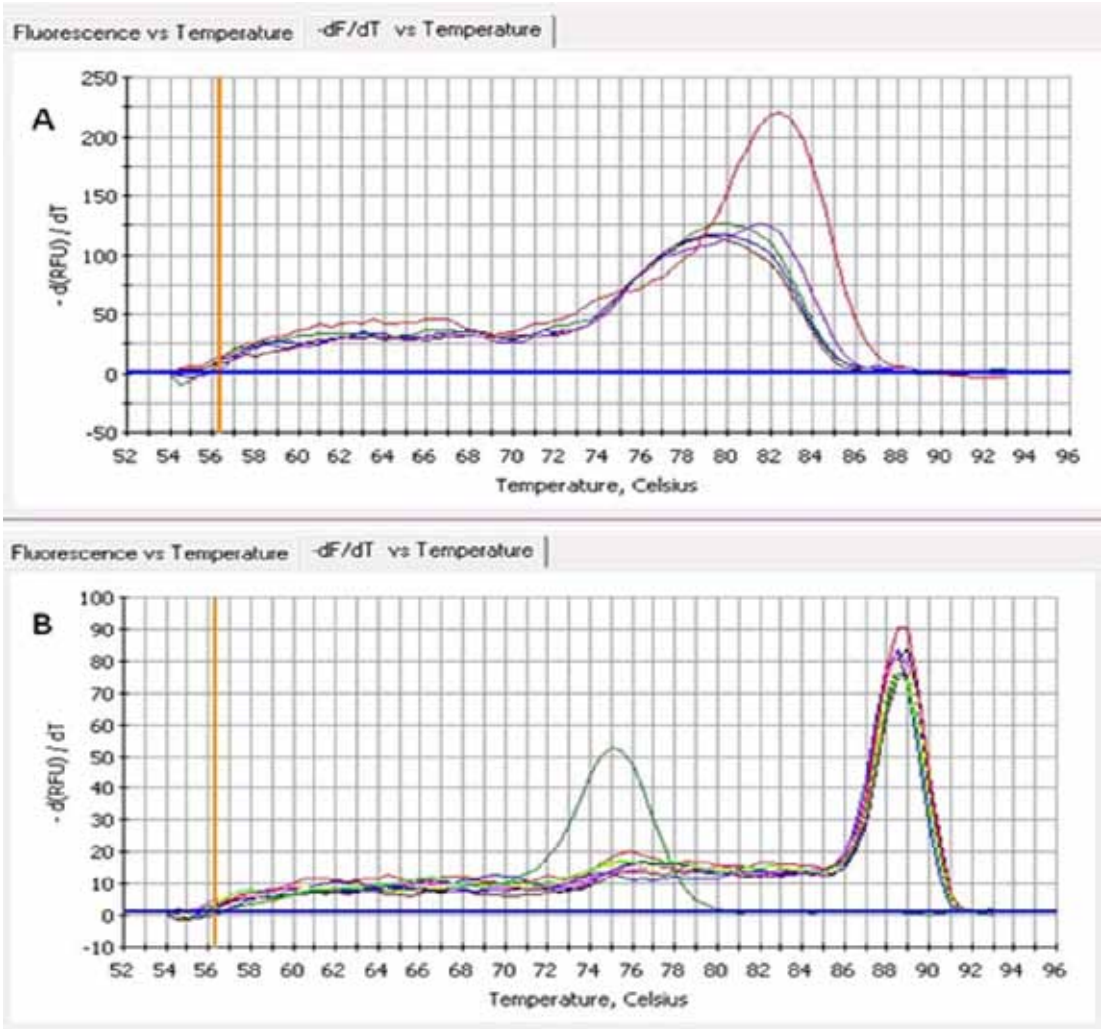

Fig. 1. Melting curve for the receptor TLR4: A – at 33 µM; B – at 10 µM primers concentration.
The green "peak" in part B is the negative control without template

Table 1. Fold change in gene expression of the receptors TLR4 and TLR6 (fold difference) compared to non-stimulated control cells and standardized against β-actin

	TLR4	TLR6
Non-stimulated cells	1.0	1.0
Cells stimulated with LPS	1.9	1.9
Cells stimulated with glucan from *Saccharomyces cerevisiae*	1.6	1.5
Cells stimulated with glucan from *Pleurotus ostreatus*	2.1	2.1

Presently, there are no available *in vivo* or *in vitro* studies dealing with the influence of pleuran on the gene expression of TLR receptors in swine. An increased expression of porcine TLR was recorded only after stimulation of alveolar macrophages, immature and mature dendritic cells with synthetic particular β-glucan and zymozan (2).

In our study we successfully standardized the conditions of qRT-PCR. The results obtained will be used in future experiments intended for testing the influence of higher concentrations of β-glucans or other potential immunomodulatory substances on IPEC-J2 cells not only with regard to expression of TLR receptors, but also expression of their adaptor signal molecules or selected cytokines.

ACKNOWLEDGEMENT

This study was carried out within the project "INFEKTZOON – the centre of excellence for animal infections and zoonoses", ITMS code 26220120002, and was supported by the operation programme Research and Development, financed by the European fund for regional development and by VEGA grant No. 1/0727/09 – Study of the effect of beta-glucans as modifiers of the biological response of farm animals.

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Selected papers from the 54th STUDENT SCIENTIFIC CONFERENCE with international participation, held at the University of Veterinary Medicine and Pharmacy in Košice on May 4, 2011.