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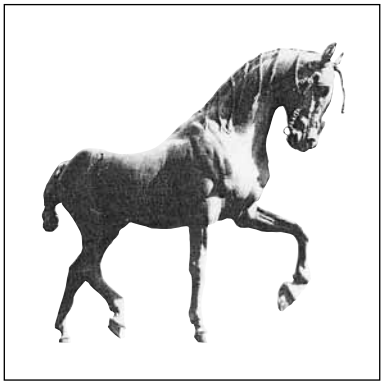

53rd STUDENT SCIENTIFIC CONFERENCE

April 15th, 2010

The aim of the 53rd Student scientific conference (ŠVOČ) organised in the academic year 2009/2010 was to present results of scientific investigations carried out by undergraduate students and young scientists. The papers were presented in the following four sections:

1. Preclinical – 2. Clinical
3. Hygiene of food and the environment
4. Young scientists

UNIVERSITY OF VETERINARY MEDICINE AND PHARMACY IN KOŠICE

FOLIA VETERINARIA, 54, 2: 57–60, 2010

HISTORY OF THOROUGHBRED BROODMARES REVEALED BY MITOCHONDRIAL DNA (mtDNA) ANALYSIS

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ABSTRACT

The Thoroughbred racehorse has been bred for over 300 years. The pedigrees of all Thoroughbreds are held in the General Stud Book (GSB). The female lines of the Thoroughbred can be traced to foundation stock, which according to GSB pedigrees date to the middle of the 17th century. With the use of modern DNA techniques, it is possible to place these maternal ancestral lines in a global context. In general, the results suggest that the Thoroughbred maternal line descends from the Far East. Therefore, the history of man and horse must be considered together as this provides historical explanations for the introduction of Thoroughbred stocks in England in the mid 17th century.

Key words: Far East; Genghis Khan; King Charles II; mtDNA; Thoroughbred

INTRODUCTION

The Thoroughbred, first bred in the 17th and 18th centuries in England, has become one of the most valuable livestock commodities in the world. The first recording of the pedigrees of the Thoroughbreds were taken and established in the General Stud Book (GSB) in 1791 AD (12). William Towers, the man entrusted with compiling the GSB had to contend with breeders who were happy to pass off their horses as being more fashionably bred than they actually were (12). Some of the errors in the pedigrees described in the earliest volumes of the GSB linger today (2). This first volume contains pedigrees dating as early as 1657 (12).

Thoroughbreds have been designated to certain family numbers, according to a scheme proposed by Australian racing pundit Bruce Lowe in the 1800's (7). Lowe divided the Thoroughbreds into different female families, according to the number of classic winners prior to 1890 in England (Derby, Oaks, 2000 Guineas, 1000 Guineas, and St. Leger). Hence, family 1 had the most classic winners; family 2 had the second most, and so on. The family number details the female line that an individual descends from, which is referred to as the female tail-line. Thus, every Thoroughbred in the world today comes from one of these 43 female families.

Mitochondrial DNA (mtDNA) was first demonstrated to be maternally inherited (i.e. from mother to offspring) in horses by Hutchinson *et al.* (3). Analysis of mtDNA sequences is thus often used to assess female ancestral relationships. Based on this approach, a recent study performed by Hill *et al.* (2) suggested that only 30 founder mares have contributed 94% of the modern maternal lines. They found that the putative founders for 25 Thoroughbred family lines screened could be attributed to 12 unique mtDNA sequence types (termed "haplotypes"). This was the first study to use molecular markers to assess the accuracy of pedigrees held within the GSB volumes. However, this study did not probe deeper to assess the potential origins of the Thoroughbred lines in relation to European and Asian wild horse populations and breeds.

The purpose of this study was to use mtDNA to reconstruct the genealogical history of Thoroughbreds in relation to other breeds.

MATERIAL AND METHODS

Blood samples were taken and the DNA extracted using standard kits. The polymerase chain reaction (PCR) was used to amplify the target regions of DNA, using primers described by Hill *et al.* (2), and the procedures described by Jansen *et al.* (4). PCR products

were sequenced using an ABI 3730 automated sequencer, following a cycle sequencing reaction. Chromatographs were corrected and sequences aligned to one another and to published sequences.

289 published sequences were downloaded from GenBank (a publicly accessible database that holds sequence data), from the studies of Mc Gahern *et al.* (8, 9), Hill *et al.* (2), Kim *et al.* (5), L o p e s et al. (6) and J u r a s and C o t h r a n (unpublished) to allow the Thoroughbred to be placed in a global context to other breeds. These sequences represented 18 wild horse populations and breeds. Europe was represented by sequences from Irish Draught, Kerry Bog Pony (both from Ireland), Pura Raza Espanol, Lusitano (both from the Iberian peninsular in Spain), and Zemaitukai (an ancient population from Lithuania) breeds, as well as four ancient horse sequences sampled from wild horse fossils from Ireland and England (ranging in age from 1, 200–32, 255 years ago). The Kazakhstan region of central Asia was represented by the Mesenskay (Northern Russia), Orlov (Caspian Sea area), and Akhal-Teke (Afghanistan) breeds. The Far East was represented by sequences from Mongolia, Guan Mountain (Eastern China), Yakut (North-Eastern Russia), Vyaskaya (Northern Russia), Cheju (North Korea) and Yunnan (China).

Each unique sequence type from the downloaded samples was assigned a haplotype number (HAP1-59); Thoroughbred samples were labelled with the haplotype designations used by Hill *et al.* (2) (HAPA-L). The dataset were condensed into a file containing haplotype designations and the population in which they were found. Relationships among haplotypes were visualised by reconstructing a genealogical tree, using a minimum evolution optimality criterion (which is based on reconstructing a pair wise distance matrix of different sequences).

RESULTS

For new sequences, all family designations matched those proposed by Hill *et al.* (2), including the conclusion that families 7 and 22 share a female ancestor. Figure 1 shows a genealogical tree tracing ancestry of the thoroughbred lines in relation to other populations. The tree is rooted with sequences from Mongolia (Far East; haplotype 58) and shows haplotype designations, along with letters indicating in what regions that particular sequence type is found ("E" for Europe; "FE" for Far East; and "CA" for Central Asia). Thoroughbred haplotypes are boxed. Thoroughbred sequences can be divided into 5 distinctive groups (clades labelled I to V on Fig. 1). Thoroughbred haplotypes A, E, F, G, H, I, J, K are shared with samples from other populations (Fig. 1), whereas haplotypes B, C, D and L are only found in Thoroughbreds. All except one of the ancient horse samples (including samples from Lithuania, Ireland, and Przelwalski's horse) are most closely related to Thoroughbred haplotype A (which is only found in family 9); the other ancient European sample (from England) is identical to Thoroughbred haplotype G, which is also found in samples from other European populations, the Far East, and Central Asia. Thoroughbred haplotypes B-G (Clade IV; Fig. 1) are very similar to one another in sequence and are closely related to haplotypes found in other European populations, the Far East and Central Asia. Haplotype K shares a common ancestor with these samples but is quite divergent in sequence. Haplotype L (Clade III) is the only Thoroughbred sequence that is not closely related to a haplotype from the Far East; it appears to have its roots in Central Asia and Europe. Haplotype A (Clade) is most closely related to other European haplotypes and to one found in Central Asia and Russia (Hap3) but the next closest relatives to this clade are from the Far East (haplotypes 7, 8, 9). Thoroughbred haplotypes H and I (Clade V) are closely related to one another and to haplotypes from all three geographic regions. Haplotype J (Clade II) is not closely related to any of the other Thoroughbred haplotypes but is again closely related to those from each of the other geographic regions. All of the haplotypes that form the base of the tree are from the Far East (from Mongolia, Russia and China), which would be interpreted as an indication that they are the ancestral sequences for all modern horse populations and breeds studied.

DISCUSSION

The foundation mares were established between c. 1650–1718 (12). If these dates are to be believed, it is remarkable that it is possible to trace them back this far. However, little research has been performed to trace the foundation mares further back before these dates. The maternal inheritance of mtDNA and its rate of evolution make it ideal for reconstructing relationships among matrilines and can be used as a forensic tool to uncover historical patterns. A recent study used this approach to examine relationships of Irish horse populations to many breeds from around the globe. Since its domestication some ~4,000 years ago (4) the horse has been used widely by humans. Its high mobility and association with humans up to recent times has allowed for mixing of horse breeds, which "has led to an obscuring of the genetic structure within the species" (9). Mc Gahern *et al.* (9) states that the obscuring of the genetic structure is most often seen in European breeds, whereas for more isolated populations, such as those in eastern Asia, historical relationships tend to be clearer. Mc Gahern *et al.* (9) considered samples taken from the Mongolian, Akhal-Teke, Guan Mountain, Mesenskay, Orlov, Vyatskaya, and Yakut populations to be isolated. Recognizing these potential limitations, the purpose of this study was to combine all available data on mtDNA variation in horses to identify potential source populations for modern Thoroughbreds and to put these relationships into the context of human activities in Europe.

From the sharing of haplotypes and the genealogy (Fig. 1), it appears that most of the Thoroughbred families can be traced to the Far East, including Mongolia, Russia and the Guan Mountains of China. Thoroughbred clades II–V include haplotypes of Far Eastern origin and the closest relatives to clade I (haplotype A; family 9) are also from these regions. All Thoroughbred families are also closely related to individuals from the Kazakhstan region of Central Asia and to other European breeds. With the advent of modern transportation, the transport of horses across the globe has become possible. However, Mongolia has been geographically and politically

isolated for centuries. Therefore, it is more likely that the samples from this region used by McGahern *et al.* (8, 9) represent ancestral populations for modern Thoroughbreds rather than Thoroughbred lines being taken to Mongolia or other parts of the Far East, in more recent times. To evaluate this hypothesis, the genealogy in Fig. 1 is drawn from the perspective of Mongolian haplotypes being ancestral. Since all of the other basal sequences are also from the Far East (i.e. they are closely related to sequences from Mongolia), this suggests that all modern breeds and populations could have arisen from maternal ancestors in this region. Genghis Khan (born c. 1162) invaded Mongolia's modern day political giant China in c. 1211, and subdued the Ch'in Empire (10). After Khan's death in c. 1227, his successors continued invading west across Asia and into Europe (10). It was his grandson Batu, who directed the invasion of Europe; Kiev in c. 1240, Poland and Hungary followed and in c. 1241 a Christian army was annihilated at Legnica (western Poland) (10).

It seems plausible that horses used by the Khan's invasion of Western Europe were left behind and inter-bred with local populations within Europe. Breeding stock from these European populations were taken to perpetuate the Thoroughbred in England, which just happened to have their ancestry rooted in Mongolia and the Far East. The personal memoirs of Captain Byerley describe how in 1688 he brought back several breeding mares and one of the foundation sires, Byerley Turk, as part of the spoils of war following the defeat of the Turks at Buda, Hungary (1). This adds weight to the notion that horses brought from Western Europe had Mongolian and Far Eastern maternal ancestors, due to the fact that Khan's army ransacked Hungary in c. 1241 (10). Another possibility is that the mares were brought over from the East for the sole purpose of being bred to produce faster horses. Horses could have been brought over to England in the 1600's from the East. It only takes a little research to show that there were major links between England and the East during this time. The monarch at the time of the re-establishment of the royal studs was King Charles II. The link comes via his marriage. In 1662 he married Catherine of Braganza, daughter of the King of Portugal (11). The marriage dowry included "...the port and island of Bombay (India), the port of Tangier, and about £330,000 in sugar, money, and Brazilian mahogany" (11). Thus the port of Bombay was handed to the British through this marriage. From historical records it was known that Genghis Khan and his successors had dealings in India (9). Horses could have been taken to India, interbred with populations there, and subsequently taken to England. It should also be noted that Babur, a direct descendant of Genghis Khan, founded the Mughal Empire in India when he invaded India from Afghanistan in the 1520's (10). This reinforces the idea that horses could have been brought from Mongolia to India to be used in that country.

CONCLUSION

Comparison of mtDNA sequences can be a powerful tool to uncover historical events, through the tracing of ancestral relationships among extant populations. For the Thoroughbred families considered in this study, there was a preponderance of ancestors with Asian origins. The molecular data gathered here, along with historical evidence (including the personal memoirs of Captain Byerley) together support a hypothesis that the foundation stock for many of the modern Thoroughbred families were brought by Genghis Khan and his men travelling across Asia and into Eastern Europe. This conclusion is based on the finding that the majority of the Thoroughbred families examined in this study share the same mtDNA haplotype as breeds from intermediate areas between the Far East and Europe. Horses brought by the invaders could have interbred with local horse populations or Mongolian horses could have been left to form their own herds. More thorough sampling of horses in this region and consideration of nuclear genes that would also reflect paternal relationships could shed more light on the exact origins of the Thoroughbred lines.

REFERENCES

1. Haralambos, K. M., 1990: *The Byerley Turk: Three Centuries of the Tail Male Racing Lines.* The Kenilworth Press Ltd, London, 168 pp.

2. Hill, E. W., Bradley, D. G., Al-Barody, M., Ertugrul, O., Splan, R. K., Zakharov, I., Cunningham, E. P., 2002: History and integrity of the thoroughbred dam lines revealed in equine mtDNA variation. *J. Anim. Genet.,* 33, 287–294.

3. Hutchinson, C. A., Newbold, J. E, Potter, S. S., Hall, E. M., 1974: Maternal inheritance of mammalian mitochondrial DNA. *J. Nature,* 251, 536–238.

4. Jansen, T., Forster, P., Levine, M. A., Oelke, H., Hurles, M., Renfrew, C. *et al.*, 2002: Mitochondrial DNA and the origins of the domestic horse. *Proc. Natl. Acad. Sci. U.S.A.,* 99, 10905–10910.

5. Kim, K. I., Yang, Y. H., Lee, S. S., Park, C., Ma, R., Bouzat, J. L., Lewin, H. A., 1999: Phylogenetic relationship of Cheju horses to other horse breeds as determined by mtDNA D-loop sequence polymorphism. *J. Anim. Genet.,* 30, 102–108.

6. Lopes, M. S., Mendoca, D., Cymbron, T., Valera, M., da Costa-Ferreira, J., da Camara Machado, A., 2005: The Lusitano horse maternal lineage based on mitochondrial D-loop sequence variation. *J. Anim. Genet.,* 36, 196–202.

7. Lowe, C. B., 1914: *Breeding Racehorses by the Figure System,* 2nd edn., **W. Allison,** ed., The field and Queen (Horace Cox) Ltd, UK, 308 pp.

8. McGahern, A. M, Edwards, C. J., Bower, M. A., Hefferman, A., Park, S. D. E., Brophy, D. G. *et al.,* 2006a: Mitochondrial DNA sequence diversity in extant Irish horse populations and in ancient horses. *J. Anim. Genet.,* 37, 498–502.

9. McGahern, A., Bower, M. A. M., Edwards, C. J., Brophy, P. O., Sulimova, G. *et al.,* **2006b:** Evidence for biogeographic patterning of mitochondrial DNA sequences in Eastern horse populations. *J. Anim. Genet.,* 37, 494–497.

10. Parker, G, Stone, N., Overy, R., Barraclogh, G., 2001: *The Times: History of the World,* 4th edn., Times Books, Harper Collins Publishers, London, UK. 192 pp.

11. Pearson, H., 1960: *Charles II.* The Windmill Press Ltd., Surrey, UK, 274 pp.

Fig. 1. A genealogy of relationships between Thoroughbred horses and individuals from populations and breeds from around the world

The tree is rooted with a Mongolian sequence (Hap58) and shows branch lengths proportional to the degree of differentiation between sequences (each unique sequence is designated as a different haplotype). Thoroughbred haplotypes are boxed and clades (groups of haplotypes sharing a common ancestor more recently with each other than with other sequences) including Thoroughbreds are designated by a roman numeral (I to V).

12. Weatherbys & Sons, 1971: *An Introduction to a General Stud Book.* Wearherbys & Sons, London.

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Selected papers from the 53rd STUDENT SCIENTIFIC CONFERENCE with international participation, held at the University of Veterinary Medicine and Pharmacy in Košice on April 15, 2010.

FOLIA VETERINARIA, 54, 2: 61–63, 2010

ACTINOBACILLUS EQUULI ENZOOTIC IN A HORSE HERD

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ABSTRACT

Normally horse lungs contain only small numbers of potential bacterial or fungal pathogens as they are typically removed by the normal protective lung mechanisms. If they are present, they are considered transient contaminations. However, if these mechanisms are disturbed or lung immunity suppressed, proliferation of such contaminants can become pathogenic to the host. The most frequent source of contaminations of the lower respiratory tract is aspiration of microorganisms that constitute the physiological microflora of the upper respiratory tract. *Actinobacillus equuli* is associated with a considerable number of infections including; peritonitis, arthritis, endocarditis, enteritis, mastitis and abortions in horses of all age categories. The bacteriological examination of transtracheal aspirates of 10 horses (Quarter Horse breed), of mean age 1.33 years, from the investigated herd, allowed us to diagnose bronchopneumonia caused by the pathogenic *Actinobacillus equuli*. The incidence of this problem in foals reached 100%, but the susceptibility to antibiotics varied. Mycological examinations demonstrated concomitant findings of moulds of the genera *Aspergillus, Rhizopus* sp., *Penicilium* sp., *Mucor* sp. and *Candida* sp. in all horses.

Key words: *Actinobacillus equuli;* bacterial bronchopneumonia; horse

INTRODUCTION

In the majority of horses we find mixed bacterial microflora consisting most frequently of Gram-positive pathogens (*Streptococcus equi, Staphylococcus aureus, Streptococcus pneumoniae*), Gram-negative pathogens (*Pasteurella caballi, Actinobacillus equuli, Escherichia coli, Klebsiella pneuioniae, Bordetella bronchiseptica*) and anaerobes (*Bacteroides fragilis, Peptostreptococcus anaerobius* and *Fusobacterium* spp.) (5). When classifying bacterial respiratory diseases in horses, the occurrence of individual pathogens is associated with four basic bacterial respiratory syndromes, namely: streptococcosis of horses; foal bronchopneumonia syndrome; inflammatory airway disease (IAD), (i.e. inflammation of respiratory tract and pneumonia); and pleuropneumonia of adult horses.

The foal bronchopneumonia syndrome is frequently found in foals up to 9 months of age. It is caused by resident bacteria which, in the case of the normal immunity of the respiratory tract, are opportunistic pathogens. The immunity of the respiratory tract may be impaired by viral infections, transport, weaning of foals, total anaesthesia or other various stressful situations. *Actinobacillus equuli*, as an ubiquitous commensal of upper respiratory tract in all animals is classified as a non-hemolytic *A. equuli* subsp. equuli (causing predominantly neonatal septicaemia known as „Sleepy foal disease“) or haemolytic *A. equuli* subsp. *Haemolyticus,* associated mostly with lower respiratory tract diseases, infections of other organ systems in older horses (peritonitis, arthritis, endocarditis, enteritis, mastitis) and abortion in horses of all age categories (5).

MATERIAL AND METHODS

To make the diagnosis, we supplemented clinical examinations with bacteriological and mycological examinations of the lung aspirates (carried out at ŠVPÚ Dolný Kubín, Testing laboratory Prešov), obtained by the TTA (transtracheal aspiration) method from 10 horses. The gender ratio was 5 mares to 5 stallions, with

the mean age being 1.33 ± 1.26 SD year (0.25–3 years) and the breed was the American Quarter Horse (AQH). Each sample was tested separately for antibiotic susceptibility using 18 antibiotics (Amoxicilín, Amoxicilín+ Klavulanová acid, Cefalexin, Cefquinome, Ceftiofur, Colistin, Doxycycline, Enrofloxacin, Erytromycin, Florfenicol, Flumequin, Gentamycin, Lyncospectin, Oxytetracyklin (OTTC), Penicilin (PNC), Streptomycin (STM), Sulfamethoxazol+ Trimetoprim, Tetracyklin (TTC)). Bacteriological and mycological protocols served as a basis for determining the antibiotic susceptibility. In addition, adequate antibiotic therapy of the affected horses was supported by mucolytic therapy.

RESULTS

Bronchopneumonia caused by the pathogen *Actinobacillus equuli* was diagnosed in the observed herd. The mean age of the horses in the herd was 6.31 ± 5.73 SD years (0.25–30 years). The incidence of the disease reached 75 % in the foals and 76.5 % in the horses up to 3 years of age. No disease was detected in horses older than 3 years (Fig. 1).

In the affected horses we observed: apathy; fever; productive coughs of various frequency and intensity; along with bilateral mucinous discharge from the nostrils. The auscultation findings were mostly unilateral and situated at the caudal lung margins. Mycological examinations showed concurrent findings of fungi of the genera *Aspergillus* (*A. fumigatus, A. niger, A. flavus*), *Rhizopus* sp., *Penicilium* sp., *Mucor* sp. and *Candida* sp. (Fig. 2). In individual horses we detected various combinations of the mentioned fungi. Uniform findings were observed only in repeatedly treated foals.

The susceptibility to the antibiotics tested varied (Fig. 3). Antibiotic therapy was supplemented with mucolytics and we also increased the iodine supply in the feed to eliminate fungi infiltration. Antibiotics were administered for 8–10 days depending on the antibiotic type and health status of the

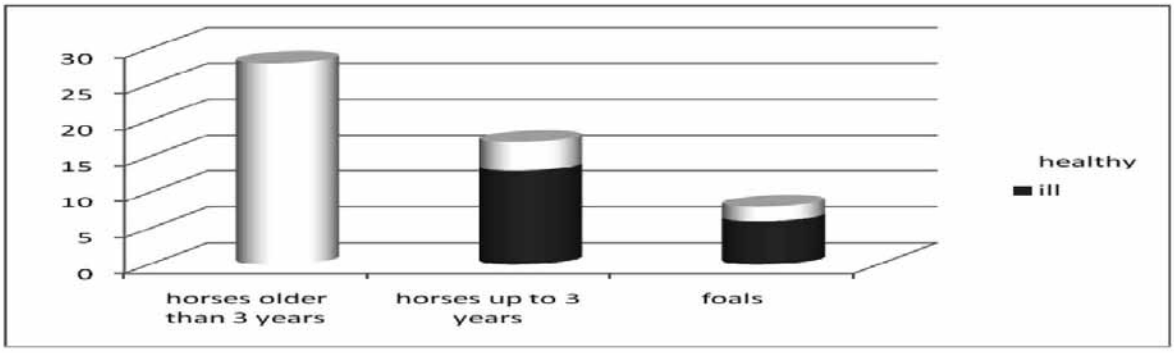

Fig. 1. Incidence of *A. equuli* in the herd according to age structure

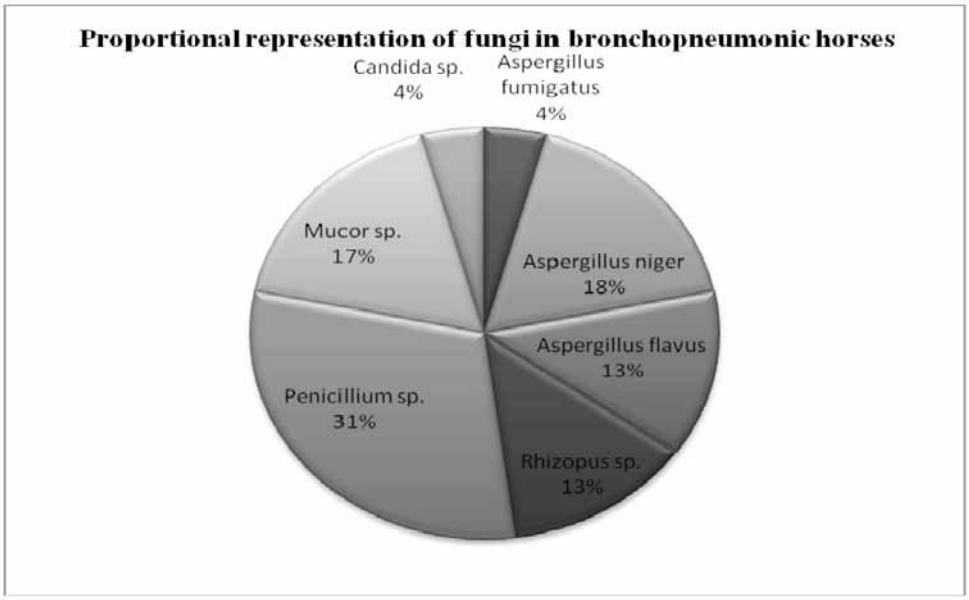

Fig. 2. Proportional representation of fungi isolated from transtracheal aspirates

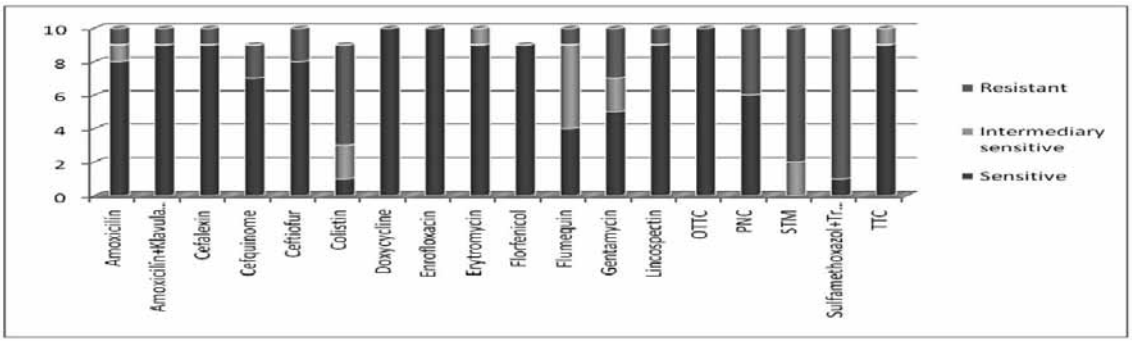

Fig. 3. Susceptibility of A. equuli to individual antibiotics.

horses. Mucolytic treatments continued up to the complete disappearance of any nasal discharge.

DISCUSSION

Actinobacilloses are observed worldwide, particularly in foals within the first 12–72 weeks of their life, and they can also induce gastrointestinal diseases accompanied with septicaemia (1). In Slovakia, the first occurrence of *Actinobacillus equuli* was recorded in the herd investigated in our study in which it caused enzootic bronchopneumonia. Affected horses were younger than 3 years of age and independent of the gender. The alleviation of clinical symptoms was evident on days 3–5 and the total convalescent period lasted 13–18 days from the beginning of the antibiotic therapy.

REFERENCES

1. Carter, G. R., Davis, E., 2007: Microbial Diseases: A through D. In *A Concise Guide to the Microbial and Parasitic Diseases of Horses, International Veterinary Information Service,* Ithaca NY (www.ivis.org).

2. Gyles, C. L., Prescott, J. F., Songer, J. G., Thoen, C. O., 2004: *Pathogenesis of Bacterial Infections in Animals,* 3rd edn., Blackwell Publishing, USA, 225–234.

3. McGorum, B. C., Dixon, P. M., Robinson, N. E., Schumacher, J., 2007: *Equine Respiratory Medicine and Surgery,* Saunders Elsevier, USA, 347–349.

4. Reed, S. M., Bayly, W. M., Sellon, D. C. 2004: *Equine Internal Medicine,* 2nd edn., Saunders Elsevier, USA, 289–336.

5. Sellon, D. C., Long, M. T. 2007: *Equine Infectious Diseases,* Saunders Elsevier, USA, 1–12.

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THERAPY AND CONSEQUENCES OF VENOMOUS SNAKE BITES

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ABSTRACT

The correct therapy is essential for saving lives after venomous snake bites. Many people, including human doctors, have used old methods that can be ineffective or even harmful. This study describes novel, correct appropriate therapy which has been validated by professionals. It consists of laical first aid which is very important to slow down the effect of poison and preserve all of the important functions of life. After arrival of the emergency squad, the appropriate medical first aid can be provided including professional pharmacological treatments. The last stage consists of the hospital treatment with a very high level of care provided by specialists. We describe also the consequences of a Lataste's viper (*Vipera latastei*) bite when the correct appropriate first aid was not provided.

Key words: first aid; Lataste's viper; snake bite consequences; therapy

INTRODUCTION

The correct therapy is essential for saving lives following poisonous snake bites.

Laical first aid. The basic first aid rule is to keep the victim calm and have them behave rationally. Any excitement and movement only worsen the intoxication. The following rule is to call the emergency squad (ES) as soon as possible. One should remember that the immobilisation of the affected site or bitten extremity is important for slowing down the intoxication. It is not recommended to apply the conventional constricting tourniquet to prevent venous return. The spreading of the poison from the bite site is slowed down by pressure immobilisation bandages (PI) according to Sutherland. If, for any reason, it is not possible to apply PI (e.g. in case of evident intoxication with a deadly neurotoxin such as in the venom of *Elapidae* snakes), one can apply in exceptional cases a tight arterial tourniquet to delay the paralysis of the respiratory muscles. It is applied above the snake bite in the torso direction. After its application, the pulse at the limb periphery must not be palpable. The tourniquet should be soft and elastic to prevent damage to the skin and nerve bundles and should be released for 15 sec every 30 min. It can be used for a maximum of 2 hours. This method can save lives under certain extreme situations. However it always increases the extent of the local damage to the affected tissue up to the possible development of gangrene. The use of this method should be considered carefully.

To treat people bitten by small European vipers, such as *Vipera berus*, it is recommended to use loose bandage instead of PI to prevent necrosis under the action of cytotoxin and proteolytic enzymes. Traditional and historical manipulation with the site of snake bite, such as "cutting and sucking", rinsing with oxidative solutions, phenols, potassium permanganate, hydrogen peroxide, etc., are ineffective and potentially dangerous.

If we expect an early arrival of the emergency squad, an increased supply of liquids is not recommended. In the opposite case, under extreme conditions and unavailability of emergency services, particularly when the person was bitten by *Viperidae snakes*, we can offer easily absorbed liquids, such as slightly sweetened water, weak or herbal tea. Drinking coffee or alcohol is dangerous and may worsen the course of the intoxication. The use of laical sedatives or analgesics is not recommended except for extreme situations. If the venom of the spitting cobras *Naja* and *Hemachatus* hits the eyes it is most important to rinse them as quickly as possible with a stream of clean water, milk or other non-toxic, non-irritant and non-infectious

fluid. The patient should be transported by emergency service or, under extreme conditions, with a minimum physical load on the afflicted person.

Medical first aid. Sedatives (the best choice being parenteral benzdiazepins) should be administered, particularly to anxious and hysterical patients. An alternative is chlorpromazine with parallel antiemetic effect, in the case of intoxication with *Viperinae venom*. The dosage is 25–50 mg.kg^{-1} b.w. for adults and 1 mg.kg^{-1} b.w. for children. Analgesics paracetamol or nonsteroidal antiflogistics with analgesic effect, (e.g. ibuprofen) may be administered for pain which can be locally severe. To prevent an allergic reaction to the venom components and development of angioneurotic oedema, parenteral corticosteroids (hydrocortisone at a dose of 2–4 mg.kg^{-1} b.w., i.v.) can be administered and supplemented with antihistaminics. It is necessary to check the PI. The wound should be disinfected locally.

In the states which require identification of venom with an identification kit when there is an uncertainty about snake species, such as in Australia, the snake bite site is not disinfected to preserve venom residues around the wound. Anaphylactic shock is treated by adrenalin at a dose of 0.3–0.5 ml.kg^{-1}, i.m. (0.3–0.5 mg diluted 1 : 1000), 0.01 ml.kg^{-1} b.w. i.m. in children, repeatedly every 5–15 min until the stabilisation of the circulation and is supplemented with volume therapy. We also administer corticosteroids, methylprednisolone 125 mg i.v. (1 mg.kg^{-1} b.w. in children), repeatedly every 6 hours, and antihistaminics, e.g. H1-receptor-antagonist. Therapy for the circulation collapse or shock must be supplemented with oxygen therapy. Rhythm and contractility disorders are treated symptomatically. The eyes affected by the spitting cobra venom, should be rinsed at the laical first aid and irrigated with clean water, saline or borax rinse. The case may be consulted with the national toxicological centre.

Hospital treatment. It includes administration of respective antiserum, symptomatic treatment or plasmapheresis and subsequent convalescence after intoxication.

The effect of *Vipera latastei* and *Vipera berus* (a little stronger venom) is comparable with the most deadly venoms of the pit viper of the genus *Bothrops*, or rattlesnakes of the genus *Crotalus*. Because of their smaller size the rattle snakes produce less venom. The LD50 for *Vipera berus* is 0.11 mg.kg^{-1} but the maximum quantity used is 10–18 mg (1).

MATERIAL AND METHODS

Observation of intoxication after a *Vipera latastei* snake bite.

RESULTS

Our observations involved a *Vipera latastei* bite on the medial surface of the right leg, just above the firmly tied hiking shoe which acted as an arterial tourniquet. The fang tip spread was 5.61 mm and drops of blood were seen in the wounds. In two hours a slightly painful dark blue haematoma developed running horizontally and parallel with the edge of the strongly tied shoe. Eight hours after the bite and a very demanding hike, we took off the shoe. The haematoma was darker, almost black and enlarged, 10 cm long, 2.5 cm below the bite and tapered in the caudal direction (almost triangular shape). After additional 2 hours from taking off the shoe the haematoma extended by 7 cm dorsally and 7 cm ventrally. Swelling was observed ventrally from the bite.

On the second day, the pain became more intensive which was reflected in the unnatural gait. In the following days, the swelling slightly diminished but the haematoma and painfulness persisted. After 10 days the pain slightly abated. In the central zone, the site of the longest action of the venom, we observed necrotic tissue and a small concave depression.

It took 6 months for the bite site to return to normal. No therapy was used in this case; not even analgesics, only the inguinal nodes were checked for enlargement. The black centre of the necrotic depression developed due to the long lasting action of the venom in one place as the leg was strongly trussed in the shoe which acted as a constricting tourniquet. Only after taking off the shoe did the venom spread to the surrounding tissues and this slowed down its destructive cytotoxic and enzymatic action. In such cases a loose bandage is recommended.

DISCUSSION

Snake bite therapy consists of laic first aid, medical first aid and hospital treatment (1).

It is necessary to remember that the best and safest approach is to use the correct appropriate therapy procedures mentioned in the reference. This can save one's life. One should not try to suck the venom from the wound. Although it is not toxic at swallowing because of its proteinaceous nature and subsequent denaturation by the digestive juices, there is a risk of its penetration into blood through micro traumas in the mouth cavity mucosa. Also it is not recommended to cut the bite wounds because it is seldom known to which side the fangs injected the venom; the fangs are bent and their length is unknown, so we may speed up the penetration of the venom into the blood. The snake bite wounds should not be wiped with irritating substances nor dirty things which could introduce to the wound not only snake venom but also secondary infectious agents. The best therapy is prevention! We should respect these animals!

In conclusion we can state with relief that the described case of snake bite ended without any long term physical or psychical implications. However, it is not always so. Unfortunately, venomous bites can have some serious consequences.

REFERENCE

1. Valenta, J., 2008: *Venomous Snakes: Intoxication, Therapy* (In Czech). 1st edn., Prague, Galen, 401 pp.

*

Selected papers from the 53rd STUDENT SCIENTIFIC CONFERENCE with international participation, held at the University of Veterinary Medicine and Pharmacy in Košice on April 15, 2010.

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PRELIMINARY ANALYSIS OF FLAVONOIDS IN PROPOLIS AND ITS NATURAL SOURCES

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ABSTRACT

The bee product propolis is a mixture of substances with a wide spectrum of effects. Because of that it has become a subject of interest with the aim of determining the composition and proportion of individual groups of substances responsible for its biological activity. The most recent results indicate that the carriers of the antibacterial, antivirotic, antimycotic and anti-inflammatory effect are due to the flavonoids, aromatic acids and their esters. The present study focused on thin layer chromatographic analysis of basic flavonoids in propolis originating from Eastern Slovakia and on the analysis of its sources – buds of some trees. The quantitative evaluation of propolis involved the spectrophotometric determinations of the biologically effective flavones and flavonoles. Thin layer chromatography on Silicagel 60 in the system chloroform : methanol : benzene with detection under UV light at 254 nm showed the presence of chryzen, tektochryzen, galangin and pinobanksin in all samples of propolis analysed in our study. Identical aglycones were found in extracts of poplar buds subjected to acid hydrolysis. Our results indicate that the flavonoids dominating in propolis from Eastern Slovakia are the same as those identified in recently analysed samples. The mean content of flavones and flavonoles in propolis was 6.05%. A follow-up of this analysis will focus on HPLC analysis of other chemical groups, particularly aromatic acids and their esters which may exhibit allergenic effects.

Key words: propolis; spectrophotometry; thin layer chromatography

INTRODUCTION

Propolis (bee glue) is a sticky material collected by bees (*Apis mellifera,* L.) from tree buds and other plant parts, acting as a protective substance against freezing temperatures, bacterial, mycotic or viral infections and other potentially harmful entities and effects. Bees collect this material from various trees, such as poplar, birch, beechnut, willow, horse chestnut, alder and various conifer, process it metabolically and mix it with wax to obtain propolis. The composition of propolis and its properties depend on the local flora and the climatic conditions of the geographical area from which the bees collect the secretions (4).

It has been generally accepted and chemically proven that resin from poplar buds, particularly from the buds of black poplar *Populus nigra (Salicaceae)* (1), is the main source of propolis in the temperate region. Thus, the European propolis contains typical poplar phenolic compounds, such as flavonoids, aromatic acids and their esters (7).

Analysis of propolis proved its complex nature and the presence of more than 300 natural compounds (6). Polyphenols (include flavonoids, phenolic acids and their ester) are considered the most important biologically active compounds in propolis due to their well known ability to inhibit specific enzymes, stimulate some hormones and neurotransmitters and capture free radicals (3).

The effectiveness of propolis was tested in Denmark on a sample of 16 000 patients suffering from various diseases and positive results were obtained in 96 % of the patients. The flavonoids and phenolic acids present in propolis have antibacterial, antifungal, antivirotic, antineoplastic, hepatoprotective, immunomodulation,

anti-inflammatory and many other positive pharmacological effects. Their use appeared beneficial in the therapy of allergies, asthma, diabetes, hypertension, increased cholesterol and other indications. Many of these pharmacological effects were associated with its antioxidant activity (5).

MATERIAL AND METHODS

We examined samples of propolis from different parts of East Slovakia Lowlands and also the buds from poplar, willow and birch trees collected from the area within 1 km around the bee hives located in the village of Čeľovce. One gram of a crushed propolis sample was used to prepare an ethanol extract 1 : 10 by weight.

In the buds, flavonoids are bound to the sugar component in the form of glycosides while propolis contains only flavonoid aglycones. Under laboratory conditions glycosides are converted into aglycones by means of acid hydrolysis.

The required sample aliquot (1 g) was transferred into a flask, wetted with 3 drops of concentrated HCl and covered with 10 ml of 96.5 % ethanol. The prepared mixture was heated under a reflux condenser for three hours at 95 °C. After filtering the solvent, it was evaporated and the residue was dissolved in 2 ml of 96.5 % ethanol.

Thin layer chromatography (TLC)

Appropriate volumes of standard solutions and propolis and buds extracts were applied to aluminium foil backing TLC Silikagel 60 F254, (20 × 20 cm) plates of layer thickness 0.2 mm (Merck, Germany). A mixture of chloroform, benzene and methanol was used as a mobile phase. To determine the less polar flavonoids, isalpinin and tektochryzen (due to the presence of metoxyl group in the molecule) the mobile phase was prepared using the above mentioned solvents at a ratio of 89 : 10 : 1, for determination of galangin, chryzen and pinobanksin, with free hydroxyl groups, at a ratio of 85 : 10 : 5. The developed plates were sprayed with Neu's reagent and the particular compounds were detected under a UV light of wavelength 254 nm.

Spectrophotometry

The content of flavones and flavonoles was determined according to Cvek (2) using Spectrophotometer Biochrom Libra S6, Biochrom Ltd. Cambridge, CB4 OF3 England. The solution was composed of 1ml ethanolic extract and 0.1 ml reagent (2 % $AlCl_3$ and 5 % glacial acetic acid in methanol), made up to 2.5 ml with 5 % glacial acetic acid in methanol. After 30 min we measured the absorbance of the solution at the wavelength of 415 nm against blanks prepared in the same way but omitting the reagent. The calibration curve was constructed using a galangin standard dissolved in ethanol to concentrations of 10 $\mu g.ml^{-1}$, 20 $\mu g.ml^{-1}$, 30 $\mu g.ml^{-1}$, 40 $\mu g.ml^{-1}$ and 50 $\mu g.ml^{-1}$ (2).

RESULTS AND DISCUSSION

Thin layer chromatography showed no marked differences in chemical composition of individual samples of propolis from various areas of the East Slovakia region. The samples contained the same compounds as the standards used (galangin, chryzen, tektochryzen and pinobanksin). Various studies indicated a lower content of isalpinin in propolis compared to other flavonoids. It is probably that this low concentration which prevented its detection by TLC. The extract of poplar buds contained flavonoids detected in propolis samples. Only galangin and chryzen – the most frequent flavonoids in propolis – were detected in buds of the birches and willows.

Spectrophotometry was used to analyse quantitatively the flavons and flavonols in propolis. The results are summarised in Table 1.

Table 1. Results of spectrophotometric examination

Sample	Bee hive location	Content of flavones and flavonoles	Way of collection
Propolis 1	Hraň	9.58 %	Special grid
Propolis 2	Sečovská Polianka	5.98 %	Special grid
Propolis 3	Čeľovce	9.6 %	Special grid
Propolis 4	Kuzmice	2.98 %	During cleaning
Propolis 5	Dargov	2.1 %	During cleaning
Poplar	Čeľovce	0.46 %	
Birch	Čeľovce	0.21 %	
Willow	Čeľovce	0.08 %	

CONCLUSION

We present the results concerning the first determination of flavonoids in propolis samples originating from the East Slovakia Lowlands. Propolis from various parts of the Zemplín region contained flavonoids, galangin, chryzen, tektochryzen and pinobanksin; all compounds with significant biological effects. The similarity of substances present in propolis and poplar buds demonstrates that the bee population uses for production of propolis predominantly exudates of buds of Populus nigra resulting in a poplar type propolis. The high effectiveness of propolis is based on the high content of biologically active compounds. The determination of additional compounds in propolis will be carried out by HPLC technique in the future.

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REFERENCES

1. Bankova, V., 2005: Recent trends and important developments in propolis research. *Evidence-based Complementary and Alternative Medicine,* 3, 29–32.

2. Cvek, J., Medic-Saric, M., Jasprica, I., Zubçic, S., Vitali, D., Mornar, A., Vedrina-Dragojevic, I., Tomic, S., 2007: Optimisation of an extraction procedure and chemical characterisation of Croatian propolis tinctures. *Phytochemical Analysis,* 5, 451–459.

3. Gomez-Caravaca, A. M., Gomez-Romero, M., Arraez-Roman, D., Segura-Carretero, A., Fernandez-Gutierrez, A., 2006: Advances in the analysis of phenolic compounds in products derived from bees. *J. Pharm. Biomed. Anal.,* 41, 1220–1234.

4. Hroboňová, K., Lehotay, J., Čižmárik, J., 2008: Determination of some phenolic acids in propolis by an HPLC method. *Journal of Liquid Chromatography & Related,* 8, 1213–1226.

5. Medić-Šarić, M., Rastija, V., Bojić, M., Maleš, Ž., 2009: From functional food to medicinal product: Systematic approach in analysis of polyphenolics from propolis and wine. *Nutrition Journal,* 1, 33–50.

6. Paulino, N., Dantas, A. P., Bankova, V., Longhi, D. T., Scremin, A., Lisboa de Castro, S., Calixto, J. B., 2003: Bulgarian propolis induces analgesic and anti-inflammatory effects in mice and inhibits in vitro contraction of airway smooth muscle. *J. Pharm. Sci.,* 3, 307–313.

7. Salomao, K., Pereira1, R. S., Campos, L. C., Borba, C. M., Cabello, P. H., Marcucci, M. C., 2008: Brazilian propolis: correlation between chemical composition and antimicrobial activity. *Advance Access,* 317–324.

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DETERMINATION OF THERMOGRAPHIC STANDARDS IN DOGS USING THE INFRARED CAMERA

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ABSTRACT

The relationship between a disease and body temperature is as old as medicine itself. From the Hippocrates time, the changes in body surface were found by application of wet mud. The Gradual development of science and improved techniques has changed the way of body temperature scanning in medicine and currently top instruments are now available. Thermography is a non-invasive visualisation method for visual imaging of skin temperatures. Animals are not exposed to any harmful action of radiation. Thermography records changes in skin temperatures that cannot be detected by a routine clinical examination. It is also very helpful at early diagnosis of pathogenic changes, diagnostics of difficult cases and their precise localisation.

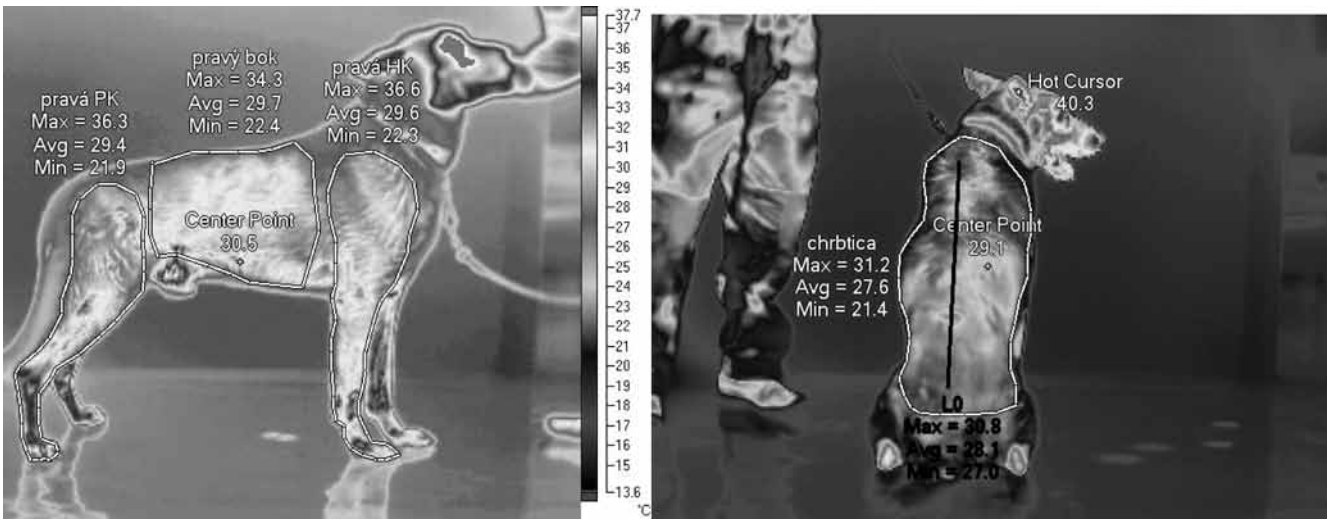
Key words: dogs; temperature standards; thermography; veterinary medicine

INTRODUCTION

In veterinary medicine it is often difficult to make a diagnosis due to several factors, such as the lack of symptoms, change of symptoms in the course of the disease, or the patient's inability to speak. The result is that the disease is not diagnosed in a timely fashion, or the lesions are localised incorrectly, which may result in incorrect therapy and excessive stress on the patient. This fact is frustrating for the veterinarian, expensive for the animal owner and a negative impact for the patient. Thermography is a suitable method of clinical examination; deviation of the temperature from the normal temperature model is used for detection and precise localisation of disorders. It is very important to have at one's disposal well documented normal temperature patterns and gradients for all animal species, scanned in a controlled environment, and only then to approach detection of pathological states.

MATERIAL AND METHODS

Healthy dogs were evaluated thermographically. Six German Shepherd dogs marked as longhaired, and 4 German shorthaired pointer dogs, a Weimar pointer, a Labrador retriever, and a Doberman were examined. We obtained overall 6 projections from each dog; i.e. in the standing position from the left, from the right, cranially and caudally, and in sitting position cranially and caudally. The thermographic evaluation focused on the region of limbs, joints and spine. A thermographic camera Fluke Ti 55/20 (FLUKE USA) was used. The camera setting – temperature range selected for medical application (–20 °C–100 °C), lens 20 mm/0.8 F, and skin emissivity 0.98. For all the thermographs the pallet "High contrast" with simplified temperature range 41.5 +2.8 °C was used. Conditions of measurement were: mean room temperature Tm = 20.6 °C; relative humidity 32 %; and the camera distance from each individual animal about 3.5 metres.



Figs. 1, 2. Demonstration of thermographs of longhaired dogs obtained by the respective software

Each dog was evaluated thermographically in an examination room under stable conditions, without any source of heat emission, direct sunshine and draught; the mean temperature of the room was 19–21 °C, and the relative air humidity was 40–60 %. The patient had to acclimatize within 20 minutes. The picture taken by the camera was processed by the available software. The software to the Fluke camera enabled us to create sufficiently precise contour polygones of individual regions on thermographs and to determine maximum, mean, and minimum temperatures in the given regions.

RESULTS

The temperature standards of selected body parts of longhaired and shorthaired dogs were obtained by individual measurements. These model temperatures were compared. We focused on temperature deviations on the left and right body sides and temperature differences between shorthaired and longhaired dogs. This helped us to obtain information on the influence of hair in the evaluations of the thermographs.

Table 1. Temperature standards of selected body regions – longhaired dogs

	Minimal (°C)	**Maximal (°C)**
SIDE	19.1–24.7	25.5–37.0
TL	15.5–22.6	28.3–36.6
PL	17.0–22.2	28.1–36.5

TL – thoracic limb, PL – pelvic limb

Table 2. Temperature standards of selected body regions – shorthaired dogs

	Minimal (°C)	Maximal (°C)
SIDE	23.5–29.9	34.0–38.1
TL	21.3–26.2	33.8–37.5
PL	21.3–26.3	33.9–37.3

TL – thoracic limb, PL – pelvic limb

DISCUSSION

Thermography is a method for the visualisation of the surface temperature of the object (5, 6), which is useful for detection of inflammation and provides reliable information about the potential localisation of a lesion (1, 4, 5). Our measurements showed that the temperature standards of shorthaired dogs were higher by as much as 4 °C, however, we suspected on such possible deviations. Although hair influences significantly emitted heat, it is possible to examine thermographically also uncut animals (e.g. show dogs); temperature models obtained by measurements on uncut dogs (uniform hair length) remain stable. Despite the fact that hair contributed to temperature decrease, the temperature standards were constant also after hair cutting (3).

CONCLUSION

Infrared thermography is an excellent supplement to clinical examination and other visualisation methods, such as radiography and ultrasonography. It records changes in

the skin temperature not detectable at routine clinical examination and is enormously helpful at early diagnosis of pathological changes and resolving diagnostically demanding cases. The use of infrared thermography opens the way for obtaining reliable information on potential localisation of the pathological process and thus for focusing the effort on suspicious regions. The right timing and use of thermography in veterinary medicine shortens the diagnostic process and minimizes the use of expensive, invasive and often painful procedures.

We are aware of the fact that our results are partial, however, application of infrared thermography in veterinary medicine in Slovakia is at the beginning. We plan to continue with these studies and use temperature models for evaluation of pathological patients, especially dogs.

REFERENCES

1. Eddy, A. L., Van Hoogmoed, L. M., Snyder, J. R., 2001: The role of thermography in the management of equine lameness. *Vet. J.,* 162, 172–181.

2. Harper, D. L., 2000: *The value of infrared thermography in the diagnosis and prognosis of injuries in animals.* www.flirthermography.com.

3. Loughin, C. A., Marino, D. J., 2007: Evaluation of thermographic imaging of the limbs of healthy dogs. *Am. J. Vet. Res.,* 68, 1064–9.

4. Turner, T. A., 2001: Diagnostic thermography. *Vet. Clin. North Am. Equine Pract.,* 17, 95–113.

5. Turner, T. A., 1991: Thermography as an aid to the clinical lameness evaluation. *Vet. Clin. North Am. Equine Pract.,* 7, 311–38.

6. Vaden, M. F., Purohit, R. C., McCoy, M. D., Vaughan, J. T., 1980: Thermography: a technique for subclinical diagnosis of osteoarthritis. *Am. J. Vet. Res.,* 41, 1175–9.

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ULTRASONOGRAPHIC EXAMINATION OF THE EYE IN HORSES AFFECTED WITH MOON BLINDNESS

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ABSTRACT

Moon blindness is presumably an immune-mediated disease of the uveal tract of horses characterised by episodes of active inflammation. The recurrent inflammation processes cause changes in the eye leading to opacity of originally transparent structures. Pathological changes of such character are always indication for ultrasonographic examination. This paper describes the ultrasonographic findings in 10 horses with recurrent uveitis with a different degree of affliction.

Key words: eye; horse; moon blindness; recurrent uveitis; ultrasonographic examination

INTRODUCTION

Recurrent uveitis or moon blindness is one of the most common ocular diseases in horses. Traditionally it is characterised by episodes of active inflammation followed by varying periods of quiescence (3). The clinical symptoms of the acute stage of moon blindness include painfulness, blepharospasms, lacrimation and photophobia. The findings may include corneal oedema and/or neovascularisation. The iris is oedematous, in miosis, and there are opacities in the anterior chamber. Inflammatory changes in the iris may lead to the development of synechiae. The aqueous humour may acquire an abnormal colour (yellow or green). In the majority of cases there is a decrease in intraocular pressure. A fundic examination may reveal chorioretinitis, subretinal exudation, optic nerve inflammation or peripapillary oedema (1).

The first mention of ultrasonographic (USG) examination of the horse eye dates back to the 1960's but presently it is considered a routine ophthalmologic examination technique. The USG image of the eye can be obtained with 8–10 MHz examination probes. Generally, patients tolerate USG very well. Local corneal anaesthesia suffices for this examination. For nervous horses, sedation and lead anaesthesia of n. auricuulopalpebralis (5 ml of 2 % local anaesthetics) is recommended (5).

MATERIAL AND METHODS

The USG examination was carried out on 10 horses diagnosed with moon blindness (8 horses were affected unilaterally and 2 bilaterally). In two horses, which were examined in the stage of acute uveitis, we used local corneal anaesthesia with Benoxi 0.4 % (oxibuprocaini hydrochloridum, UNIMED PHARMA s.r.o., Bratislava). To compare the results of the USG examination, the horses were examined ophthalmoscopically with a direct ophthalmoscope Welch Allyn 11720 (Welch Allyn, USA). USG diagnosis was made by means of a mobile ultrasonograph Aquila (výrobca firma ESOATE S. p. A., Taliansko) equipped with a 10 MHz linear probe using two-dimensional imaging mode (B-mode). During the examination the probe was placed transpalpebrally. We applied a thick layer of contact gel to both the probe and eyelashes. The probe was positioned so as to obtain gradually the image of the entire eyeball.

RESULTS

Patient No. 1: Kala, 16 years, sorrel mare, Belorussian warm-blooded, moon blindness diagnosed in April 2009, afflicted bilaterally.

• USG of the right eye: anterior uveitis, increased echogenicity of the anterior chamber, cataract, thickened iris, anterior synechia, inflammatory exudate in corpus vitreum

• USG of the left eye: anterior-posterior synechia, cataract.

Patient No. 2: Jamba, 17 years, white mare, English thoroughbred, two recurrences of moon blindness (the first one approximately two months ago) unilateral affliction.

• USG: no pathological finding.

Patient No. 3: Gin, 13 years, dark brown gelding, Furioso, 8-year history of moon blindness episodes, bilateral affliction.

• USG of the right eye: absence of anterior eye chamber, anterior-posterior synechia, lens luxation, cataract, degenerative changes of vitreous humour, retinal ablation, eyebulb phthysis

• USG of the left eye: shallow anterior chamber, anterior-posterior synechia, shortening of the anterior-posterior diameter, eyebulb phthysis

Patient No. 4: Chanson, 8 years, sorrel gelding, Slovak warm-blooded, acute stage of uveitis, unilateral affliction.

• USG: anterior uveitis, changes in echogenicity of anterior chamber, changes in subcapsular space in the zone of anterior lenticular capsule, inflammatory exudate in corpus vitreum, chorioretinitis with subretinal exudate

Patient No. 5: Juta, 14 years, white mare, English thoroughbred, 9-year history of moon blindness episodes, unilateral affliction.

• USG: shallow anterior chamber, anterior-posterior synechia, thickened iris, cataract, retinal ablation, pronounced shortening of the anterior-posterior diameter

Patient No. 6: Favory XI- 31 (Flamenco), 11 years, white gelding, Lipican, 7-year history of moon blindness episodes, unilateral affliction, euthanasia of the afflicted eye performed.

• USG: eye with serious inflammatory changes, anterior chamber absent due to synechia, hyperechogenic changes in the iris and vitreous humour, pronounced shortening of the anterior-posterior diameter and deformation of vitreous chamber.

Patient No. 7: KAM Archery Shining, 5 years, sorrel mare, quarter horse, 4 moon blindness episodes in the course of last two years, unilateral affliction.

• USG: cataracta incipient

Patient No. 8: Širkan, 8 years, dark brown gelding, trotter, 4- year history of moon blindness episodes, unilateral affliction.

• USG: thickened iris cataract, retinal ablation and shortening of the anterior-posterior diameter

Patient No. 9: Aischa, 15 years, sorrel mare, Westphalian warm-blooded, 7-year history of moon blindness episodes, bilateral affliction.

• USG of the right eye: absence of anterior chamber, strong hyperechogenic alterations of the iris, anterior-posterior synechia, cataract, luxation of lens to anterior chamber, retinal ablation, shortened anterior-posterior diameter, deformed vitreous chamber.

• USG of the left eye: no pathological findings

Patient No. 10: Mercedes, 4 years, sorrel mare, Slovak warm-blooded, first symptoms of the disease – October 2008, unilateral affliction.

• USG: anterior uveitis, posterior synechia, cataract, inflammatory degeneration of vitreus.

DISCUSSION AND CONCLUSION

Diaz (2) recommended 6–13 MHz linear probe for examination of the eye. The use of 10 MHz probe in our study appeared suitable. In agreement with the reports of other authors we were able to detect pathological changes located in all segments of the eye with the exception of corneal oedema which has been described by Krisova *et al.* (4) as an enlargement of the hypoechogenic space in the corneal stroma. The technique of palpebral imaging used in our study showed no changes of similar character.

Ultrasonography appears to be a safe and increasingly available veterinary diagnostic method for the diagnosis of ocular lesions involving opacity of normally transparent eye structures. Its use is definitely necessary for patients in which we consider the possibility of surgical therapy of moon blindness.

REFERENCES

1. Crispin, S. M., 2005: *Veterinary Ophthalmology.* 1st edn., Oxford, Blackwell Science Ltd., 299–301.

2. Diaz, O. S., 2004: Ultrasound of equine eye and adnexa and clinical applications. *Clinical Techniques in Equine Practice,* 3, 317–325.

3. Hollingsworth, S. R., 2005: *The Merck Veterinary Manual.* [online]http://www.merckvetmanual.com/mvm/index.jsp?cfile=html/bc/30300.html.

4. Krisova, S., Mezerova, J., Zert, Z., 2007: Importance of direct ophthalmology and ultrasound in the diagnostics of equine recurrent uveitis. *Medycyna Weterynaryjna,* 63, 1172–1176.

5. Trbolová, A., Mihály, M., 2003: Ultrasonographic examination of the horse eye (In Slovak). *Infovet,* 10, 9–12.

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Selected papers from the 53rd STUDENT SCIENTIFIC CONFERENCE with international participation, held at the University of Veterinary Medicine and Pharmacy in Košice on April 15, 2010.

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SIZE OF THE HOOF AS A PREDISPOSITION FACTOR TO LAMENESS IN WESTERN HORSES

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ABSTRACT

Horses of western breeds are bred predominantly for speed, agility and good manoeuverability, frequently by mating with related individuals. In some lines, particularly in reining and cutting, we could observe a side effect of such practice, namely small hooves compared to the body size or body weight. A small hoof area means higher loads on the hoof structures and can become a factor predisposing to lameness. Our study investigated the loading of the hoof in 25 Quarter Horses (QH). This may be one of the factors which might enable us to predict the development of lameness or, in lame horses, the potential return to sport activities.

Key words: hoof loading; small hoof; Western breeds

INTRODUCTION

The aim of our study was to investigate the occurrence of small hooves and hoof loading in Quarter Horses (QH) and their influence on the development of orthopaedic diseases. A small hoof compared to the body size or weight is the hoof exposed to loads higher than 5.5 kg.cm^{-2}.

For lame horses with hoof loading of ≥ 5.82 kg.cm^{-2} there is little chance for the return to sport activities. This level is considered a "grave prognosis" and restitutio ad integrum (4) has never occurred in them.

Pathological hoof states resulting in lameness can be divided to 3 groups:

1. Defects of hoof wall (cracks and crevices entering the corium), laminitis, local loosening of hoof capsule caused by hoof imbalance, corium inflammation, abscesses, aseptic corium inflammation, horn tubule, frog rot and pododermatitis verucossa.

2. Hoof bone diseases, tendonitis of the deep digital flexor tendon, ruptures of collateral ligaments, cysts and bone remodelling.

3. Diseases of the podotrochlear apparatus; capsulitis and synovitis of the joint capsule, desmitis of ligamentum ungulosesamoideum impar and collateral ligaments of the distal sesamoid bone, osteitis, osteopathy, vasculitis of arteries and fractures of the distal sesamoid bone (4).

In comparison with Arabians and ponies, Quarter Horse (QH) and the English Thoroughbred show a higher prevalence of diseases of the podotrochlear apparatus (1).

Horses participating in speed and working competitions, such as roping, cutting, reining, barrel racing and polo, may frequently run into problems involving the loss of the shoe and the associated damage of the hoof wall (4).

Lameness caused by vibrations is closely related to the small hoof (2).

Diseases of the podotrochlear apparatus are frequent in western horses and result in their elimination from sport activities. The most frequently affected are horses 6 to 7 years old (3).

MATERIAL AND METHODS

This study investigated the loading of the hoof in 25 horses of the Quarter Horse breed as one of the factors allowing one to make prognosis of the development of lameness in healthy horses and potential return to successful sport activities in lame horses. The horses were divided to two groups. Group A consisted of horses with loads on the hoof of the front limb of ≥ 5.5 kg.cm^{-2} and group

B with hoof loading < 5.5 kg.cm^{-2}. Limping horses were examined for lameness and, according to need, subjected to radiographic examination with an apparatus Chirax 70, and to USG employing Mindray 6600 equipped with 7.5 MHz linear probe.

The hoof was measured immediately below the coronary band and the number obtained was substituted in the formula X = live weight × 12.56/hoof perimeter (2). The result expresses the load on the hoof in kg.cm^{-2}. The live weight was determined by measuring thorax perimeter using a tape manufactured by Virbac and by subsequent calculation according to the formula live weight = (thorax perimeter)^2 × body weight/11900.

The arithmetic mean of two measurements was substituted to the equation for calculation of the hoof loading. The results obtained were evaluated with basic statistical methods.

RESULTS

The group A consisted of 13 horses (52 %) and the group B of 12 horses (48 %). The values obtained for groups A and B are presented in Tables 1 and 2, resp.

The mean loading and standard deviation for the investigated herd (n = 21) were as follows: front limbs 5.48 kg.cm^{-2} ± 0.552, back limbs 5.51 kg.cm^{-2} ± 0.464.

The mean loading and standard deviation for 5 lame horses (4 from group A, 1 from group B) reached 5.54 kg.cm^{-2} ± 0.318, which is by 0.06 kg (1.09 %) higher than the mean for the herd. All five horses limped on their front limbs. In 11 horses we recorded the increased loading of hooves of both front limbs and in 2 horses of one front limb.

Table 1. Horses with load on the front limbs equal to or exceeding 5.5 kg.cm^{-2} – group A

Horse No.	LFL kg.cm^{-2}	RFL kg.cm^{-2}
1	5.43	5.60
2	5.77	5.77
3	5.62* L	5.79
4	5.63	5.82* L
5	6.17	6.36
6	5.75	5.75
7	5.74	5.74
8	5.93	5.93
9	6.70	6.70
10	5.76* L	5.59
11	5.57	5.57
12	6.44	6.44
13	5.50* L	5.34

LFL – left front limb; RFL – right front limb; *L – limping limb

Table 2. Horses with load on the front limbs smaller than 5.5 kg.cm^{-2} – group B

Horse No.	LFL kg.cm^{-2}	RFL kg.cm^{-2}
1	5.02* L	4.73
2	5.08	5.22
3	4.96	4.96
4	5.05	5.05
5	5.15	5.00
6	4.98	4.83
7	5.02	4.88
8	4.93	5.08
9	5.34	5.17
10	4.94	4.79
11	5.41	5.23
12	5.19	5.34

LFL – left front limb; RFL – right front limb; *L – limping limb

DISCUSSION

The loading of the hoof higher than 5.5 kg.cm^{-2} occurs in only 2 % of sport horses (4). In our set of animals (25 horses) it occurred in 13 cases (54 %).

Frequency of lameness caused by hoof pathology is 7.5-fold more frequent in horses with small hooves (4). In our study it reached 30.76 % in the group A and 8.33 % in the group B (3.69-fold higher frequency).

The chance lame horses with hoof loading ≥ 5.82 kg.cm^{-2} for recovery is very low. No complete treatment for horses with such hoof loading was reported (4). In one such case reported in our study the lameness persisted for more than 6 months, moreover, this was not the first episode which is a bad prognosis for future sport career.

CONCLUSION

The American Quarter Horse as a dominant breed used in western sports seems to be susceptible to the development of small hooves compared to the body weight. According to authors from abroad this is the result of breeding focusing on sport performance and the fact that occasionally we meet with common ancestors on the side of both parents.

Because the number of QH horses in Slovakia is increasing due to the increasing popularity of western sports, it is probable that we will meet more frequently with animals of this breed and situations typical of it. Individuals with small

hooves require a high level of the care of hooves. Actions which minimize the negative influence caused by higher loading of the hoof structures can be mitigated by professional shoeing.

REFERENCES

1. Ackerman, N., Johnson, J., Dorn, C., 1997: Navicular disease in the horse: risk factors, radiographic changes and response to therapy. *J. Am. Vet. Med. Assoc.,* 170, 185–188.

2. Bradley, R. J., 2001: Common lameness in the cutting and reining horses. In *Proceedings 47th Ann. Conv. Am. Assoc. Equine Practnr.,* 6–11.

3. Robert, D. L., 2001: Lameness in rodeo horses. In P*roceedings 47th Ann. Conv. Am. Assoc. Equine Practnr.,* 1–5.

4. Turner, A. T., 2006: How to subjectively and objectively examine the equine foot. In *Proceedings 52nd Ann. Conv. Am. Assoc. Equine Practnr.,* 531, 532, 535.

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FOLIA VETERINARIA, 54, 2: 77–79, 2010

OBSERVATION OF OESTROUS CYCLE OF HUTSUL MARES KEPT AT THE NATIONAL STUD FARM IN TOPOĽČIANKY

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ABSTRACT

The oestrous cycle in mares is the interval between two successive oestrous ovulations. Some deviations of the oestrous cycle may occur due to various factors. Our observations of a closed herd of 21 Hutsul mares on the National stud farm (NSF) in Topoľčianky in 2009 revealed the following deviations in 13 mares:

- **Extended length of the oestrous cycle lasting 26–44 days, while the normal length is 19–21 days. Extremely long cycles, lasting 2 months, observed in two mares, attributed to embryonic mortality.**
- **Non-physiological length of oestrus in some mares, lasting 11–17 days, and even 18 days (maximum) in one mare. We also recorded a split oestrus, restored after 3 days.**
- **In one mare we observed dioestral ovulation on day 12 when a mating occurred by mistake after 12 days.**

Key words: cycle; oestrus; ovulation; mare

INTRODUCTION

A typical feature of the mating season of mares, as seasonally polyestrous breeders (1), is an oestrous cycle interrupted physiologically by gravidity or disease (4, 5, 9). It is an interval between the first and last ovulation in the respective year, which corresponds to 10–11 oestrous cycles. It is characterised by regular oestrous cycles terminated by ovulation (2, 3). The oestrous cycle is the interval between two successive oestrous ovulations. Oestrus, which is manifested externally by a receptive behaviour toward the stallion and a willingness to mate, and terminates by ovulation and the cessation of external signs (8). Ovulation occurs at the end of the growth of the dominant follicle which ruptures, releases the ovum into the oviduct and forms the corpus luteum (CL) (2). Some deviations of the oestrous cycle may occur due to various factors (6, 9).

The aim of our study was to observe and evaluate the oestrous cycle and heat in a herd of 21 Hutsul mares kept on the National stud farm (NSF) in Topoľčianky during the mating season of 2009.

MATERIAL AND METHODS

During the mating season of 2009 (from February 1st to September 30th) we observed a herd of 21 purebred Hutsul mares kept on the NSF in Topoľčianky, farm Hostianske. Seventeen mares were pregnant since the previous mating season of 2008 and 4 were non-pregnant. Their age ranged between 5 and 16 years. The mares were clinically healthy, in good condition and were fed as a group (hay supplemented with concentrates). They were housed in an open stable with unlimited access to the pastures.

Ultrasonographic (USG) rectal examinations were carried out by the instrument Aloka SSD-500 equipped with 5 MHz linear probe to confirm gravidity or examine problematic mares during the period of mating. The length of oestrus was observed by means of a test stallion at a teasing wall. The beginning of oestrus was the first day when the mare was willing to mate by showing evidence of external signs. The last day, the end of the oestrus, was the day of the disappearance of the external signs and receptive behaviour and refusal

of the stallion. Because regular rectal USG could not be conducted on the day of ovulation, it could not be determined objectively. The USG examination was repeated to prove the formation of the CL. Irregularities of the oestrous cycle were evaluated only by USG examinations and the detection of the oestrus by the stallion.

The results were processed statistically using software Statgraphics 5.0.

RESULTS AND DISCUSSION

The mating season of 2009 of the 4 nonpregnant mares at the NSF in Topoľčianky started on February 9th and ended on May 5th (the last first mating) (Table 1). Of these 4 mares only one was fertilised which indicated only 25 % successfulness. The biological beginning of the mating season of the 17 pregnant mares was defined with the help of the parturition time. The total effectiveness of all first matings was only 28.5 % (6 of 21 mares, Table 2). The beginning and length of the mating season could not be evaluated objectively in these mares because pregnancy was confirmed in all 21 of them before the end of August.

The oestrous cycle in the mares with 2 or more cycles is described below. Its length varied between 17 and 46 days. It was assumed that the 36-day cycle in mare No. 677 and 36 (707, 718, 678, 612 and 568) to 68 days (718) in the other mares could be attributed to an embryonic mortality after successful mating which lengthens the cycle considerably due to the so-called pseudogravidity, i.e. the time needed to resorb the contents. This way the embryonic mortality affects the irregularity of the oestrous cycle occurring between the two cycles with physiological length and ovulation (7). More mounts during one oestrus may contribute to the development of post-service endometritis and subsequent embryonic mortality (1). Dioestral ovulation was recorded on day 12 in mare No. 673 when it was subjected to mating by a mistake after 12 days. A so-called split oestrus was observed in mare No. 710. Oestrus recovery was recorded after 3-day and ended successfully. Its total length was 18 days and 9 mounts were required.

Onset of the first post-partum oestrus in 17 mares fertilised in the previous season ranged between 8 and 12 days post-partum (mean 10.12 ± 4.3 days). The length of oestrus was 3 to 11 days with 2 to 6 mounts. The mean length of the first post-parturient oestrus was 6.2 ± 2.2 days with 3.6 ± 1.3 mounts per oestrus. The successfulness was only 35.3 % (Table 3). In the herd with 21 mares the oestrus lasted 6.95 ± 3.42 days (range 3 to 18 days). The number of mounts required per oestrus was 2 to 9 (average 4.04 ± 4.48). This is excessive compared to the recommendations of authors from abroad. The number of mounts needed to ensure one pregnancy in the herd was 2 to 23 with the average number of mounts 8.09 ± 6.35 per one pregnancy in the herd (Table 4). Twenty one pregnancies in the herd were ensured by 42 oestrous cycles, i. e. successfulness of oestrus with mating was 50 % per herd.

Table 1. Onset of the oestrus – nonpregnant mares – NSF Topoľčianky, 2009

Mare No.	Date of the 1st oestrus	Fertile (+/-)
568	February 9, 2009	-
673	February 11, 2009	-
729	April 7, 2009	+
707	May 8, 2009	-

Table 2. Occurrence of the first oestrus – NSF Topoľčianky, 2009

Month/year	Number of heats	Fertile (+)	Effectiveness (%)
02/09	5	1	20
03/09	0	-	-
04/09	2	1	50
05/09	3	0	0
06/09	7	2	28
07/09	3	1	33
08/09	1	1	100

Table 3. Post-partum oestrus – 17 mares, NSF Topoľčianky, 2009

Onset of the 1st post-parturient oestrus			Oestrus length (days)/ number of mounts			Successfulness of the 1st post-parturient oestrus
Earliest (day)	Latest (day)	Mean (day)	Shortest	Longest	Mean	
8	12	10.12 ± 4.3	3/2	11/6	6.2 ± 2.2 day 3.6 ± 1.3 mounts	35.3 %

Table 4. Oestrus characteristics – 21 mares, NSF Topoľčianky, 2009

	Lowest	Highest	Mean
Length of oestrus in days/ number of mounts	3/2	18/9	6.95 ± 3.42
Number of mounts per oestrus	2	9	4.04 ± 4.48
Number of mounts per pregnancy	2	23	8.09 ± 6.35

CONCLUSION

Our observations of a closed herd of 21 Hutsul mares on the National stud farm (NSF) in Topoľčianky in 2009 revealed the following deviations in 13 mares:

- Abnormal length of the oestrous cycle (26–44 days, 2 months in 2 mares) most likely attributed to embryonic mortality which affected markedly the luteal phase.
- Non-physiological length of oestrus in some mares 11–18 days, split oestrus in one mare, restored after 3 days.
- Dioestral ovulation in one mare on day 12.

Monitoring of the oestrous cycle and the limited number of matings per 1 optimum mount per one oestrus in optimum time appears to be more advantageous and an effective approach.

REFERENCES

1. Gintre, O. J., 1992: *Reproductive Biology of the Mare.* Equiservices Publishing, 642 pp.

2. Hura, V. *et al.*, 2000: Autumn period and its possible use in breeding Hutsul mares after finishing of the mating period (In Slovak). In *Proceedings of the International conference Horse Breeding in the New Millenium,* Nitra, 94–96.

3. Hura, V., Hajurka, J., Kačmárik, J., Valocký, I., Macák, V., Novotný, F., 2001: Ovarian activity of Hutsul mares in winter months (In Slovak). In *Proceedings of the International scientific conference on the occasion of 50th anniversary of establishment of the Ist Internal clinic,* UVM Košice, 215–217.

4. Chvátal, O. J., et al., 2001: Observation of ovarian activity in mares throughout the year (In Czech). In *Proceedings of the International scientific conference on the occasion of 50th anniversary of establishment of the Ist Internal clinic,* UVM Košice, 211–213.

5. Kudlač, E., 1995: Oestrous activity in mares and its potential management (In Czech). In *Proceedings of the IIIrd annual conference of ČHS: Reproduction of horses,* Brno, 1–5.

6. Loy, R. G., 1967: How the Photoperiod Affects the Reproductive activity in Mares. *Mod. Vet., Pract.,* 47–49.

7. Mckinon, A. O., 1992: *Equine Reproduction.* Lea & Febiger, Pennsylvania, 1137 pp.

8. Palmer, E., 1978: Control of the oestrus cycle of the mare. *J. Reprod. Fertil. Suppl.,* 54, 495–505.

9. Sharp, D. C., 1988: Transition into the breeding season: Clues to the mechanism of seasonality. *Equine Vet. J.,* 20, 159–160.

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THE PREVALENCE OF DIROFILARIASIS IN DOGS IN EASTERN SLOVAKIA

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ABSTRACT

This study focused on the prevalence of *Dirofilaria* spp. in dogs in the risky regions of Eastern Slovakia and on monitoring of the climate conditions suitable for the development of this parasite. Microfilaria were detected in the blood by a modified Knott's test. In positive cases, the individual species were differentiated by a histological staining technique and the PCR. To monitor climate conditions in the two observed regions we obtained mean daily temperatures from 9 climate stations, recorded by the Slovak hydrometeorological institute (SHMU) over the past 10 years. We determined the number of dirofilaria generations that could complete its development in these regions over a certain time period. Our study proved that dirofilariasis is a topical problem in Eastern Slovakia, particularly in the districts of the Košice Region, with a prevalence figure as high as 20.88%.

Key words: *Dirofilaria immitis; Dirofilaria repens;* dirofilariasis; dog; prevalence; Slovakia

INTRODUCTION

Dirofilariasis is a vector transmitted parasitic disease of carnivores caused by the zoonotic species of the genus *Dirofilaria*, namely *Dirofilaria immitis* and *Dirofilaria repens*. Until recently, the disease occurred in Europe mostly in the southern regions with warmer climates. However, with dogs travelling abroad, global warming and the related increase in the dispersal of vectors, Slovakia is today one of the countries with the occurrence of this disease.

D. immitis and *D. repens* are transmitted through more than 60 mosquitoes of the family *Culicidae,* which is essential for completion of the dirofilaria life cycle. The development of the dirofilaria larvae in mosquitoes is affected by the environmental temperature, which should not decrease below 14 °C, and have a sufficient number of days with such temperatures (1, 2).

Dirofilaria immitis causes heartform infections. *Dirofilaria repens* induces a subcutaneous form of dirofilariasis, the pathogenicity of which is frequently undervalued (6).

MATERIAL AND METHODS

The research was carried out on 157 dogs older than one year: 37 from Košice Region districts; 21 from Michalovce district; 23 from Trebišov district; 50 from Vranov nad Topľou district; and 16 from Prešov district.

On the basis of a questionnaire we divided the dogs into groups according to gender, age, use, length of hair and breed. We tried to determine whether any of these factors played a role in the occurrence of dirofilariasis.

Samples of blood for examination were withdrawn either from the vena cephalica antebrachii or the vena saphena and collected in sterile tubes with the addition of K3-EDTA.

Dirofilaria species were diagnosed by means of a modified Knott's test for detecting the presence of microfilaria in the blood (3). To differentiate between the different species (*Dirofilaria repens* and *Dirofilaria immitis*) in the blood of the positive dogs, we used the histochemical staining (commercial kitu Leucognost SP® MERCK) (4) as well as the PCR molecular-genetic methods (5).

In collaboration with SHMÚ, we checked mean daily temperatures recorded over the past 10 years by 9 climate stations located in the relevant districts and identified the climate conditions suitable for the completion of the life cycle of *Dirofilaria species*. This served as a basis for the determination of seasonality and the number of dirofilaria generations in individual years. The temperature of 14 °C was considered the threshold temperature for the development of dirofilaria in the mosquito vectors.

RESULTS AND DISCUSSION

Dirofilariasis was proved in 21 out of the 157 examined dogs (13.38 %). Twenty dogs suffered from subcutaneous dirofilariasis and in one dog we recorded a mixed infection with both species.

Nineteen positive dogs showed no clinical symptoms. Two positive dogs died. One of them was not subjected to postmortem examination and examination of the other identified cardiovascular failure due to large number of adult worms in the heart and pulmonary vessels which was determined as the cause of its death.

The prevalence of dirofilariasis in the Prešov Region reached 3.03 % and in the Košice Region 20.88 %. The largest numbers of positive dogs were recorded in Trebišov (39.13 %) and Michalovce (29.03 %) districts. The prevalence in the district Vranov nad Topľou was 4 %, in the Košice Region districts 2.7 % and in the Prešov district 0 %. Our results confirmed those of the epidemiological studies which indicated that dirofilariasis has spread to the countries of Eastern Europe (1).

Dirofilariasis was diagnosed most frequently in watchdogs (33.33 %) and less in companion (9.52 %) and hunting dogs (2.63 %).

The prevalence of dirofilariasis was the highest in the 5–7 years age category (25 %). Lower but approximately the same prevalence was recorded in age categories 3–5 years (14.58 %) and 7 and more years (13.64 %). The lowest prevalence was observed in dogs younger than 3 years (9.86 %).

The majority of positive dogs were short-haired breeds (17.35 %) while in long-haired breeds the prevalence was only 9.30 %. None of the thick-haired dogs were positive. The division of breeds according to size showed the highest prevalence of dirofilariasis in giant (29.03 %) and large breeds (22 %), much lower in medium (1.92 %) and zero prevalence in small breeds.

The investigation of the mean temperatures in Eastern Slovakia districts showed that the development of the dirofilaria in the vector and its transmission had a seasonal character with the highest occurrence in the summer. In the Prešov Region dirofilaria could develop from early May till late September. In the Košice Region the suitable period lasted from early May till early October. In the eastern territory, during the periods when the temperature was above 14 °C and persisted for the number of days sufficient for development of larvae to infectious L3 form in the mosquitoes, the temperature ranged (in the past 10 years) between 18.89 °C and 20.63 °C. On the basis of the respective temperature information and calculation of the accurate number of suitable days, we determined that 3 generations of dirofilaria could develop annually in the Prešov Region, and 3–4 generations in the Košice region.

Our results indicate that dirofilariasis is a relevant problem in Eastern Slovakia, particularly in the Košice Region districts where its prevalence reached 20.88 %.

The investigation of the risk according to the individual criteria showed that dirofilariasis was most frequently diagnosed in watchdogs and male dogs older than 3 years and they were at a higher risk for this disease. The higher risk group included giant and large breeds and short-haired dogs. The transmission of dirofilariasis in the Slovak Republic has been affected considerably by people choosing to travel with their dogs to endemic areas without previous preventive premedication. After returning home these infected dogs became an infection reservoir in Eastern Slovakia.

Climate conditions, particularly temperature, precipitation and relative humidity, in the Košice and Prešov Regions are ideal for development and transmission of dirofilaria. In the Eastern Slovakia districts, D. repens is the dominant species.

With regard to the zoonotic character of the disease one should also mention the risk to the health of the human population.

REFERENCES

1. Genchi, C., 2009: Climate and *Dirofilaria* infection in Europe. *Vet. Parasitol.,* 163, 286–292.

2. Genchi, C., Rinaldi, L., Cringoli, G., 2007: *Dirofilaria immitis* and *Dirofilaria repens* in dog and cat and human infections. In *Mappe Parassitologiche 8,* **Genchi, C., Rinaldi, L., Cringoli, G.,** (Eds.), Rolando Editore, Naples, 211 pp.

3. Knott, J., 1939: A method for making microfilarial survey on day blood. *Trans. R. Soc. Trop. Med. Hyg.,* 33, 191.

4. Peribañez, M. A., Lucientes, J., Arce, S., Morales, M., Castillo, J. A., Gracia, M. J., 2001: Histochemical differentiation of *Dirofilaria immitis, Dirofilaria repens* and *Acanthocheilonema dracunculoides* microfilariae by staining with a commercial kit, Leucognost-SP®. *Vet. Parasitol.,* 102, 173–175.

5. Rishniw, M., Barr, S. C., Simpson, K. W., Frongillo, M. F., Franz, M., Dominguez Alpizar, J. L., 2006: Discrimination between six species of canine microfilariae by a single polymerase chain reaction. *Vet. Parasitol.,* 135, 303–314.

6. Svobodová, V., Svobodová, Z., Beladičová, V., Valentová, D., 2005: First cases of canine dirofilariasis in Slovakia: a case report. *Vet. Med. Czech,* 50, 510–512.

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QUALITY OF PORK MEAT AFTER FEEDING LINSEED AND VITAMIN E

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ABSTRACT

Consumers have been more and more interested in food that has a favourable effect on their health and improves their quality of life. One of the possibilities of how to prevent diseases (especially cardiovascular) is the consumption of polyunsaturated fatty acids (PUFA), mainly n-3 fatty acids. The goal of our study was to observe the possibility of increasing n-3 PUFA in meat by the supplementation of linseed and vitamin E. Also, the oxidative stability of such improved meat during storage in a refrigerator (4 °C, 11 days) was observed. Linseed feeding has a direct effect on the composition of fatty acids in pork fat. In the experimental group the proportion of n-3 PUFA significantly increased and that of saturated fatty acids decreased compared to the control. In the experimental group the oxidation processes during storage were slightly higher than those in the control group, but the vitamin E added inhibited significantly the oxidation processes.

Key words: linseed; lipid oxidation; n-3 PUFA; pork; Vitamin E

INTRODUCTION

The human organism is able to produce for itself all fatty acids that are needed, except for two: linoleic acid (LA), n-6 fatty acid, and α-linolenic acid (ALA), n-3 fatty acids. These should be ingested by food, and therefore they are called "essential fatty acids". Deficiency in essential fatty acids manifests itself by: a decreased function of the immune system; deceleration of growth; impaired reproduction; increased inflammatory states; and occurrence of different diseases (6).

The feeding of animals is important for the control of the overall meat quality. In pigs, as monogastric animals, it is relatively easy to change the composition of fatty acids of the adipose tissue through a change in the feed rations. Both the amount and composition of fatty acids significantly influence the quality of the adipose tissue of pigs (1). Linseed is a rich source of α-linolenic acid (C18 : 3, n-3) and its feeding to pigs results in the increase in the concentration of n-3 acids in the animal meat. Vitamin E is the most effective fat soluble antioxidant and it is able to protect unsaturated fatty acids in cellular membranes which are important for the membrane's function and structure.

The aim of our study was to observe the effect of feeding linseed (6 %) and vitamin E (360 mg.kg^{-1}) on the composition of fatty acids and the oxidation stability of the meat produced.

MATERIAL AND METHODS

Sixty pigs (crossbred Slovak white improved x Landrace) were involved in the experiment and divided into two groups. Group 1 (control) was fed a traditional feed mixture (TF). Group 2 (experimental) was fed TF with the addition of linseed (Linum usitatissimum L, variant Flanders) in the dose of 6 % and vitamin E in the dose of 360 mg.kg^{-1} TF. Pig fattening in the groups lasted for 60 days to the slaughter weight of 110–120 kg. After slaughter processing, the samples of rump musculature and cutlet (m. longissimus dorsi) were collected from each pig. Samples were packed into polyethylene wrappers and stored in a refrigerator at 4 °C.

The fatty acids were determined as their methylesters by gas chromatography using an apparatus GC-6890 N (Agilent Technologies) according to Čertík *et al.* (2). Oxidation of fat was de-

termined using the thiobarbituric acid (TBA) method according to Marcinčák *et al.* (4). Individual determinations were carried out on days 1, 3, and 11 of the samples storage in the refrigerator.

Statistical processing of the results was performed using software Graph Pad Prism 3.0 (1999). Results are expressed as the arithmetical mean (x) and standard deviation (SD). The differences between the groups were evaluated by the Student *t*-test, and $P < 0.05$ was considered as a statistically significant difference.

RESULTS AND DISCUSSION

The composition of fatty acids in pork fat of the experimental and control groups is presented in Table 1. The feeding of linseed as a source of PUFAs has a direct effect on the composition of fatty acids in pork fats (6). In the experimental group the portion of essential fatty acids in the fat component of the meat increased, and the portion of oleic acid decreased (C 18 : 1).

Linseed of variation Flanders is a significant source of α-linolenic acid. In the experimental group, the meat fats contained 3–4-times higher portion of α-linolenic acid (C 18 : 3, n-3) than that in the control. Also, an increase in the portion of eikosapentaenoic (C 20 : 5, n-3) and docosapentaenoic acids (22 : 5, n-3) in the rump musculature as well as in the cutlets was significant. Therefore, it is possible to influence the essential fatty acid by feeding (3). An increase in docosahexaenoic acid (DHA) can be reached, above all, by the addition of fish oil into the feed rations (6).

The results of the changes in fats during storage of samples in the refrigerator (4 °C) are presented in Table 2. Storage of the samples in the refrigerator caused an increase in the amount of degraded products of fat oxidation in both groups of samples. In the samples of the rump and the cutlet of the experimental group, the values were higher than those in the control. However, the differences between the control and experimental groups were not statistically significant ($P > 0.05$). The higher intake of fats in the feed resulting in the higher portion of fats in the musculature of the experimental group, had no statistically significant effect on the increase in the values of the degrading changes in the meat fat. The feeding of vitamin E contributed significantly to the lower oxidation of the experimental group meat (5).

Table 1. Composition of fatty acids in pork fat

Fatty acid (%)	Cutlet Exp. group	Rump Exp. group	Cutlet Control	Rump Control
C 16:0	23.79	23.650	22.661	22.409
C 18:0	12.800	11.398	12.321	12.206
C 18:1-9	42.268	41.300	45.533	46.807
C 18:2-9c, 12	7.038	7.356	6.546	6.209
C 18:3-6, 9, 12	0.109	0.097	0.174	0.184
C 18:3-9, 12, 15	**1.508**	**1.979**	**0.416**	**0.432**
C 20:0	0.275	0.230	0.213	0.218
C 20:2-11c, 14	0.345	0.32	0.405	0.406
C 20:3-8, 11, 14	0.096	0.123	0.114	0.085
C 20:4-5, 8, 11, 14	0.473	0.705	0.651	0.322
C 20:5-5, 8, 11, 14, 17	**0.125**	**0.156**	**0.086**	**0.090**
C 22:1-13	0.027	0.024	0.043	0.028
C 22:5-7, 10, 13, 16, 19	**0.234**	**0.300**	**0.026**	**0.026**
C 22:6-4, 7, 10, 13, 16, 19	0.037	0.046	0.041	0.030
Sum of saturated FA	38.784	37.338	36.951	36.647
Sum of unsaturated FA	61.216	62.662	62.457	62.678
Sum of essential FA	9.620	10.762	8.106	7.323

Boldface type indicates significant differences $P < 0.05$

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Table 2. Determination of TBA (mg.kg^{-1}) in samples of meat stored by cooling (4 °C) for 11 days

		Day 1	Day 7	Day 11
Exp. group	Cutlet	0.106 ± 0.033	0.264 ± 0.041	0.312 ± 0.020
	Rump	0.116 ± 0.024	0.287 ± 0.041	0.304 ± 0.055
Control	Cutlet	0.075 ± 0.020	0.155 ± 0.023	0.283 ± 0.016
	Rump	0.105 ± 0.011	0.119 ± 0.010	0.232 ± 0.042

REFERENCES

1. Bryhni, E. A., Kjos, N. P., Ofstad, R., Hunt, M., 2002: Polyunsaturated fat and fish oil in diets for growing-finishing pigs: effect on fatty acid composition and meat, fat, and sausage quality. *Meat Science,* 62, 1–8.

2. Čertík, M., Sláviková, L., Masrnová, S., Šajbidor, J., 2006: Enhancement of nutritional value of cereals with γ-linolenic acid by fungal solid state fermentations. *Food Technology and Biotechnology,* 44, 75–82.

3. Guillevic, M., Kouba, M., Mourot, J., 2009: Effect of a linseed diet on lipid composition, lipid peroxidation and consumer evaluation of French fresh and cooked pork meats. *Meat Science,* 81, 612–618.

4. Marcinčák, S., Sokol, J., Turek, P., Popelka, P., Nagy, J., 2006: Determination of malone dialdehyde in pork meat by solid phase extraction and HPLC (In Slovak). *Chemické Listy,* 100, 528–532.

5. Nam, K. T., Lee, H. A., Min, B. S., Kang Ch. W., 1997: Influence of dietary supplementation with linseed and vitamin E on fatty acid, α-tocopherol and lipid peroxidation in muscles of broiler meat. *Anim. Feed Sci. Technol.,* 66, 149–158.

6. Raes, K. De., Smet, S., Demeyer, D., 2004: Effect of dietary fatty acids on incorporation of long chain polyunsaturated fatty acids and conjugated linoleic fatty acids in lamb, beef pork meat, a review. *Anim. Feed Sci. Technol.,* 113, 119–221.

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DETECTION OF THYLOSINE AND OXYTETRACYCLINE RESIDUES IN HONEY

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ABSTRACT

The sensitivity of selected testing bacterial strains of the STAR method were investigated in order to select the most suitable testing microorganisms for the determination of the minimal inhibitory concentrations of the thylosine and oxytetracyline residues in honey. At the same time, the residues of thylosine and oxytetracycline in honey were observed using Premi®Test, which, after its modification, was used as an alternative method for the residue determination by the microbiological plate methods (STAR method). Unlike the plate methods, the Premi®Test eliminates the effect of the natural inhibitory substances contained in honey. The results showed that the minimal inhibitory concentrations of the antibiotics tested were either lower (oxytetracycline) or comparable (thylosine) with those found with the selected test bacterial strains with the STAR method.

Key words: honey; oxytetracycline; Premi®Test; STAR method; tylosín

INTRODUCTION

In beekeeping, tetracyclines and thylosine are used to treat effectively bacterial diseases of honey bee brood, such as American foulbrood caused by the *Paenibacillus larvae* and European foulbrood caused by *Melissococcus pluton*. The use of these drugs in the prevention and treatment of the diseases can lead to residues in the honey. The presence of residues can induce an increased resistance of microbial strains in honey consumers, and in some cases lead to allergic, or toxic reactions in hypersensitive individuals.

With regard to an increasing concern related to residues in honey in the European Union, the use of antibiotics and sulphonamides for prevention and treatment of bacterial diseases of honey bees was banned. In general, the maximal residual limits (MRL) were set by the Regulation of Commission No. 2377/90 (2), based upon the requirements of the EC Directive No. 96/23 (1996) (5), but these values are not given for honey. This, however, does not mean that they are not used illegally. Therefore, it is necessary to have at our disposal the methods enabling qualitative and quantitative analysis of these substances also in honey, developed and validated in agreement with the Decision of Commission No. 2002/657/EC (4). At present, the application of these regulations in relation to antibiotics has not been harmonised in all member states. Some countries do not allow the presence of any residues of antibiotics above the level of the corresponding detection limits of the methods used; other countries apply the so-called action limit on the level of 50 $ng.g^{-1}$ (7). In the USA, oxytetracycline (OTC) has been used since the fifties to prevent and treat bacterial diseases of bee brood and adult bees. This led to working up the rules for its official use and determination of MRL also for honey (3). In some other countries, such as the Republic of Korea, MRL of 300 $\mu g.kg^{-1}$ is valid for OTC in honey (1).

In our study, we used standard solutions of thylosine and oxytetracycline and diluted them in honey (fortification) to obtain concentrations needed for determination of detection limits of both the STAR method and Premi®Test.

MATERIAL AND METHODS

STAR method

The following selected test strains were used with the STAR method: pH 7.4 agar with the strain *Bacillus stearothermophilus* (Bs); pH 8 agar with the strain *Kocuria varians* (Kv); and pH 6 agar with the strain *Bacillus cereus* (Bc). The honey was diluted using phosphate buffer (pH 6.0) at the ratio of 1 : 1, heated to 80 °C and maintained at this temperature for 10 minutes. Standard solutions of thylosine and oxytetracycline and fortified samples of honey were applied to each of individual discs. The plates prepared were incubated at 55 °C for at least 12–15 hours (Bs), at 37 °C for at least 24 hours (Kv), and at 30 °C for at least 18 hours (Bc). After incubation the discs were examined for the presence of clear regular zones. The positivity was indicated by zones bigger than 2 mm.

Premi®Test: after weighing 2 g of honey previously heated to 40 °C, 5 ml of the extracting solution of acetonitril/acetone were added and the mixture homogenised for 45 seconds on the vortex and for another 5 minutes in an ultrasonic bath. The extract was centrifuged at 4 100 r. p. m., at 4 °C for 10 minutes. The supernatant was evaporated to dryness at 40–45 °C, and dissolved in 250 µl of peptone water. After inoculation and incubation we observed the colour of the lower two thirds of the solid agar medium. Yellow colouring of the medium indicated an absence of residues, or their presence under the detection limit, while a violet colour indicated the presence of residues above the detection limit.

RESULTS AND DISCUSSION

The mean and minimal inhibitory zones induced by the presence of thylosine residues are presented in Table 1. The detection limit of 70 µg.kg^{-1} of selected test strains obtained with the STAR method was consistent with that of the Premi®Test.

The mean and minimal inhibitory zones induced by the presence of oxatetracycline residues are presented in Table 2. The detection limit for oxytetracycline determined by the Premi®Test was 75 µg.kg^{-1} and that obtained with STAR method was 90 µg.kg^{-1}.

Table 2. Mean (average ± SD) and minimal inhibitory zones (mm) of OTC residues

Concentration (µg.kg-1)	*Bacillus cereus*		Premi®Test
	Standard	Honey	
100	3.38 ± 0.17 3.12	2.33 ± 0.16 2.15	+
90	NA	2.057 ± 0.05 2.02	+
75	NA	0	+
50	0	NA	-

NA – non-analysed

All persons responsible for honey processing have to take necessary measures, especially by performing their own control, to make sure that their products do not contain the antibiotic residues. Producers can put on the market only the products obtained from bees that were not administered any banned substances or banned medications or were not treated illegally.

The ideal screening method should detect the presence of all licensed antimicrobial substances on or under the MRL level. The method should by robust, fast, simple, and applicable to the wide scale of food matrices (6). Most of the detection methods are based on the use of plate methods out of which the STAR method is the most widespread. On the one hand the plate methods are simple but on the other

Table. 1 Mean (average ± SD) and minimal inhibitory zones (mm) of tylosin residues

Concentration (µg.kg^{-1})	*Bacillus stearothermophilus*		*Kocuria varians*		Premi®Test
	Standard	Honey	Standard	Honey	
100	2.29 ± 0.34 2.05	3.07 ± 0.20 2.78	4,02 ± 0,65 3,27	2.48 ± 0.07 2.41	+
75	NA	3.35 ± 0.31 3.13	NA	NA	+
70	NA	2.66 ± 0.21 2.37	NA	2.44 ± 0.24 2.21	+
50	1.96 ± 0.13 1.84	1.81 ± 0.20 1.55	2.35 ± 0.61 1.89	2.09 ± 0.10 1.98	-

NA – non-analysed

hand they require long incubation period and cannot detect many antibiotics reliably. The Premi®Test is based upon the inhibition of the growth of Bacillus staerotermophillus and is sensitive to many antibiotics. A great advantage of using the Premi®Test in comparison with the plate methods is its slow demands on time and equipment of the microbiological laboratory.

REFERENCES

1. Moonsun, J., Insook, R. P., 2008: Quantitative detection of tetracycline residues in honey by a simple sensitive immunoassay. *Anal. Chim. Acta,* 626, 180–185.

2. Council Regulation (EEC) No. 2377/90 laying down a Community procedure for the establishment of maximum residue limits of veterinary medicinal products in foodstuffs of animal origin.

3. Pena, A., Pelantova, N., Lino, C. M., Silveira, M. I. N., Solich, P., 2005: Validation of an analytical methodology for determination of oxytetracycline and tetracycline residues in honey by HPLC with fluorescence detection. *J. Agric. Food Chem.,* 53, 3784–3788.

4. Commission Decision 2002/657/EC concerning the performance of analytical methods and the interpretation of results.

5. Council Directive 96/23/EC on measures to monitor certain substances and residues thereof in live animals and animal products.

6. Steady, S., Sharmany, M., Tarbiny, J. A., Gibsony, E., 2004: Meeting maximum residue limits: an improved screening technique for the rapid detection of antimicrobial residues in animal food products. *Food Addit. Contam.,* 21, 216–221.

7. Viñas, P., Balsalobre, N., López-Erroz, C., Hernández-Córdoba, M., 2004: Liquid chromatography with ultraviolet absorbance detection for the analysis of tetracycline residues in honey. *J. Chromatogr. A,* 1022, 125–129.

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OBSERVATION OF IONIZING RADIATION AND CHROMIUM INTERACTIONS IN *POECILIA RETICULATA*

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ABSTRACT

The experiment focused on the interactions of ionizing radiations and potassium dichromate in relation to morphological changes in the Guppy *(Poecilia reticulate)*. Potassium dichromate was added to aquarium water in concentration of 50 mg.l^{-1} and the fish were irradiate with gamma rays at a dose of 20 Gy. The microscopic changes observed corresponded to macroscopic findings, clinical symptoms and changes described in mammals and birds.

Key words: gamma radiation; histological changes; *Poecilia reticulate*; potassium dichromate

INTRODUCTION

In the past decades extensive industrial and agricultural production has caused considerable environmental damage. This involves high accumulation of various chemical elements and compounds in sediments, soil and water which can affect negatively the health of humans and other animals. The aim of this study was to investigate histological changes related to the interaction of chromium and ionizing radiation.

MATERIAL AND METHODS

The study was conducted on the fish *Poecilia reticulate*, reared in a laboratory from fish obtained from an aquarium shop. The fish were reared under constant conditions: water temperature 24 °C, 3.9 °N, pH 7.04, artificial aeration, water filtration, photoperiod 12 h per day (3, 4). They were fed live and dry feed. Randomly selected experimental fish were divided to 3 groups (each in 15 l aquarium) kept under identical conditions. Group 1 served as the control. Exposure to chromium (Group 2 and 3) was ensured by adding potassium dichromate ($K_2Cr_2O_7$, 50 mg.l^{-1}) to aquarium water. Fish in Group 3 were irradiated with gamma rays at a dose of 20 Gy (^{60}Co-source, Chisostat, dose input 0.850286 Gy.min^{-1}).

Samples for histological examination were processed by conventional method. Whole killed fish were fixed in 10 % neutral formalin and embedded in paraffin. Histological sections of thickness 7 µm were stained with haematoxylin-eosin and examined under light microscope Jenamed equipped for microphotography.

RESULTS AND DISCUSSION

Fig. 1 shows the cross section of the kidney (mezonephros) of fish exposed to chromium only. It shows round renal corpuscle and urinary tubules. Medulla spinalis is seen in the upper right corner and muscles with extended interstitial spaces in the lower right part of the figure.

Fig. 2 shows the renal cross section after combined exposure to radiation and chromium. There are several urinary tubules with marked morphological changes. On the lower left corner there is considerably damaged vessel.

Fig. 3 shows the details of a renal corpuscle of a Guppy exposed to chromium without evident morphological changes. It consists of a glomerulus composed of fenestrated capillaries on the surface of which there are cells of the internal leaf of Bowman's capsule, the so-called podocytes. The outer leaf consists of cells of unilamellar tilelike epithelium.

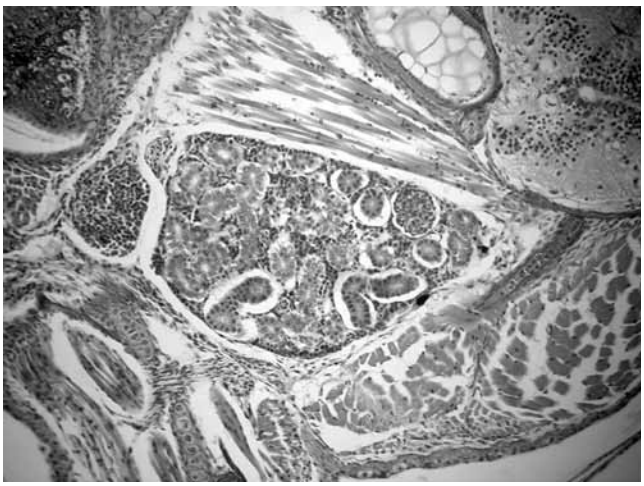

Fig. 1. Renal cross section (chromium)

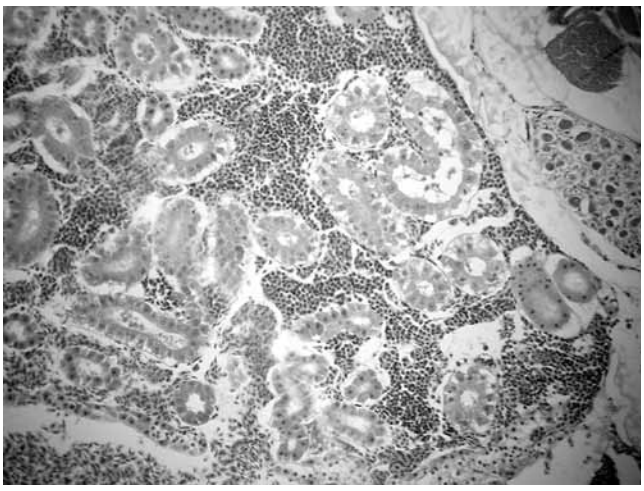

Fig. 2. Renal cross section (chromium and irradiation)

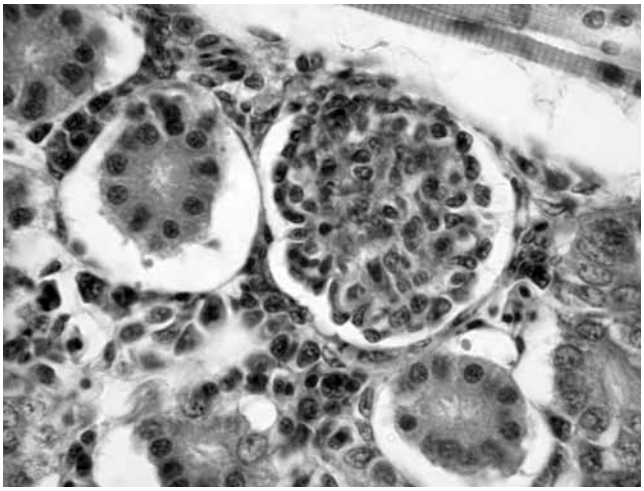

Fig. 3. Detail of renal corpuscle (chromium)

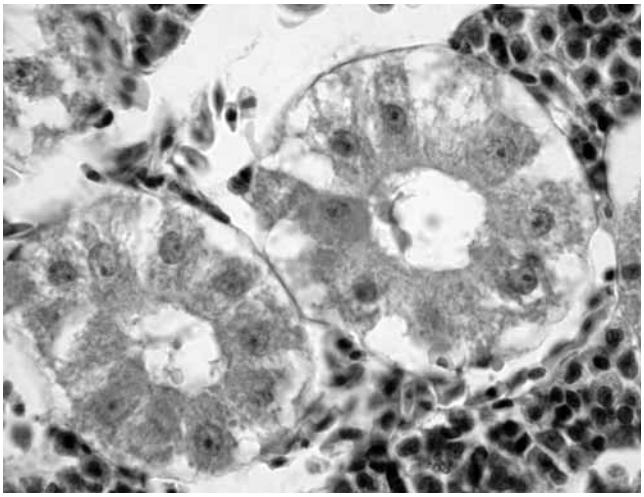

Fig. 4. Detail of renal tubules (chromium and irradiation)

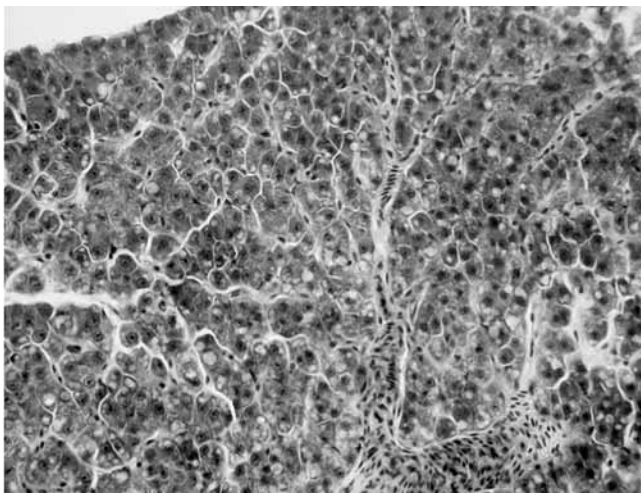

Fig. 5. Liver of control fish

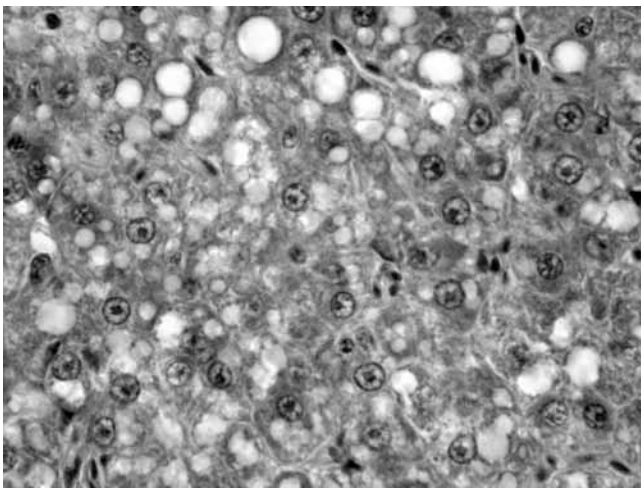

Fig. 6. Liver of fish exposed to chromium

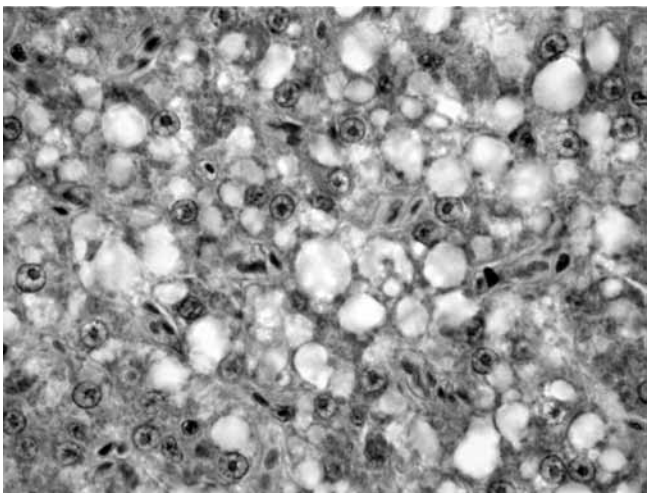

Fig. 7. Liver (chromium and irradiation)

Fig. 4 is a detailed picture of urinary tubule's cross section with marked morphological changes after exposure to chromium and irradiation. The brush border is discontinuous and markedly damaged. The cytoplasm of the cells is noticeably vacuolated, particularly close to the basal membrane. The integrity of the cells is disturbed, they contain erythrocytes with nuclei.

The microscopic observation of the liver of control fish (Fig. 5) revealed liver cells with typical polyhedric shape and a centrally located nucleus with a conspicuous nucleolus. Small fat globules were present in the cytoplasm.

The nuclei of liver cells of fish exposed to chromium (Fig. 6) were round and the cytoplasm contained fat globules of varying size. The hepatocytes of fish exposed to chromium and ionizing radiation (Fig. 7) had shrunken nucleus and the cytoplasm contained many fat globules which tended to coalesce and form extensive conglomerates.

Microscopical changes in *Poecilia reticulate* corresponded to macroscopic findings, clinical symptoms and changes described in mammals and birds (1, 2, 5).

ACKNOWLEDGEMENTS

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REFERENCES

1. Bencko, V., Cikrt, M., Lener, J., 1995: *Toxic Metals in Living and Working Environment of Humans* (In Czech). Grada Publishing, s. r.o., 282 pp.

2. Beňová, K., Cigánková, V., Dvořák, P., Hromada, R., 2006: The influence of ionizing radiation on the morphology of *Poecilia reticulata*. *Folia Veterinaria,* 50, Suppl. S21–S22.

3. ČSN 46 6807, 1974: Acute Toxicity Test on Fishes, 7 pp.

4. Dvořák, P., 2004: Selected Specificity of Aquarium Fish Disease (In Czech). *Bulletin VÚRH,* Vodňany, 40, 101–108.

5. Slanina, Ľ., Sokol, J. *et al.*, 1991: *Vademecum of Veterinary Surgeon* (In Slovak), Príroda, Bratislava, 1182 pp.

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MONITORING OF SELECTED CHEMICAL AND MICROBIOLOGICAL PARAMETERS IN SURFACE WATER IN NNR TAJBA

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ABSTRACT

The only natural living population of European Pond Turtle (*Emys orbicularis*) in Slovakia qualified the National Nature Reserve (NNR) Tajba to become location of national importance. We monitored seasonal changes in the quality of water and potential influence of agricultural activities on this location. Samples of water, taken in monthly intervals between January and December 2009, were examined for basic parameters of water quality: pH, N_{total}, N_{NH3}, chemical oxygen demand (COD_{Cr}) and total count of micro-organisms. Even with this relatively small number of samples we detected some influence of agriculture on water quality in NNR Tajba. This allowed us to assume that the seasonal increase in the content of N_{total} and N_{NH3} was directly related to seasonal character of agricultural production. This could be resolved by extending the NNR location insofar as to prevent penetration of manure residues and other chemicals into surface water in NNR Tajba.

Key words: ammonia N; chemical oxygen demand; European Pond Turtle; total N; pH; surface water; total count of micro-organisms

INTRODUCTION

National Nature Reserve (NNR) Tajba is in the cadastre of the village Streda nad Bodrogom. It spreads over 273 600 m^2 and was declared NNR in 1966 as a sanctuary of the European Pond Turtle (*Emys orbicularis*). NNR Tajba is the only location in Slovakia where lives a stable, regularly reproducing population of this endangered species. In the past, part of the area was contaminated with oil spill. After the spill this terrapin was observed only in the parts unaffected by contamination. Today the territory is subject to scientific research and enjoys 5th degree of protection. Despite this protection, pollution of surface water in this territory has not been fully prevented which could affect the population of turtles.

The aim of our study was to monitor changes in the quality of surface water in the protected area from chemical and microbiological point of view.

MATERIAL AND METHODS

Samples of water were taken in monthly intervals from January till December 2009. Each sample was examined to determine its chemical and microbiological profile. We focused on parameters important for evaluation of the quality of water. The results obtained were compared with standards for surface water (1). Each sample was first examined qualitatively to determine the presence or absence of individual components and in case of positive result we determined quantitatively the level of the respective parameter. Determinations were carried out in duplicate and the results are reported as means.

Chemical analysis

pH was determined potentiometrically using a pH meter (HACH EC 30). For determination of total nitrogen (N_{total}) a non-conserved and unfiltered sample was digested with sulphuric acid and hydrogen peroxide at 480 °C (HACH Digesdahl, Model 23130-21) to convert N_{total} to ammonia form which, after alkalisation, was distilled into diluted sulphuric acid and determined by alkalimetric titration. Ammonia nitrogen (N_{NH3}) was determined by distillation (Parnas-Wagner apparatus), absorption in volumetric solution of sulphuric acid and alkalimetric titration. Determination of chemical oxygen

demand (COD_{Cr}), was carried out with potassium dichromate in a strongly acidic environment at 140 °C for 2 hours (HACH, COD Reactor) and the consumption of potassium dichromate was determined spectrophotometrically (HACH, DR-4000).

Microbiological examination

A 0.1 ml aliquot of sample was inoculated onto meat peptone agar in a Petri dish and cultivated at 36 °C for 24 hours (total count of bacteria – TCB).

RESULTS

Results of chemical and microbiological examination of samples of surface water from NNR Tajba, are presented in Tables 1 and 2.

DISCUSSION

Changes in the quality of water in National Nature Reserve Tajba indicated not only seasonal influence but also potential effect of agricultural activities. The level of pH in surface water should be in the range 6.0–8.5 (1). Our results ranged between 6.37 and 7.65. The shift to the alkaline zone is related to intensive photosynthetic assimilation of micro-organisms (2). Starting from March, we observed a gradual increase up to November when the temperature decreased together with the activity of micro-organisms and the pH level decreased, too.

Total nitrogen is all nitrogen bound in organic and inorganic compounds present in the analysed sample (2). According to legislative provisions the limit for N_{total} is 9.0 $mg.l^{-1}$. Our results obtained in individual months varied by more than 200 %. The abrupt increase in total N in the period of March – June indicated potential effect of agricultural activities in the respective territory. Ammonia can occur in water in two forms NH_3 and NH_4^+, depending on pH (2). According to water standard ammonia in surface water should not exceed 1 $mg.l^{-1}$. This level was not exceeded in the period from January to October. In November – December we observed an abrupt increase in ammonia which can be explained by dying of microflora at first freezing temperatures. Comparison of our results with those obtained in 1992 (3) shows that ammonium level decreased considerably. This may be associated with elimination of an existing in the nineties, the wastewaters from plant PNZP (3). COD_{Cr} or the chemical oxygen demand is a measure of organic substances present in water (3). It is a summary parameter that can indicate contamination of surface water even with oil and sewage spill and run-off from agricultural facilities. According to the standard the limit for surface water is 35 $mg.l^{-1}$. Our results exceeded considerably this limit which can be related to the high bacterial counts in our samples. TBC was several fold higher compared to running surface water with more intensive water exchange. The results of total bacterial counts corresponded to fluctuating temperatures. Our results indicated that quality of surface water in NNR Tajba does not comply with the criteria for surface water set by the respective Governmental

Table 1. Level of chemical parameters in January – December 2009

	28th Jan	19th Feb	25th March	27th April	27th May	17th June	30th July	19th Aug	10th Sep	22th Oct	5th Nov	10th Dec
pH	6.71	6.37	7.28	7.21	7.23	7.65	7.06	7.04	7.32	7.35	7.52	6.88
						$mg.l^{-1}$						
N_{total}	>1	>1	24.65	8.96	22.41	22.41	2.24	2.24	11.21	6.72	4.48	11.20
N_{NH3}	>1	0.13	0.74	0.62	1.22	1.17	0.93	0.97	>1	>1	1.12	2.62
COD_{Cr}	233	235	84	>1	112	189.3	192.2	372.8	1366	>1	>1	124.5

Table 2. Total bacterial count in January – December 2009

	28th Jan	19th Feb	25th March	27th April	27th May	17th June	30th July	19th Aug	10th Sep	22th Oct	5th Nov	10th Dec
						CFU						
TBC	2.0×10^4	2.3×10^3	1.4×10^3	1×10^0	1.5×10	1.8×10^3	4.9×10^3	1.2×10^4	3.0×10^2	1.5×10^3	2.0×10^4	4×10^3

TBC – total bacterial count

Ordinance. One of possible solutions that could limit the influence of agriculture on Tajba reserve area is extension of the protected area so manure and other chemicals could not run directly into the dead distributary. The quality of water should be monitored also with regard to its importance for European Pond Turtle. This topic is open for further research.

REFERENCES

1. Governmental Ordinance of SR, No. 296/2005 Coll. which introduces requirements on the quality and qualitative goals and limit values of parameters of pollution of wastewaters and special waters. Effective of 21st June, 2005.

2. Ondrašovič, M., Para, Ľ., Ondrašovičová, O., Vargová, M., Kočišová, A., 1996: *Veterinary Care About the Environment* (In Slovak). DataHelp, Košice, 109 pp.

3. Rigler, R. 1993: European Pond Turtle (*Emys orbicularis*), its keeping and population living in Eastern Slovakia (In Slovak). *Terarista,* 4, 3–8.

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PREVALENCE OF *DIROFILARIA* SPP. IN DOMESTIC AND FREE LIVING CARNIVORES IN EASTERN SLOVAKIA

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ABSTRACT

Dirofilariasis is a zoonotic parasitic disease which, up to the most recent time, was characteristic of Southern European countries, particularly those in the Mediterranean area. However, many factors, such as, climatic changes, global warming, increased abundance of vectors due to frequent floods and increased mean total precipitations and also increased travelling of owners with pets without prophylaxis, has resulted in the spreading of this disease from the endemic Southern European regions to a northernly direction. The first occurrence of dirofilariasis in Slovakia was recorded in Bratislava district in 2005. In 2007 this disease was, for the first time, diagnosed in a man in our territory, with a subcutaneous localisation of the parasite. Free living carnivores, particularly the red fox (*Vulpes vulpes*), are suitable hosts of filariae and can serve in nature as an important reservoir of the parasite for vectors which subsequently infect dogs and possibly also humans. In our study we examined 213 dogs and 38 foxes from the Košice and Prešov regions. Of all these animals, the diagnosis of dirofilariasis was confirmed in 19 dogs (8.41%) and 21 foxes (55.26%).

Key words: *Dirofilaria immitis*; *Dirofilaria repens*; dog; fox; Eastern Slovakia

INTRODUCTION

Owners travelling with their animals, without taking any adequate prophylactic measures, belong among the ideal means for the spreading of novel infections or well-known infections that had not occurred in certain territories for many years.

„Emerging Infectious Diseases“ is a fixed phrase designating a large group of infectious diseases, the incidence of which is monitored intensively on a worldwide scale from both veterinary and human points of view. These diseases, which are spreading, are related to the migration of humans and animals and are associated with global climatic changes (3). This group includes also diseases, of dogs and less frequently of cats but also of free-living carnivores, caused by the zoonotic species of the genus Dirofilaria. *Dirofilaria immitis* parasitizes the pulmonary arteries, right ventricle and atrium and thus induces serious cardiovascular conditions. The less pathogenic *Dirofilaria repens* parasitizes the cutis and subcutis.

In the Slovak territory dirofilariasis was first diagnosed in 2005. *Dirofilaria repens* was diagnosed in 6 of 15 dogs examined in the region of Bratislava. Of another 7 examined dogs, originating from the district Komárno, four were infected with the species *D. repens* and in addition, two other dogs were found to have a mixed form of the infection (*D. repens* and *D. immitis*) (6). Presently, the incidence of this disease appears to be increasing constantly. This is not a negligible veterinary problem and due to the zoonotic potential of the agent, it also constitutes a human medical problem as well.

The aim of our study was to carry out an epizootological survey of dirofilariasis in domestic and free living carnivores in the Eastern Slovakia territory and to determine the prevalence of the disease agent. Although our investigations of this parasitosis focused more on dogs, one of our objectives was to point out other possible reservoirs of the disease involving most likely free living carnivores, particularly foxes. It is exactly the increasing population of foxes and their more frequent close association with villages and towns that can increase the probability and risk of transmission of dirofilariasis to domestic animals and humans.

MATERIAL AND METHODS

We examined the blood of 213 dogs using samples collected in collaboration with a number of practicing veterinarians and dog breeders from various regions of Eastern Slovakia. For our examinations, 3 ml of venous blood were collected into tubes containing EDTA which enabled the later processing of the samples.

A modification of the Knott technique (2) was used as a rapid diagnostic method for detection of the microfilariae in the blood. Differentiation between microfilariae of the individual species can be made by histochemical staining based on their patterns of acid phosphatase activity. In the microfilariae of *Dirofilaria immitis*, the activity of acid phosphatase was found in the anal and excretion pore zones while *D. repens* exhibited this activity only in the anal pore zone. A commercial staining kite Leucognost SP® (Merck) was used for this purpose (4). The PCR analysis with species specific primers was employed as a very specific method suitable for detection of the agent and confirmation of the species differentiation (5).

We also examined 38 red foxes hunted down in the districts of Košice, Vranov above Topľa and Stropkov. The Knott technique was used to examine the blood obtained from the heart. The presence of adult nematodes was determined by helminthological dissections. DNA for the PCR method was isolated from the spleen using commercial kits.

RESULTS AND DISCUSSION

Of the 213 examined dogs, dirofilariasis was diagnosed in 19 animals (8.92%). Mono-infection with *D. repens* was confirmed in 17 dogs which means a 7.98% prevalence. Mixed infections of *D. repens* and *D. immitis* was diagnosed only in two dogs (0.94%).

The diagnosis of dirofilariasis in foxes is rather complicated. The presence of the adult filariae was not confirmed and microscopical examination of blood obtained from cadaver hearts by the method of Knott appeared unsuitable due to low detection power. The only reliable diagnostic method capable of diagnosing dirofilariasis in free living carnivores is the PCR. Of the 38 examined foxes, *D. repens* diagnosis was confirmed in 21 animals which means a 55.26% prevalence. Mixed infection or mono-infection with *D. immitis* was not observed.

The principal cause of the occurrence of dirofilariasis in Slovakia is most likely the staying of dogs abroad and their return to Slovak territory without effective prevention. However, presently we recorded this disease also in dogs which never travelled, i.e. they acquired the infection in our territory. Our examination showed that *D. repens* is the predominant species in Slovakia which was confirmed by our examination of foxes. The causative agent of the cardiac form, *D. immitis*, was diagnosed only in two dogs but even this was a mixed infection together with *D. repens*. The low prevalence of *D. immitis* may be explained on the basis of studies by Genchi *et al.* (1995) who investigated the interrelationship between both dirofilariae species and indicated that spreading of *D. immitis* was suppressed in the territories with high prevalence of *D. repens* (1). Our investigations proved that there are suitable conditions for the spreading of this parasitic disease in Slovakia and confirmed the potential role of red foxes to act as a reservoir for this zoonosis.

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REFERENCES

1. Genchi, C., Solari Basano, G., Bandi, C., DI Sacco, B., Venco, L., Vezzoni, A., Cancrini, G., 1995: Factors influencing the spread of heartworms in Italy: interaction between *Dirofilaria immitis* and *Dirofilaria repens*. In *Proceedings of Heartworm Symposium '95.* American Heartworm Society, Baravia, IL, 65–71.

2. Knott, J., 1939: A method for making microfilarial survey on day blood. In *Transactions of the Royal Society of Tropical Medicine and Hygiene,* 33, 191.

3. Mišoňová, P., Nágl, I., Svobodová, V., 2003: Issues of dog dirofilariasis in the Czech Republic (In Czech). *Veterinářství,* 53, 2003S, 449–453.

4. Peribañez, M.A., Lucientes, J., Arce, S., Morales, M., Castillo, J.A., Gracia, M.J., 2001: Histochemical differentiation of *Dirofilaria immitis*, *Dirofilaria repens* and Acanthocheilonema dracunculoides microfilariae by staining with a commercial kit, Leucognost-SP®. *Vet. Parasitol.,* 102, 173–175.

5. Rishniw, M., Barr, S.C., Simpson, K.W., Frongillo, M.F., Franz, M., Dominguez Alpizar, J.L., 2006: Discrimination between six species of canine microfilariae by a single polymerase chain reaction. *Vet. Parasitol.,* 135, 303–314.

6. Svobodová, V., Svobodová, Z., Beladičová, V., Valentová, D., 2005: First case of canine dirofilariosis in Slovakia: a case report. Vet. Med.-Czech., 50, 510–511.

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THE OCCURENCE OF ANTIBODIES AGAINST CANINE HERPESVIRUS IN INFECTIOUS TRACHEOBRONCHITIS OF DOGS

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ABSTRACT

Infectious tracheobronchitis of dogs, also called Kennel cough, is a multifactorial, highly contagious respiratory disease of dogs. One of the viruses involved in the development of this disease is canine herpesvirus (CHV), although it may not be directly responsible for the clinical symptoms. This study investigated 20 clinically unhealthy dogs and 10 healthy dogs for the prevalence of antibodies to CHV and also to determine the parameters of non-specific immunity in these dogs. The dogs positive for antibodies to CHV showed alterations in the parameters of non-specific immunity.

Key words: antibodies; canine herpesvirus; infectious tracheobronchitis; non-specific immunity

INTRODUCTION

Infectious tracheobronchitis of dogs is a multifactorial, highly contagious respiratory disease of dogs characterised by paroxysmal cough, fever, lethargy and nose and/or eye discharges (1). The viral agents associated with this disease include; canine parainfluenza virus type 2 (CPiV-2), canine adenovirus type 2 (CAV-2), canine distemper virus (CDV) and canine herpesvirus (CHV) (2). Canine herpesvirus (CHV) participates in infectious tracheobronchitis only partially and it is not a primary disease agent (4). Transplacental infections results in aborted or mummified foetuses. In newborns, the infection is associated with a systemic haemorrhagic diathesis. In older dogs, the disease is inapparent, most frequently affects the genital apparatus and respiratory system and develops due to reactivation of the virus from its latent state. Reactivation is initiated mostly by immunosuppression which is associated with the Kennel cough complex (3).

MATERIAL AND METHODS

The group of sick animals (S) consisted of 20 dogs with clinical symptoms of the respiratory system. Ten clinically healthy animals served as controls (C).

Qualitative detection of antibodies to CHV was carried out by means of the Canine Herpes Virus antibody ELISA test (B.V. European Veterinary Laboratory).

Immunological analysis

Phagocytic activity of neutrofils was determined by the evaluation of the ingestion of 2-hydroxyethyl methacrylate particles (MSHP, diameter 1.2 µm, ARTIM Prague) (9). Phagocytic index of neutrophils was expressed as the ratio of ingested neutrophils and total number of neutrophils. Metabolic activity of the leukocytes was determined by iodonitrotetrazolium test modified by Mareček and Procházková (5). Blastic transformation of lymphocytes was evaluated by ELISA BrdU (colorimetric) test using phytohaemagglutinin PHA-P (Sigma, USA) at concentration 20 µl.ml^{-1}. Chemotactic activity was determined by the method of migration of polymorphonuclear cells under agarose.

RESULTS AND DISCUSSION

Both groups of dogs were subjected to serological examination for qualitative proof of antibodies to CHV. The results are presented in Table 1. In the S group, the positivity for CHV antibodies was detected in 12 out of 20 dogs which corresponded to a prevalence of 60 %. The prevalence of antibodies to CHV in various dog populations reported in the literature, reached 88 % in England, 45.8 % in Belgium and 39.3 % in the Netherlands (6, 7, 8). No antibodies to canine herpesvirus were detected in the dogs from our group C.

Table 1. Qualitative proof of presence of antibodies to CHV in the group of sick dogs (S) by means of Canine Herpes Virus antibody ELISA method

Dog No.	1	2	3	4	5	6	7	8	9	10
Ab	–	+	+	–	–	+	+	+	–	+
Dog No.	11	12	13	14	15	16	17	18	19	20
Ab	+	–	+	+	+	–	+	+	–	–

Ab – antibodies, (-) – negative for antibodies, (+) – positive for antibodies

Table 2. Comparison of the level of parameters of non-specific immunity in sick (S) and clinically healthy dogs (C)

	Le ($\times 10^3$)	FANe (%)	FINe	IMA	SI	CHI
Group S (n = 20)	18.125 ± 9.33	72.44* ± 21.25	13.28** ± 7.53	2.69 ± 0.70	1.06* ± 0.39	1.15* ± 0.27
Group C (n = 10)	9.5 ± 3.5	58,3 ± 5.7	7.2 ± 5.7	2.21 ± 1.06	2.90 ± 1.2	1.89 ± 0.26

Le – leukocytes, FANe – phagocytic activity of neutrophils, FINe – phagocytic index of neutrophils, IMA – index of metabolic activity, SI – stimulation index, CHI – chemotactic index, * – $P \leq 0.05$; ** – $P \leq 0.001$, S – sick dogs, C – healthy dogs

The results of the parameters of non-specific immunity are presented in Table 2. We recorded an increased number of leukocytes, a significant increase in phagocytic activity of neutrophils ($P < 0.05$) and the index of phagocytic activity ($P < 0.001$) in the group of sick dogs. The stimulation index in group S was significantly lower in comparison with group C ($P < 0.05$). Our examination indicated the presence of immunosuppression which is an important factor in the pathogenesis of canine herpesvirus disease (2). The localisation of infection in the respiratory system or genital tract of older, immunosuppressed individuals plays an important role in the transmission of the disease to the foetuses or newborn animals.

CONCLUSION

Our study investigated the prevalence of antibodies to canine herpesvirus in dogs during the acute infection of the respiratory system. Qualitative serological examination by indirect ELISA showed that 60 % of the sick dogs were positive for antibodies to CHV. The immunological examinations preformed, indicated immunosuppression which plays an important primary role in infections caused by or associated with canine herpesvirus.

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REFERENCES

1. Ford, R. B., 2004: Infectious tracheobronchitis. In **King, G. L.:** *Textbook of Respiratory Disease in Dogs and Cats,* Elsevier, USA, 665 pp.

2. Greene, C. E. *et al.*, 2006: *Infectious Diseases of the Dog and Cat.* Third edition, W. B. Saunders, Philadelphia, 1387 pp.

3. Greene, C. E., Carmichael, L. E., 2006: Canine herpesvirus infection. In **Greene, C. E. *et al.*:** *Infectious Diseases of the Dog and Cat.* Third edition, W. B. Saunders, Philadelphia, 2006, 1387 pp.

4. Haanes, E. J., Tomlinson, C. C., 1998: Genomic organization of the canine herpesvirus US region. *Virus Res.* 53, 151–162.

5. Mareček, D., Procházková, J., 1986: *Selected Diagnostic Methods in Medical Immunology* (In Czech). Avicenum, Prague, 219 pp.

6. Reading, M. J., Field, H. J., 1999: Detection of high levels of canine herpesvirus-1 neutralising antibody in kennel dogs using a novel serum neutralisation test. *Res. Vet. Sci.,* 66, 273–275.

7. Rijsewijk, F. A., Luiten, E. J., Daus, F. J., Van der Heijden, R. W., Van Oirschot, J. T., 1997: Prevalence of antibodies against canine herpesvirus 1 in dogs in the Netherlands in 1997–1998. *Vet. Microbiol.,* 65, 1–7.

8. Ronsse, V., Verstegen, J., Onclin, K., Guiot, A. L., Aeberle, C., Nauwynck, H. J., 2002: Seroprevalence of canine herpesvirus-1 in the Belgian dog population in 2002. *Reprod. Dom. Anim.,* 37, 299–304.

9. Větvička, V., Fornusek, I., Kopeček, J., Kamínková, J., Kašpárek, I., Vránová, M., 1982: Phagocytosis of human leukocytes: a simple micro method. *Immunol. Lett.,* 5, 97–100.

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ENZOOTIC PNEUMONIA OF PIGS – LABORATORY AND FIELD DIAGNOSTICS IN PIG HERDS

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ABSTRACT

This study focused on the diagnostics of enzootic pneumonia of pigs (EPO) in selected pig herds. Due to the characteristic mycoplasma lesions found in the lungs of affected animals, pathological-anatomical dissection can be used as one of the field methods for diagnosis of this disease directly on the farm or in the abattoir. We investigated biological material from 10 pigs which demonstrated clinical symptoms of respiratory disease. *Mycoplasma hyopneumoniae*, the causative agent of EPO, was diagnosed in nasal swabs using the nested PCR reaction which is sufficiently sensitive, rapid, and relatively inexpensive. This method allowed us to detect the presence of *M. hyopneumoniae* in a single nasal swab sample. To support the diagnosis, we selected the ELISA method for the detection of specific antibodies against *M. hyopneumoniae*. The presence of antibodies was demonstrated in 50 % of the examined sera.

Key words: enzootic pneumonia of swine; ELISA; *Mycoplasma hyopneumoniae*; nested PCR

INTRODUCTION

Currently, respiratory diseases are considered the most important health problem of modern pig production. *Mycoplasma hyopneumoniae* is a primary infectious agent causing enzootic pneumonia of pigs (EPO) which is frequently complicated by the presence of Pasteurella multocida and Actinobacillus pleuropneumoniae resulting in a complex disease termed respiratory syndrome of pigs. Definitive diagnosis of respiratory disease is based on: a combination of anamnesis data; clinical examinations; laboratory examinations; and the findings in pathological-morphological dissections carried out on the farm or in the abattoir. EPO affects pigs at the age of 3 months. EPO is a worldwide problem responsible for high economic losses in pig herds. It is usually a mild, slow, chronic disease manifested mostly by decreased weight gains. Losses due to secondary bacterial infection and stress occur mostly at the age of 4–6 months of age (2, 5).

MATERIAL AND METHODS

Samples for laboratory PCR examination were taken from 10 pigs of various ages with clinical symptoms of respiratory disease. They were obtained by nasal swabbing with cotton-wool swabs wetted with transport medium. For the ELISA method, intended to prove the presence of antibodies to *M. hyopneumoniae*, we used samples of venous blood collected for preparation of serum from the same animals from which we obtained the nasal swabs. To prove the presence of antibodies to *M. hyopneumoniae* we used the blocking ELISA method, (commercial diagnostic kit supplied by OXOID). To isolate DNA from the nasal swab samples we used the commercial NucleoSpin® Tissue kit (MACHEREY-NAGEL) suitable for isolation of DNA from samples of tissue, cell cultures

and various types of biological material. For amplification we used primers marked as Hp1, Hp3, Hp4 a Hp6: Hp1 (5′TTT TAG TTC GCT AAA ATA TTT AGT AGC A 3′), Hp3 (5′ TCT GTC ATC TCG TTA GCC TCG 3′), Hp4 (5′ TTT TAT TCA AAG GAG CCT TCA 3′), Hp6 (5′ GTC TTA GTC ACT TTT GCC ACC 3′). For the first PCR reaction we selected a combination of primers Hp1/Hp3 and for the second PCR reaction we used a combination of Hp4/Hp6. The size of the target sequence after the first PCR reaction was 967 bp and after the second PCR reaction 660 bp. In the PCR reaction we used Taq-Purple DNA Polymerase PCR Master Mix containing 5 mM $MgCl_2$ (PPP master mix supplied by Top-Bio). All samples were adjusted to the volume of 20 µl which contained 0.5 µl of each primer used, 7 µl ddH_2O, 10 µl PPP master mix, 2 µl DNA. An aliquot equal to 1 µl of the product of the first PCR reaction was used in the second, the so-called nested PCR reaction.

RESULTS

The present study focused on randomly selected pig herds in Slovakia by examining samples from 10 pigs. Our results showed that in one animal (sample No. 7) we diagnosed the presence of *M. hyopneumoniae* in the nasal swab by the nested PCR reaction. Examination by the ELISA method detected specific antibodies to *M. hyopneumoniae* in 50 % of the pigs (5 pigs, samples No. 2, 5, 7, 8 and 10) and 2 samples (20 %) provided dubious results.

DISCUSSION

Serological tests are frequently used to monitor the health status of pig herds. Antibodies to *M. hyopneumoniae* can be detected by the ELISA test or by the less frequently used complement fixation test.

Antibody profiling can meet with difficulties due to the differences in the ELISA tests (1, 3). They involve the following examples: inability of serological tests to differentiate natural infection from vaccination; absence of correlation between various measurements of antibody titres; variations in detection of antibodies against different strains of *M. hyopneumoniae* (6); and considerable variability of the time needed for seroconversion in animals. Serological tests detect the onset of seroconversion and not the beginning of infection. Under natural conditions, Morris *et al.* (4) detected seroconversions in a herd 3 weeks after the contact of animals with the disease agent but the peak titre of antibodies was reached around the 11th week post infection. With the PCR methods, an important aspect is the potential contamination of the samples. Considering the fact that we amplified the *M. hyopneumoniae* DNA of the live and dead organisms, we faced the question whether or not the tested pigs carried active infections. Because *M. hyopneumoniae* adheres to the ciliary epithelium of the respiratory tract, tracheobronchial swabs or bronchoalveolar lavages appear more suitable samples for the detection of *M. hyopneumoniae* by the PCR technique.

Comparison of the nested PCR, blocking ELISA and immunofluorescence showed that the nested PCR was the most sensitive method for the detection of *M. hyopneumoniae* infections. The nested PCR appears to be a suitable method for confirmation of *M. hyopneumoniae* infection on pig farms (7).

The majority of information on the prevalence of EPO and the economical losses associated with this disease was based on evaluation of pathological-anatomical changes observed in the abattoirs. Our results confirmed long-term observations of the occurrence of EPO in pig herds in neighbour states but also outside of Europe.

REFERENCES

1. Ameri-Mahadabi, M., Zhou, E. M., Hsu, W. H., 2005: Comparison of two swine *Mycoplasma hyopneumoniae* enzyme-linked immunosorbet assay for detection of antibodies from vaccinated pigs and field serum samples. *J. Vet. Diagn. Invest.,* 17, 61–64.

2. Betts, A. O., 1952: Respiratory diseases of pigs. V. Some clinical and epidemiological aspects of virus pneumonia of pigs. *Vet. Rec.,* 64, 283–288.

3. Erlandson, K. R., Evans, R. B., Thacker, B. J., Wegner, M. W., Thacker, E. L., 2005: Evaluation of three serum antibody enzyme-linked immunosorbent assay for *Mycoplasma hyopneumoniae, J. Swine. Health. Prod.,* 13, 198–203.

4. Morris, C. R., Gardner, I. A., Hietala, S. K., Carpenter, T. E., Anderson, R. J., Parker, K. M., 1995: Seroepidemiology study of natural transmission of *Mycoplasma hyopneumoniae* in a swine herd. *Prev. Vet. Med.,* 21, 323–337.

5. Ross, R. F., 2003: Mycoplasmas diseases of swine. In **Straw, B. E., D'Allaire, S., Mengeling, W. L., Taylor, D. J.:** *Diseases of swine 1.* (In Slovak), Hajko & Hajková, Bratislava, 357–366.

6. Strait, E. L., Erickson, B. Z., Thacker, E. L., 2004: Analysis of *Mycoplasma hyopneumoniae* field isolates. In *Proc American Association of Swine Veterinarians,* Des Moines, Iowa, 95–96.

7. Verdin, E., Saillard, C., Labbé, A., Bové, J. M., Kobisch, M., 2000: A nested PCR assay for detection of *Mycoplasma hyopneumoniae* in tracheobronchiolar washings from pigs. *Vet. Microbiol.,* 76, 31–40.

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QUANTIFICATION OF ANTIRABIES ANTIBODY LEVELS IN SERA OF VACCINATED HUMANS

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ABSTRACT

The highly specific and sensitive immunoenzymatic methods are the methods most frequently used for the detection of antirabies antibodies. This study presents the results of the detection and quantification of antibodies obtained by ELISA in comparison with the results from conventional methods (virus neutralization test on mice, virus neutralization test *in vitro* – RFFIT, FAVNt). The working procedure and the evaluation of the results are described. We recommend the monitoring of the effects of immunization in vaccinated humans or, at least, in professional personnel exposed in high risk workplaces.

Key words: ELISA; rabies; RFFIT; serum; vaccine

INTRODUCTION

Rabies is an acute neurotrophic viral disease manifested as a fatal encephalitis in humans. Protection is achieved by pre-exposure and post-exposure vaccinations (2). Detection and quantification of antirabies virus antibodies is important for the evaluation of immunity or for the determination of the effectiveness of the antirabies vaccines (3). The most sensitive method for the determination of antirabies antibody levels in the sera of vaccinated humans is the virus neutralization test (VNT) in mice (1) which serves as the WHO reference method, but it is economically and time demanding.

The ELISA test is practically the most frequently used laboratory method for detection and quantification of biologically active substances at very low concentrations (4). However, the humoral response is not the same in all vaccinated individuals; in some we repeatedly detect lower levels of antirabies antibodies. These lower levels are seen in a group of immunoincompetent or immunosupressed people. With regard to the seriousness of rabies, it is recommended to monitor the effectiveness of the vaccinations, particularly in professionally exposed people.

The aim of this study was to compare the detection of antirabies antibodies in the sera of pre-exposure vaccinated people using two different methods, i. e. RFFIT and ELISA.

MATERIAL AND METHODS

In order to compare the detection and quantification of antirabies antibodies, we used sera from 8 professionals working in biological, research and pharmaceutical laboratories. They were subjected to target vaccination against rabies using commercially available human antirabies vaccines (i.e. vaccine VERORAB which is a lyophilised inactivated vaccine prepared in a Vero cell line). Blood samples were taken from these pre-exposure vaccinated people on days 0, 14 and 28. They were revaccinated one year after primovaccination and their sera were obtained on day 28 after revaccination. The sera obtained from the blood were inactivated at 56 °C for 30 min and examined by two methods, i. e. ELISA and RFFIT.

The titres of antirabies antibodies, determined by RFFIT in the sera are expressed in international units ($IU.cm^{-3}$) and the titres obtained by ELISA are expressed in equivalent units ($EU.\ cm^{-3}$).

Table 1. Detection of antirabies antibody titres by ELISA and RFFIT tests in the sera from people subjected to pre-exposure vaccination against rabies

		ELISA ($EU.cm^{-3}$)	RFFIT ($IU.cm^{-3}$)
1	0-sampling	0.211	0.17
	Primovaccination	3.914	3.25
	Revaccination	3.855	3.20
2	0-sampling	0	0.10
	Primovaccination	1.368	0.83
	Revaccination	1.515	0.95
3	0-sampling	0.100	0.09
	Primovaccination	2.106	1.85
	Revaccination	2.812	2.25
4	0-sampling	0.110	0.07
	Primovaccination	2.741	2.30
	Revaccination	2.835	2.35
5	0-sampling	0.015	0.01
	Primovaccination	3.001	2.54
	Revaccination	3.825	3.10
6	0-sampling	0.030	0
	Primovaccination	1.114	0.81
	Revaccination	1.315	0.95
7	0-sampling	0	0
	Primovaccination	0.718	0.45
	Revaccination	0.920	0.52
8	0-sampling	0.112	0.10
	Primovaccination	4.851	3.45
	Revaccination	6.308	4.25

RESULTS

Table 1 shows the results of the examinations of sera from people subjected to pre.-exposure vaccination for the levels of antirabies antibodies by RFFIT and ELISA test. The vaccination scheme was the same but their humoral response differed.

DISCUSSION

The most important requirements for the use of vaccines in human and veterinary immunoprophylaxis is their safety and reliability in inducing immunity towards the respective infectious disease.

The antibody response to viral infection arises approximately 7–21 days after infection. First is the production of macroglobulins IgM followed by the immunoglobulins IgG. Soon after the appearance of IgG antibodies, the titre of IgM antibodies begins to decrease. Therefore, in the majority of cases, 2–3 samples of blood are collected at different stages of the disease.

To ensure international standardization of some serological techniques (VNT, RFFIT, ELISA, RAT), an international standard has been used – a reference serum with a declared number of international units in 1 cm^3 ($IU.cm^{-3}$).

Our comparison showed that the slightly increased level of antirabies antibodies found may also occur in non-vaccinated individuals, but they do not reach the required protective level of 0.5 $IU.cm^{-3}$ (RFFIT) or 1.0 $EU.cm^{-3}$ (ELISA).

In conclusion, we recommend the monitoring of the effects of immunization in vaccinated humans or at least in professional personnel exposed in high risk workplaces. In the indicated cases, one should combine the determination of the level of antirabies antibodies with examinations of cellular immunity.

Our study compared the *in vitro* methods (ELISA, RFFIT) for the determination of antirabies antibodies. An important aspect of the determination is whether the level of antirabies antibodies in the vaccinated people reaches the required protective level.

Because ELISA determines not only the virus neutralization antirabies antibodies but all antirabies antibodies, it is less suitable for the determination of seropositivity or seronegativity of sera. The RIFFIT method appears more suitable because it detects the actual virus neutralization antibodies.

If we are unable to judge whether seropositivity or seronegativity is involved; the best approach is to repeat the examinations or use another suitable method.

REFERENCES

1. Atanasiu, P., 1973: Quantitative assay and potency test of antirabies serum and immunoglobulin. *Laboratory Techniques in Rabies,* Geneva, WHO, 314–318.

2. Seghal, S., 1997: Medical and veterinary aspects of rabies prevention and control. In **Dobet, B., Meslin, F.X. (eds.):** *Proceedings of the Third International Symposium on Rabies Control in Asia,* Paris, Meslin Elseiver, 140–144.

3. Ondrejková, A., Ondrejka, R., Beníšek, Z., Franka, R., Švrček, S., Maďar, M., Bugarský, A., 2002: Comparison of the detection and quantification of rabies antibodies in canine sera. *Vet. Med.,* 47, 218–221.

4. Süli, J., Beníšek, Z., Švrček, Š., Ďurove, A., Závadová, J., 1994: Quantification of anti-rabies antibodies level in sera of immunized subjects (In Slovak). *Bratislava Medical Journal,* 95, 73–74.

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SUSCEPTIBILITY OF YEASTS OF THE ORDER CANDIDA TO SELECTED PHYTO- AND CHEMOTHERAPEUTICS

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ABSTRACT

Essential oils have been used in supportive therapy of mycoses. This study tested the susceptibility of yeasts (*Candida* spp.) to antimycotics, chemo- and phytotherapeutics, by the Etest and the disc diffusion method. Good antimycotic effectiveness was exhibited by itraconazol, ketoconazol and voriconazol. Essential oils and grapefruit-containing commercial preparation failed to produce sufficient effects.

Key words: antimycotics; *Candida*; grapefruit essential oil; susceptibility

INTRODUCTION

The order *Candida* (*C.*) is represented by yeasts which, as commensal micro-organisms, are part of the microflora of healthy humans and other animals. The predisposition factors of the development of yeast infections include failure of the immunological defences and disorders of chemical and physical skin balance. *C. albicans* is considered the principal infectious agent of animal candidoses (3). In addition to treatment with local (topical antimycotics) or systemic (systemic antimycotics) medications, essential oils have been tested as a prospective group of natural substances which can serve as new prototypes of antifungal agents (2, 5).

The aim of this pilot study was to validate the effectiveness of selected antimycotics and natural substances against yeasts of the order *Candida*.

MATERIAL AND METHODS

The susceptibility of yeasts to antimycotics was carried out by two methods: i. e. the disc diffusion method (DDM) and the Etest.

The DDM is based on the use of antimycotic discs (Hi-Media Laboratories Pvt. Ltd., Mumbai, India): amphotericin (Ap) 100 IU/disc, flucoonazol (Fu) 10 μg/disc, ketoconazol (Kt) 10 μg/disc, itraconazol (It) 10 μg/disc, voriconazol (Vo) 1 μg/disc and the Etest test involves the following strips (AB bioMérieux, Sweden): amphotericin (Ap) 0.002–32 μg.ml^{-1}, ketoconazol (Kt) 0.002–32 μg.ml^{-1}, itraconazol (It) 0.002–32 μg.ml^{-1}, fluconazol (Fu) 0.016–256 μg.ml^{-1}, voriconazol (Vo) 0.002–32 μg.ml^{-1}. A modified disc diffusion method according to Ajvazjan (1) was used to check the effectiveness of 33 % grapefruit essential oil (Calendula, SR) and commercial preparation X (Switzerland) of identical concentration.

The susceptibility was tested on 19 isolates of yeasts (*C. albicans* – 10, *C. glabrata* – 9) obtained from animals with diagnosis of mycotic infections. The quality was checked with a standard strain *Candida albicans* CCM 8320 (Czech Collection of Microorganisms, Brno, CR). A pure culture was used to prepare a suspension corresponding to a density of 0.5 on the McFarland scale (approx. 1.106 h.j.ml^{-1}) which was inoculated with a sterile swab onto plates with nutrient Sabouraud agar with chloramphenicol (Hi-Media Laboratories Pvt. Ltd., Mumbai, India). Onto the surface of the inoculated agar we applied aseptically Etest strips, commercial antimycotic discs and discs of 6 mm diameter impregnated with grapefruit essential oil and commercial preparation. The plates were incubated at 37 °C for 48 hours.

The results for chemotherapeutics were interpreted using the criteria recommended by the producer. The positive effect of phytotherapeutics was indicated by zones exceeding 15 mm.

RESULTS AND DISCUSSION

The testing of the effectiveness of the antimycotics (or susceptibility of pathogens) is a necessary precondition of targeted therapy. Table 1 shows the size of the zones of inhibition of isolates produced by the antimycotics, when tested by DDM, and the number and percentage of susceptible strains. The values of the minimum inhibitory concentrations (MIC) and susceptibility of isolates to antimycotics, obtained by Etest, are presented in Table 2.

Amphotericin has a wide spectrum of effectiveness and resistance to this antibiotic is rare (6) which was confirmed also by our data. The DDM and Etest proved the antifungal effects of the ketoconazol despite the resistance of some isolates. Our testing with the DDM test revealed the effectiveness of intraconazol on 90 % of the isolates of *C. albicans* and 33 % of the isolates of *C. Glabrata*, while the Etest demonstrated its effectiveness against 60 % of the isolates of *C. albicans*. However, yeasts of the species *C. glabrata* were resistant to intraconazol. Voriconazol was effective against 40 % of the isolates of *C. albicans*, when tested with DDM. In the study carried out by Okabayashi *et al.* (7), voriconazol was more effective than itraconazol. Our study showed similar results only in the case of the isolates of *C. glabrata* (89 % of susceptible strains).

Few studies are available on the effectiveness of grapefruit essential oil. Cvetnić and Knezević (4) tested the effectiveness of ethanol extracts of grapefruit seeds and pulp against the strain *C. albicans* and the measured zones of inhibition ranged between 9 and 13 mm. Our tests showed that the mean size of the zones of inhibition of *C. albicans* strains reached 11.9 mm for 33 % essential oil and 11.6 mm for the commercial preparation. With the isolates of *C. glabrata* the 33 % essential oil produced a 12.7 mm zone of inhibition and commercial preparation a 10.5 mm zone. On the basis of the interpretation criteria (15 mm) established according to the control strain, the effectiveness of these preparations was determined to be insufficient.

Table 1. The size of the zones of inhibition (mm) obtained with the disc diffusion method and number and % of susceptible strains

Antimycotics	*C. albicans* Range (mm)	Susceptible strains		*C. glabrata* Range (mm)	Susceptible strains	
		n	%		n	%
Amphotericin	16–27	**8**	**80**	9–16	7	77
Fluconazol	12–40	4	40	10–19	0	0
Itraconazol	13–40	9	90	13–19	3	33
Ketoconazol	19–40	9	90	15–40	8	88
Voriconazol	14–40	4	40	10–16	1	11
Grapefruit oil	10–15	1	10	10–22	3	33
Preparation X	10–12	0	0	10–11	0	0

CONCLUSION

The increase in the resistance of infectious agents, which has become a human and veterinary problem of global importance has stimulated the investigations of antimicrobial properties of various plant substances (8).

Insufficient effectiveness of the tested commercial preparation and grapefruit-based essential oils of identical concentration justifies the opinion that other essential oils and plant extracts should be subjected to *in vivo* and *in vitro* testing of their effectiveness.

ACKNOWLEDGEMENT

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REFERENCES

1. Ajvazjan, B. D., 1978: *Fungicidal Activity of Disinfectants. Veterinary Disinfection of Animal Housing Objects* (In Russian). Moscow, 60–64.

2. Bakkali, F., Averbeck, S., Averbeck, D., Idaomar, M., 2008: Biological effects of essential oils – a review. *Food Chem. Toxicol.,* 46, 446–475.

3. Brito, E. H. S., Fontenelle, R. O. S., Brilhante, R. S. N., Cordeiro, R. A., Sidrim, J. J. C., Rocha, M. F. G., 2009: *Candidiases in veterinary medicine: mycological, clinical and therapeutical Aspect* (In Spanish). Ciência Rural, Santa Maria, 39, 2655–2664.

Table 2. Values of the MIC (μg.ml^{-1}) determined by Etest and number and % of susceptible strains

Antimycotics	*C. albicans* Range (μg.ml^{-1})	Susceptible strains		*C. glabrata* Range (μg.ml^{-1})	Susceptible strains	
		n	%		n	%
Amphotericín	0.125–0.5	5	50	0.016–1.5	7	78
Fluconazol	1–64	1	10	0.064–64	3	33
Itraconazol	0.064–4	6	60	0.25–32	0	0
Ketoconazol	0.032–1	7	70	0.125–3	4	44
Voriconazol	0.094–>32	4	40	0.064–32	8	89

4 Cvetnić, Z., Vladimir-Knezević, S., 2004: Antimicrobial activity of grapefruit seed and pulp ethanolic extract. *Acta Pharm.,* 54, 243–250.

5. Čonková, E. *et al.*, 2008: *Veterinary Pharmacology. Special Part* (In Slovak). 1st edn. University of Veterinary Medicine, Košice, 225 pp.

6. Krčméry, V., Haber J., 1999: *Current Pharmacotherapeutics. Systemic Antimycotics* (In Slovak). Osveta, Martin, 39, 52, 53.

7. Okabayashi, K., Imaji, M., Osumi, T., Murakami, Y., Maruyama, H., Kano, R., Hasegawa, A., Watanabe, T., 2009: Antifungal activity of itraconazole and voriconazole against clinical isolates obtained from animals with mycoses. *Jpn. J. Med. Mycol.,* 50, 91–94.

8. Woods-Panzaru, S., Nelson, D., McCallum, G., Ballard, L. M., Millar, B. C. *et al.*, 2009: An examination of antibacterial and antifungal properties of constituents described in traditional Ulster cures and remedies. *Ulster Med. J.,* 78, 213–215.

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STANDARDISATION OF THE DGGE METHOD FOR PRION PROTEIN GENE POLYMORPHISM ANALYSIS IN CATTLE

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ABSTRACT

Denaturing gradient gel electrophoresis (DGGE) is an effective laboratory method capable of detecting mutations in the examined DNA sample. Five pairs of primers were proposed for the analysis of complete prion protein gene coding region in cattle. A set of 20 samples was used to standardize conditions of PCR reactions and DGGE analysis for primers 3, 4 and 5. For primer 5 we identified one profile and for primers 3 and 4, three different profiles. The determination of nucleotide composition of individual profiles and identification of point mutations will require a sequencing method.

Key words: cattle; DGGE; polymorphism; prion protein

INTRODUCTION

Polymorphism of the prion protein (PrP) gene coding region affects incubation time and/or susceptibility to transmissible spongiform encephalopathies (prionoses, TSE) in sheep (3) and humans (1). None of the more than 60 polymorphisms (6) identified in the prion protein PrP gene coding region has been associated with the occurrence of bovine spongiform encephalopathy (BSE). A method of denaturing gradient gel electrophoresis (DGGE) was successfully used to identify polymorphism of prion protein (PrP) gene coding region in sheep (3) and goats (7). The aim of this study was to introduce the DGGE method for analysis of polymorphism of prion protein PrP gene coding region in cattle.

MATERIAL AND METHODS

Standardization of the DGGE method was carried out on a set of 20 samples of genomic DNA of cattle obtained from blood leukocytes by isolation according to Sambrook *et al.* (5). All samples were subjected to PCR reaction using primers PrP-CDS-F/R (4) resulting in a product of 1280 base pairs in length. This product included complete prion protein PrP gene coding region of cattle (795 base pairs) and served as a template for all subsequent PCR reactions in this study.

The first step in the introduction of the complex method was the selection of primers for amplification of DNA fragments suitable for DGGE analysis. The primers were selected by means of software MELTingeny 1.0 (INGENY International BV, the Netherlands) to the nucleotide sequence AJ298878 in the GenBank. Of the five primer pairs proposed for analysis of the complete protein coding region, the third, fourth and fifth primers were successfully standardized in this study. Each of the primers was designed in a way so as to amplify the DNA fragment with one temperature domain. The second step consisted in the use of software DNAStar (DNASTAR Inc., USA) to determine approximately the amplification properties of the primers. In the third step we had to determine the most suitable conditions of PCR reactions with the designed primers. For each primer we tested three different concentrations of $MgCl_2$ in the reaction mixture at various amplification temperatures. For this purpose we used the gradient thermocycler Techne TC-512 (Techne Inc., USA). All products were analysed in 1% agarose gel in 1x TAE buffer solution after staining with GelRedTM Nucleic Acid Gel Stain (Biotium, USA) and visualisation under a UV lamp. We used 100 bp DNA ladder (BioLabs Inc., USA) as a molecular weight standard.

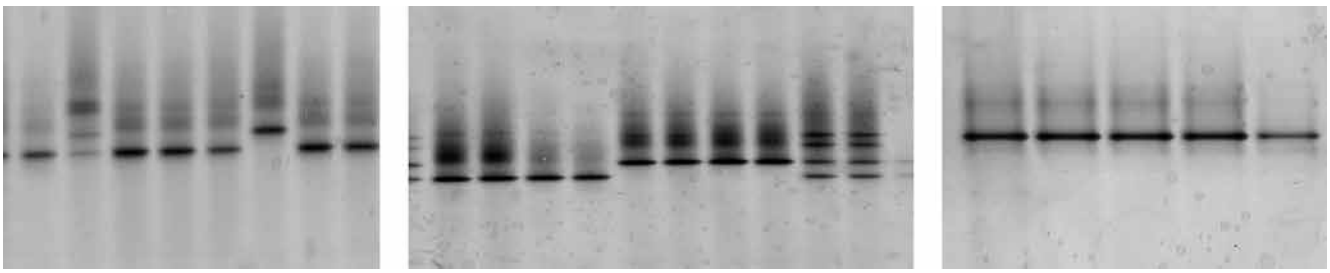

Fig. 1. Results of DGGE analysis of primers 3, 4 and 5

The DGGE method for analysis of prion protein (PrP) gene coding region in cattle was standardised employing an electrophoretic apparatus Ingeny PhorU (INGENY International BV, the Netherlands). The PCR products, amplified at various temperatures and concentrations of $MgCl_2$, were analysed in polyacrylamide gel containing denaturing components urea and formamide in the range 0–80 %. Electrophoresis was conducted at voltage 120 V and temperature 60 °C for 16 hours in 0.5x TAE buffer solution. The gels were stained with silver according to Bassam *et al.* (2).

RESULTS

When determining PCR conditions, the optimum concentration of $MgCl_2$ in the reaction mixture was 1.5 mM per one sample for all primers. The most suitable annealing temperature for primer 3 was 72 °C and for primers 4 and 5 the temperature of 56 °C.

The main aim of this study was to determine the most suitable range of concentrations of denaturing components (i. e. urea and formamide) in polyacrylamide gel that enables distinct separation of the analysed fragments. To analyse PCR products prepared by using primer 3 we selected polyacrylamide gel containing denaturing components in the range 40–80 %. To analyse the products prepared with primers 4 and 5, gel with urea and formamide gradient of 20–60 % was sufficient (Fig. 1).

Standardization of DGGE conditions for individual primers on a set of 20 samples showed three different profiles for primers 3 and 4 and one profile for primer 5. Sequencing of representative samples will be required to determine accurate nucleotide composition of the individual profiles.

DISCUSSION AND CONCLUSION

DGGE is an analytical method which allows one to detect relatively rapidly and effectively any mutation even in one nucleotide of the examined sample. It provides unique profiles characteristic of nucleotide composition of examined samples. In case of known nucleotide composition of the respective profile it is not necessary to subject all examined sample to economically demanding sequencing.

The present study successfully standardized conditions for the DGGE method for three pairs of primers intended for analysis of polymorphism of prion protein PrP gene coding region in cattle. This method will be used to examine 300 DNA samples originating from BSE-negative animals and 24 samples from BSE-positive cattle from the Slovak territory.

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REFERENCES

1. Alperovitch, A., Zerr, I., Pocchiari, M., Mitrova, E., de Pedro Cuesta, J., Hegyi, I. *et al.*, 1999: Codon 129 prion protein genotype and sporadic Creutzfeldt-Jakob disease. *Lancet,* 353, 1673–1674.

2. Bassam, B. J., Caetano-Anollés, G., Gresshoff, P. M., 1991: Fast and sensitive silver staining of DNA in polyacrylamide gels. *Anal. Biochem.,* 196, 80–83.

3. Belt, P. B., Muileman, I. H., Schreuder, B. E., Bos-de Ruijter, J., Gielkens, A. L., Smits, M. A., 1995: Identification of five allelic variants of the sheep PrP gene and their association with natural scrapie. *J. Gen. Virol.,* 76, Part 3, 509–517.

4. Hreško, S., Mojžiš, M., Tkáčiková, Ľ., 2009: Prion protein gene polymorphism in healthy and BSE-affected Slovak cattle. *J. Appl. Genet.,* 50, 371–374.

5. Sambrook, J., Fritsch, E. F., Maniatis, T., 1989: *Molecular Cloning.* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, USA, 9.16.

6. Sander, P., Hamann, H., Pfeiffer, I., Wemheuer, W., Brenig, B., Groschup, M. H. *et al.*, 2004: Analysis of sequence variability of the bovine prion protein gene (PRNP) in German cattle breeds. *Neurogenetics,* 5, 19–25.

7. Tkáčiková, Ľ., Bhide, M., Imrichová, D., Mikula, I. Sr., 2006: The analysis of prion protein gene polymorphism in Slovakian white shorthaired goats. *Folia Veterinaria,* 50, 146–150.

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COLOSTRAL NUTRITION IN INTENSIVE CATTLE BREEDING

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ABSTRACT

Newborn Holstein calves (n = 80) were divided into 2 treatment groups receiving the first colostrums by different methods of colostral feeding. Animals of group I (n = 33) were fed 2 l of colostrum from their dam using a nipple bottle at different times post partum. Animals of group II (n = 47) were drenched with 3–4 l of colostrums. The serum concentrations of the total immunoglobulins 3–5 days post partum were higher in the drenched calves (19.2 ± 11.4 U ZST) compared with bottle-fed calves (16.9 ± 11.5 U ZST). Out of the calves tested, 61% in group I and 45% in group II absorbed sufficient immunoglobulins from the colostrum to achieve adequate levels of passive immunity. It was concluded that the proper application of colostrum by a drencher is a suitable useful method for providing an adequate colostral supply to newborn calves.

Key words: calves; colostrum; immunoglobulins

INTRODUCTION

Both the prenatal development of calves and the complicated syndesmochorial placenta do not enable the passage of specifically efficient substances and immunoglobulins (Ig), and therefore calves are born agammaglobulinemic. Colostrum as the first secretion of the mammary gland after calving has a protective as well as a nutritional function. It is an irreplaceable source of immunoglobulins, vitamins, mineral substances, essential amino acids and fatty acids, as well as hormones and enzymes (1). Regarding the fact that colostrum is the only source of passive immunity in new-born calves, the impairment of colostral nutrition may significantly increase the mortality and morbidity of calves (13).

The level of immunoglobulins as well as the amount of ingested colostrum is influenced by: the age; breed; health status; dry period length; amount of secreted colostrum; and the season (10, 8, 4). Evaluation of the specific weight according to the linear relationship between specific weight and content of immunoglobulins in colostrum is successfully used for the determination of colostrum quality (3). The passive transfer of colostral immunoglubulins is influenced by: the amount of ingested Ig in colostrum; time and way of administration of the first colostrum; genetics; physiological; and climatic effects (4, 9). Colostral Ig are transported through the mucosa of the small intestine in the first days after birth, and through the lymphatic system they are transported into the blood (11). The way of feeding at the time of suckling (i.e. from the udder, suckling through the nipple (bottle-fed), and more recently via forced colostrum ingestion by the oesophageal probe) significantly influences the total intake as well as the absorption of the immunoglobulins and thus contributes to the overall resistance of the calves (2).

The aim of this study was to observe the effects of the amount and way of administration of the first colostrum on the levels of serum immunoglobulins, growth intensity, and losses due to mortality under breeding conditions.

MATERIAL AND METHODS

New-born calves were divided into 2 groups (control and experimental). New-born calves were fed colostrum of their own dams in the morning and evening. In the control group the calves obtained two litres of colostrum by suckling through the nipple. In the experimental group the calves were drenched on the average with 3–4 l of colostrum depending upon their weight (in the recommended

dose of 90 ml.kg^{-1} body weight). On day 3 to 5 after birth the values of immunoglobulins were determined from collected blood using the turbidimetric test (Zinc Sulphate Turbidity – ZST). The growth intensity during milk and at the change from milk to green nutrition was calculated according to the records of the weights at birth and at weaning.

RESULTS

The level of serum immunoglobulins

Out of the total number of 80 examined calves the mean value of immunoglobulins of 18.04 ± 11.6 U ZST was found. Out of the total number examined, the optimum levels of immunoglobulins above 20 U ZST were reached in 34 % of the calves. In the experimental group of drenched calves in 47 calves the mean value of serum imunoglobulins was 19.17 ± 11.4 U ZST. In the control group ingesting colostrum by suckling from bottles 33 calves were examined and the level of serum immunoglobulins reached on the average was 16.9 ± 11.5 U ZST. The difference in the values found in serum immunoglobulins was not statistically significant.

Division of calves according the level of immunoglobulins

The optimal saturation of colostral immunoglobulins above 20 U ZST was found in 35 % of the examined calves fed using the probe, and in 30 % of the calves ingesting colostrum through the nipple. The mean values of serum immunoglobulins in these groups were; at feeding by the probe 32.1 ± 8.54 U ZST ; and at free intake by suckling from the nipple 31.9 ± 8.84 U ZST. A slight degree of immunodeficiency in calves within the range of values of serum immunoglobulins 15–20 U ZST was found in 26 % of drenched calves, and 15 % of calves after colostrum intake by suckling from the nipple. Significantly reduced levels of immunoglobulins under 15 U ZST were confirmed in 39 % of the drenched calves and in 54 % of the bottle-fed calves.

Table 1. The mean values of Ig in relation to serum Ig level

	> 20 U ZST	15–20 U ZST	10–15 U ZST	< 10 U ZST
Parameter	x ± s	x ± s	x ± s	x ± s
Probe	32.1 ± 8.54	17.98 ± 1.42	11.67 ± 1.25	6.59 ± 2.68
Nipple	31.89 ± 8.84	17.69 ± 1.01	12.3 ± 1.32	5.01 ± 2.48

U ZST – Units of Zinc Sulphate Turbidity

The level of serum immunoglobulins and time of administration of the first colostrum

A negative correlation was observed (using precise evaluations) between the time of feeding after birth and serum levels of immunoglobulins. The examination results (independent of the amount of ingested colostrum) confirm the indirect correlation with a high degree of regression correlation r = 0.80 1 (Fig. 1) between the time of administration of the first colostrum and level of serum immunoglobulins.

Table 2. Relationship between serum immunoglobulin level and the time of administration of colostrum

Probe				Nipple			
n	Level Ig	Time (hr)	Intake (l)	n	Level Ig	Time (hr)	Intake (l)
14	28.9 ± 9.6	2.2 ± 0.9	3.44	14	27.6 ± 11.3	2.6 ± 1.5	2.0
13	15.9 ± 6.8	5.1 ± 0.9	3.15	13	15.7 ± 4.9	4.8 ± 0.8	2.0
8	9.54 ± 4.8	8.2 ± 1.0	3.44	8	4.56 ± 2.4	8.6 ± 1.5	2.0

The animals were arranged into groups according to the time of the administration of the colostrum; i. e. up to 3 hours, 3–6 hours and more than 6 hours after birth (Table 2). The highest level of immunoglobulins was confirmed in calves ingesting colostrum within 3 hours after their birth. In this group all the calves reached a high level of serum immunoglobulins with the mean value at the colostrum drenching, or bottle feeding on the level of 28.9 ± 9.6, or 27.6 ± 1.5 U ZST, respectively. On the contrary, the lowest level of immunoglobulins was found in calves ingesting the first colostrum after 8 hours after birth. At the colostrum intake by the probe and suckling from the nipple, the values of immunoglobulins were found on the average to be 9.5 ± 4.8 and 4.5 ± 2.4 U ZST, respectively ($P < 0.05$).

In this last group, all the calves showed a significant deficiency of serum immunoglobulins where the values did not exceed the level of 15 U ZST resulting in a high risk of mortality.

The effect of colostrum intake on the growth intensity and losses due to mortality

Out of the total number of calves observed within the groups, mortality in the period of milk and passage to green nutrition represented 8.9 %. Colostral nutrition, besides its protective function at postnatal saturation of immunoglobulins, meets also the nutrition function by the supply of essential nutrients supporting the metabolism, growth, and non-specific resistance of the organism. At the forced intake of higher doses of colostrum administered by the probe, 39 % of the calves had the underthreshold values of immunoglobulins and weight gain on the average was 627 g/day with mortality of 2 % of calves during pre-weaning period. In the group of calves receiving the uniform dose of 21 of the first colostrum by bottle-feeding, the underthreshold values

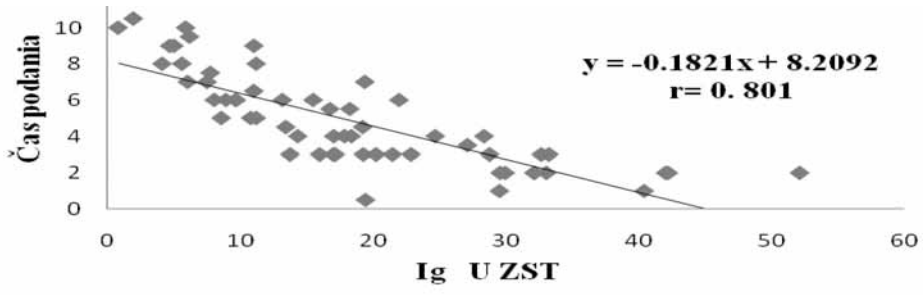

Fig. 1. Serum Ig content compared with the time of colostrum administration

Vertical axis: the time of colostrum feeding after birth, in hours

of serum immunoglobulins were confirmed in 54 % of the animals with 16.5 % losses due to mortality, and an average weight gain of 584 g daily during the period of milk consumption. The difference, even if not statistically significant, represented an increase in weight gain of 2.65 kg during the pre-weaning at the same intensity of feeding.

DISCUSSION

Bottle-fed calves were administered 2 l of colostrum daily that represented a general dose for the intake of the first colostrum, which calves spontaneously ingested during 15 minutes of suckling. Such a dose is sufficient for the saturation of serum immunoglobulins, provided that it is administered within the first 3 hours after birth (11). Our results with forced intake of high doses of colostrum administered into the rumen by the probe in comparison with those with colostrum suckling confirmed higher saturation of serum immunoglobulins in the calves on the average. Also seen by the probe method were: a lower portion of calves with the threshold values of immunoglobulins; higher growth intensity; and lower losses in calves due to mortality. Such control of the Ig level confirmed that absorption of immunoglobulins was not disrupted, which is in harmony with the finding of Hopkins and Quigley (5). They confirmed the same absorption of immunoglobulins at administration of 4 l of colostrum at one as well as 2 administrations. In contrast, Lee *et al.* (7) found the reduced absorption and lower concentration of serum immunoglobulins by the colostrum drenching method compared to suckling from the nipple. The results of our examinations found that the forced intake of colostrum through a probe is a suitable and safe way of administration of colostrums that ensures passive immunity to new-born calves, which is in harmony with the results of Kaske *et al.* (6)

CONCLUSIONS

Under breeding conditions in a considerable number (n = 80) of examined calves, we observed the effect of administration of the first colostrum by suckling through the nipple and forced administration using the probe on the levels of serum immunoglobulins measured turbimetrically on days 3–5 after birth. Higher levels of serum immunoglobulins (19.2 ± 11.4 U ZST) were recorded in drenched calves at forced intake of the first colostrum at the dose of 3–4 l in comparison with the intake from the nipple (16.9 ± 11.5 U ZST). Significantly reduced levels of immunoglobulins under 15 U ZST were confirmed in 39 % of the drenched calves and 54 % of the bottle-fed calves after the colostrum intake by suckling from the nipple. The results confirm an indirect correlation and a high degree of regression correlation (r = 0.801) between the time of the first colostrum administration and level of serum values of immunoglobulins. The forced intake of higher doses of the first colostrum by the probe represents a suitable way for ensuring passive immunity, reduction of mortality and increase in growth intensity during the milk nutrition of calves.

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REFERENCES

1. Blum, J. W., Hammon, H., 1999: Endocrine and metabolic aspects in milk-fed calves. *Domest. Anim. Endocrinol.,* 17, 219–230.

2. Brignole, T. J., Stott, G. H., 1980: Effect of suckling followed by bottle feeding colostrum on immunoglobulin absorption and calf survival. *J. Dairy Sci.,* 63, 451–456.

3. Fleenor, W. A., Stott, D. H., 1980: Hydrometer test for estimation of immunoglobulin concentration in bovine colostrum. *J. Dairy Sci.,* 63, 973–977.

4. Guy, M., McFadden, T. B., Cockrell, D. C., Besser, T. E., 1994: Regulation of colostrums formation in beef and dairy cows. *J. Dairy Sci.,* 77, 3002–3007.

5. Hopkins, B. A., Quigley, J. D., 1997: Effects of method of colostrum feeding and colostrum supplementation on concentrations of imunoglobulins G in the serum of neonatal calves. *J. Dairy Sci.,* 80, 979–983.

6. Kaske, M., Werner, A., Schuberth, H. J., Rehage J., Kehler, W., 2005: Colostrum managment in calves: effects of drenching vs. bottle feeding. *J. Anim. Physiol. Anim. Nutr.,* 89, 151–157.

7. Lee, R. B., Besser, T. E., Gay, C. C., McGuire, T. C., 1983: The influence of method of feeding on IgG concentrations acquired by calves. In *Proceedings 4th International Symposium on Neonatal Diarrhea.* VIDO, Saskatoon, Saskatchewan, Canada, 373–377.

8. Pritchett, L. C., Gay, C. C., Besser, T. E., Hancock, D. D., 1991: Management and production factors influencing immunoglobulin G1 concentration in colostrum from Holstein cows. *J. Dairy Sci.,* 74, 2336–2341.

9. Quigley, J. D., Drewry, J. J., 1998: Nutrient and immunity transfer from cow to calf pre- and postcalving. *J. Dairy Sci.,* 81, 2779–2790.

10. Shearer, J., Mohammed, H. O., Brenneman, J. S., Tran, T. Q., 1992: Factors associated with concentrations of immunoglobulins in colostrum at the first milking post-calving. *Prev. Vet. Med.,* 14, 143–154.

11. Stott, G. H., Marx, D. B., Menefee, D. B., Nightengale, G. T., 1979: Colostral immunoglobulin transfer in calves I. period of absorption. *J. Dairy Sci.,* 62, 1632–1638.

12. Vajda, V., Slanina L., 1980: Dynamics of immunoglobulins in calves in the industrial system of breeding (In Slovak). *Vet. Med. Czech,* 25, 527–536.

13. Vajda, V., 1997: *Dietetics and nutrition prevention of health disorders in calves* (In Slovak). Habilitation thesis, University of Veterinary Medicine, Košice, 188 pp.

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CLINICAL SYMPTOMS AND ALTERATION OF LIVER PROFILE PARAMETERS IN HYPERTHYREOID ANIMALS

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ABSTRACT

Sixty four clinical records of cats with hyperthyreosis diagnosed in 2002–2008 were processed for a retrospective study. The most frequently observed symptoms were weight losses and polyphagia. Thyroid gland dysfunction can lead to secondary hepatopathy. The majority of patients (62.5%) showed increased activity of alanine aminotransferase and 38.5% had increased activity of alkaline phosphatase. The non-specific clinical symptoms, such as, apathy, anorexia and somnolence, that could be ascribed to secondary damage to the organs, such as liver, or classified as apatic hyperthyreosis, were observed in 26% of the animals.

Key words: alkaline phosphatase ALP; alanine aminotransferase ALT; hepatopathy; hyperthyreosis; thyroxine

INTRODUCTION

The liver performs many functions affecting the activity of other organs and, conversely, diseases of other organs may cause secondary damage to the liver. Secondary (reactive) hepatopathies are a relatively frequent complications of other primary diseases. Changes in the activity of liver enzymes in the blood serum allow one to monitor the reaction of hepatocytes to endogenous and exogenous insults. The aim of this study was to determine the proportion of patients with altered activity of the liver enzymes alkaline phosphatase (ALP) and alanine aminotransferase (ALT) at the time of the initial diagnosis of hyperthyreosis. We recorded: the mean age of patients at the time the hyperthyreosis was diagnosed; frequency of its occurrence in the respective breeds; and the presence of other than the common symptoms of hyperthyreosis that could be related to the development of secondary hepatopathy in the afflicted animals.

MATERIAL AND METHODS

The criterion for inclusion of a patient in the study was a serum thyroxine (T4) level above 60 nmol.l^{-1}. T4 levels between 55–60 nmol.l^{-1} were considered dubious and such patients were included in the study if the repeated sampling provided result above 60 nmol.l^{-1}. The reference levels for the activity of enzymes in the relevant laboratory were 12–130 U.l^{-1} for ALT and 14–111 U.l^{-1} for ALP. The clinical symptoms of hepatopathy (or non-specific with respect to hyperthyreosis) were: anorexia; lethargy; and somnolence. According to the clinical symptoms and the activity of ALT, the patients were divided to the following basic groups: A1(increased ALT and in some cases also ALP), A2 (normal ALT and ALP), B1 (increased ALT and mostly also ALP) and B2 (normal ALT and ALP). Patients with no records of the ALT activity were included in the groups A3 and B3.

RESULTS AND DISCUSSION

Results processed in this study are presented in Table 1.

Of the 64 hyperthyreoid cats studied, 57 were European shorthaired cats (95%), 5 European longhaired cats, 1 Siamese and 1 Persian cat. No breed predisposition to this disease was observed. The mean age of the animals was 13 years and 9 months. According to other authors the disease affects

Table 1. T4 level and the corresponding activities of ALT and ALP at the time of the first diagnosis of hyperthyreosis

Patient No.	T4 nmol.l^{-1}	ALT U.l^{-1}	ALP U.l^{-1}	Patient No.	T4 nmol.l^{-1}	ALT U.l^{-1}	ALP U.l^{-1}
1 - A1	238	304	357	**6 - A2**	150	46	60
2 - A1	66.9	195		**7 - A2**	150	96	50
3 - A1	174	292	110	**9 - A2**	150	69	59
8 - A1	76.8	423.8	24.45	**10 - A2**	127	94	86
11 - A1	150	735	294	**12 - A2**	80	82	51.2
14 - A1	90	267	128	**23 - A2**	152	83	57
29 - A1	59.2	346	84	**26 - A2**	130	110.7	87.7
35 - A1	235	223	70	**27 - A2**	77.2	32	56
37 - A1	163	176	97.8				
38 - A1	80.2	237	144.3				
40 - A1	300	350	41				
42 - A1	150	328	212				
43 - A1	150	132	122				
48 - A1	150	302	178				
51 - A1	150	230	210				
58 - A1	148	167.3	143.4				
59 - A1	180	137.7	109.6				
63 - A1	150	274	104				
Patient No.	**T4 nmol.l^{-1}**	**ALT U.l^{-1}**	**ALP U.l^{-1}**	**Patient No.**	**T4 nmol.l^{-1}**	**ALT U.l^{-1}**	**ALP U.l^{-1}**
15 - B1	150	284	48	**17 - B2**	150	110	
16 - B1	109	138	213	**25 - B2**	61.8	67	60
32 - B1	82	265	75	**30 - B2**	150	113	116
36 - B1	150	138	156	**44 - B2**	77.2	38.1	109.2
45 - B1	150	171	50	**49 - B2**	116	46	58
46 - B1	150	215	191	**53 - B2**	91.6	74.7	98.1
55 - B1	123	252.6	145	**57 - B2**	73.7	58	105
64 - B1	212	705	165.9	**correl**		0.37	0.24

A1 – increased ALT and in some cases also ALP; A2 – normal ALT and ALP
B1 – increased ALT and mostly also ALP; B2 – normal ALT and ALP

cats of medium age (5, 8). The youngest age in which the diagnosis was made in our study was 6 years and 4 months; the oldest one was 19 years and 11 months. Our study revealed that the seriousness of the clinical symptoms decreased following the initial diagnosis. Feline hyperthyroidism was also investigated by Bruyette (3) and Shiel and Mooney (9) which indicates the fact that increasing attention has been paid to this disease; particularly the middle age cats compared to the previous practice of diagnosing the diseases in the earlier stages. In the 1990's, the oldest age in which feline hyperthyreosis was diagnosed was by two years higher than today (2, 10).

The most frequently observed hyperthyreosis symptoms, concordant with the literary data (6) are: loss of weight; cachexia and polyphagia; and less frequent are: diarrhoea; polyuria/polydipsia; tachycardia; heart rhythm disorders; hypertension; deterioration of hair quality; alopecia; and hyperactivity. The symptoms were observed in 74 % of animals (group A), and 26 % of the animals (group B – 17 animals) showed symptoms not typical for hyperthyreosis (anorexia, lethargy, somnolence). There may be signs of secondary hepatopathy which, in some animals (47 %) of this group (group B1 – 8 animals), correlated with markedly altered activity of liver enzymes. In additional animals (group B2 – 7 animals, 41 %) the activities of ALT and ALP were within the acceptable range but their results were not available for 2 animals (11.7 %). Besides secondary hepatopathy, such clinical symptoms may be induced by the so-called apathetic hyperthyreosis which affects approximately 10 % of the hyperthyreoidic cats (6). The aetiology of apatic hyperthyreosis may be based on secondary damage to other organs.

Of the animals included in group A (no signs of hepatopathy), 18 showed an increased activity of ALT (A1). Altered ALT and ALP in hyperthyreoid cats without clinical symptoms of hepatopathy corresponded with the data of Berent *et al.* (1). Altered ALT, ALP and serum bile acids (SBA), without apparent clinical symptoms of hepatopathy, correlated with the histopathological changes in the liver of hyperthyreoid cats (3). The potential causes of liver damage in hyperthyreoid cats are: malnutrition; hepatic anoxia; congestive heart failure; infections; and the direct toxic thyroxine action on the liver (7).

Of the patients in which the activity of ALT was determined at the time of diagnosis of hyperthyreosis (41 patients), ALT values exceeded the reference level in 62.5 % of the animals and the extent of the increase correlated directly with T4 level ($r = 0.37$). Comparison according to multiples of increase (i. e. in relation to the reference range) showed a decrease in the correlation coefficient from 0.37 to 0.32. Gunn-Moore (6) reported an alteration of ALT in 85 % of the animals. The activity of ALP was increased in 38.5 % of the cats and there was a direct correlation ($r = 0.32$) between ALP and T4. Bruyette (3) reported in his study the alteration of ALP activity in 53 % of the patients. Foster and Thoday (4) recorded a significant correlation between the T4 concentration and ALP activity, T4 concentration and activity of liver isoenzyme ALP, but not between concentration of T4 and the activity of bone isoenzyme in hyperthyreodic cats.

CONCLUSION

Long-term untreated hyperthyreosis results in secondary damage to other organs. In addition to presumed symptoms of hyperthyreosis, relatively large proportion of patients showed also non-specific signs of liver damage or apathic hyperthyreosis. In the majority of hyperthyreoid patients we detected increased serum activity of ALT as a manifestation of suspected secondary hepatopathy. With regard to the above described correlation between increased ALT and histopathological changes we recommend to use the ALT activity as a parameter suitable for identification of secondary hepatopathy regardless of the presence of clinical symptoms.

REFERENCES

1. Berent, A. C., Drobatz, K. J., Ziemer, L., Johnson, V. S., Ward, C. R., 2007: Liver function in cats with hyperthyroidism before and after I-131 therapy. *Journal of Veterinary Medicine,* SN 0891-6640.

2. Broussard, J. D., Peterson, M. E., Fox, P. R., 1995: Changes in clinical and laboratory findings in cats with hypertyroidism from 1983 to 1993. *J. Am. Vet. Med. Assoc.,* 206, 302–305.

3. Bruyette, S. D., 2004: Choosing the best tests to diagnose feline hyperthyroidism. *Vet. Rev.,* 99, 956–961.

4. Foster, D. J., Thoday, K. L., 2000: Tissue sources of serum alkaline phosphatase in 34 hypertyroid cats: Qualitative and quantitative study. *Res. Vet. Sci.,* 68, 89–94.

5. Frenais, R., Rosenberg, D., Burgaud, S., Horspol L. J. I., 2009: Clinical efficacy and safety of a once daily formulation of carbimazole in cats with hyperthyroidism. *Journal of Small Animal Practice,* 50, 510–515.

6. Gunn-Moore, D., 2005: Feline Endocrinopathies. *Small Animal Practice,* 35, 171–210.

7. Ingbar, S. H., Braverman, L. E., 1986: The thyroid: *A Fundamental and Clinical Text.* 5th edn., J. B. Lippincott, Philadelphia, PA, 839 pp.

8. Sassnau, R., 2006: Epidemiological investigation on the prevalence of feline hyperthyroidism in an urban population in Germany. *Tiererztliche Praxis Ausgabe Kleintiere Heimtiere,* 34, 450–455.

9. Shiel, R. E., Mooney, C. T., 2007: Testing for Hyperthyroidism in Cats. *Vet. Clin. Small Anim.,* 37, 671–691.

10. Thoday, K. L., Mooney, C. T., 1992: Historical, clinical and laboratory features of 126 hypertyroid cats. *Vet. Rec.,* 131, 257–263.

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THE INFLUENCE OF *SALMONELLA ENTERICA* PT4 AND *ENTEROCOCCUS FAECIUM* EF55 ON THE PROLIFERATIVE ACTIVITY AND VILLUS HEIGHT IN THE SMALL INTESTINE OF EXPERIMENTALLY INFECTED CHICKENS

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ABSTRACT

The aim of this study was to explain the influence of *Enterococcus faecium* EF55 (EF) on proliferative activity of enterocytes and villus height in the small intestine of chickens experimentally infected with *Salmonella Enterica* PT4 (SE). Morphometric analysis showed that the lowest villi were recorded in the SE groups and the highest in the EF groups. Significant difference between SE and EF groups ($P < 0.05$) were observed in the duodenum (D) at the 2nd sampling. Immunohistochemical analysis of the proliferative activity of the cells in the jejunal mucosa (J) detected the lowest activity in the SE groups ($P < 0.001$) and the most intensive proliferation of enterocytes in the EF groups ($P < 0.001$). These results indicate a positive influence of the EF on morphometric and regenerative properties of small intestine mucosa after experimental infection with *Salmonella Enterica* PT4.

Key words: chickens; *Enterococcus faecium* EF55; PCNA; *Salmonella Enterica* PT4; intestine

INTRODUCTION

Salmonelloses are among the most common zoonoses worldwide. The intestinal mucosa is the portal of entry to the body for many pathogens, including representatives of the genus *Salmonella*. Probiotics offer possible protective functions to preserve the integrity of the intestinal mucosa. (1).

Many *Salmonella* species adversely affect the villus height and the area of enterocyte brush border. One of the positive properties of probiotics is the inhibition of pathogens by means of the production of bacteriocins and the competition for intestinal surfaces; thus providing "protection" of the intestinal mucosal structures against bacterial adhesion (2).

The small intestine epithelium consists of continuously regenerating cells. Contrary to mammals, the proliferation of intestinal epithelium in poultry occurs also along intestinal villi as well as in the crypts (5). The proliferating cell nuclear antigen (PCNA) is a generally accepted marker of cell proliferation (4).

The aim of this study was to investigate the proliferative activity of enterocytes and changes in the small intestine villus height in chickens experimentally infected with *S. Enterica* PT4 on day 4 of life and administered *E. faecium* EF55 in their rations

MATERIAL AND METHODS

The experiment was carried out on 60 1-day old ISA Brown chickens. They were divided to 3 groups, 20 chicks in each: 1 – control (K); 2 – infected with single dose of *S. Enterica* PT4 (SE), 1.108 CFU in 0.2 ml PBS, on day 4 of the experiment; 3 – administered probiotic *E. faecium* EF 55 (EF), 1.109 CFU (head.day-1). On days 7 (1st sampling) and 21 (2nd sampling), samples of duodenum (D) and jejunum (J) were taken from decapitated chickens from each group, fixed in 10 % formalin, embedded in paraffin using common histological technique and were subjected to morphomet-

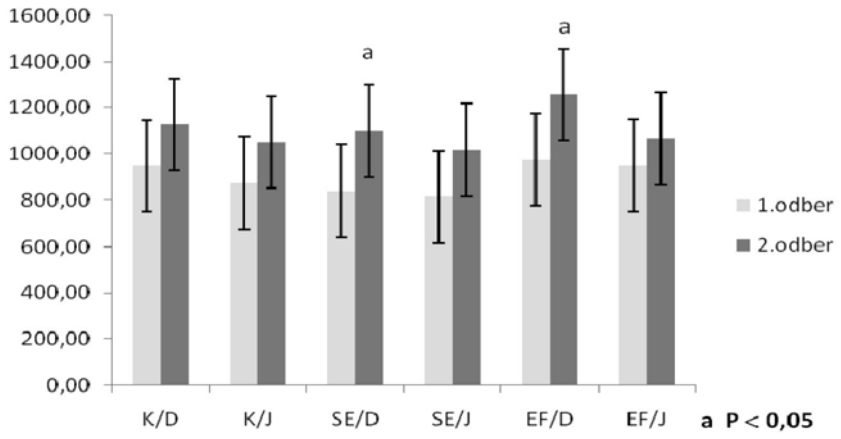

Fig. 1. Height of villi in μm (sampling 1 and 2)

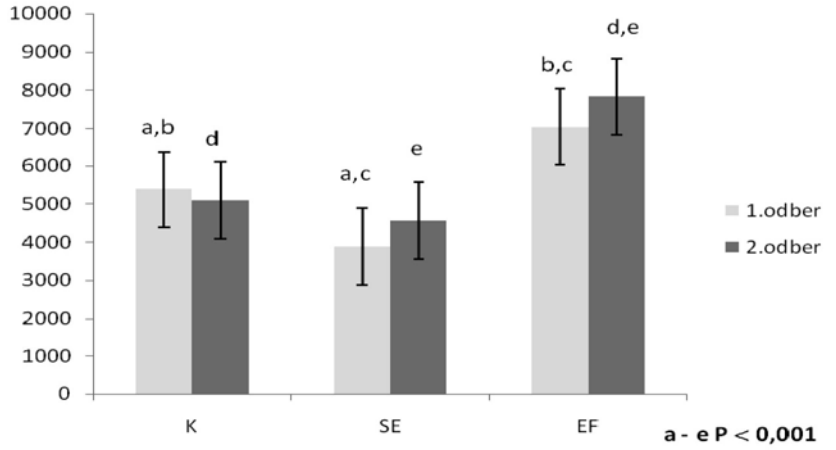

Fig. 2. Number of PCNA positive cells (sampling 1 and 2)

ric analysis (15 measurements, statistically processes by Anova) at magnification × 4, namely from the villus base up to their apical end (software NIS-Elements AR/BR v. 3.0). Samples (J) were processed also immunohistochemically to detect PCNA protein using murine anti-PCNA antibody (Clone PC 10, DACO A/S Glostrup, Denmark and Animal Reseach Kitu. The PCNA positive cells (15 measurements, statistical processing by Anova) were quantified by means of a calibrated eyepiece grid LTD 0.25 mm^2 IdxGrD (Ch. Gröpl Electronmikroskopy, UK), magn, × 100.

RESULTS

Morphometric analysis detected higher villi at the second sampling in all groups. Comparison between groups showed that the lowest villi were observed in the SE groups and the highest in the EF groups, with a significant difference between them at the second sampling in the duodenum (Fig. 1).

Immunohistochemical analysis of the PCNA protein in the jejunal villi at both samplings showed the lowest proliferative activity in the SE groups ($P < 0.001$) and the most intensive proliferation of enterocytes in the EF groups ($P < 0.001$) (Fig. 2).

DISCUSSION AND CONCLUSIONS

Salmonellosis is a bacterial disease of animals and humans caused by individual salmonella serovars. One of the ways how to positively affect the clinical course of salmonellosis or initiate its prevention is the supplementation of rations with probiotics. Their positive effects include the capability of affecting the morphological development of the intestinal epithelium (2) which is very important for the good development of a protective mucosal barrier against this bacterium (3).

The results of the present study indicate that supplementation of the probiotic bacterium *E. faecium* EF 55 induced a beneficial growth of the duodenal and jejunal villi compared to control animals; thus increasing their resorptive surface and increased proliferative activity of cells in the jejunum. These results point to the positive influence of the probiotic strain (EF) on morphometric and regenerative properties of the small intestinal mucosa. The intestinal mucosa treated this way appears to reduce the negative influence of *Salmonella Enterica* PT4.

REFERENCES

1. FAO/WHO, 2002: *Guidelines for the Evaluation of Probiotic in Food.* Joint FAO/WHO Working Group report on drafting guidelines for the evaluation of probiotics in food. London, Ontario, Canada, April 30 and May 1, 2002, 1–11.

2. Herich, R., Kokinčáková, T., Lauková, A., Levkutová, M., 2010: Effect of preventive application of *Enterococcus faecium* EF55 on intestinal mucosa during salmonellosis in chickens, *Czech J. Anim. Sci.,* 55, 42–47.

3. Herich, R., Levkut, M., 2002: Lactic acid bacteria, probiotics and immune system, Review Article. *Vet. Med. Czech,* 47, 169–180.

4. Paunescu, T., Mittal, S., Protic, M., Oryhon, J., Korolec, V., Joachimiak, A., Woloschak, G. E., 2001: Proliferating cell nuclear antigen (PCNA): ringmaster of the genome. *Int. J. Radiat. Biol,* 77, 1007–1021.

5. Uni, Z., Platin, R., Sklan, D., 1998: Cell proliferation in the chicken intestinal epithelium occurs both in the crypt and along the villus. *J. Comp. Physiol.,* 168, 241–247.

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MONITORING AND PRACTICAL PROTECTION OF BATS OVERWINTERING IN PREFAB HOUSES IN KOŠICE

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ABSTRACT

This study describes the practical experience with protection and resolving of problems arising from the wintering of bats in prefabricated apartment houses in Košice territory in the period of 2009 and 2010. During this period we captured and successfully released into the environment 79 bats of three insectivorous bat species.

Key words: Košice; monitoring; prefab houses; protection; wintering bats

INTRODUCTION

It has been proven that 28 species of insectivorous bats of two families, *Rhinolophidae* and *Vespertilionidae,* occur in the Slovak Republic territory (2). The individual bat species show considerable ecological differences manifested in their requirements on hiding places, way of hunting for food, type of favourite food, etc. The species most frequently populating prefabricated apartment houses in Slovakia include: common pipistrelle (*Pipistrellus pipistrellus*), serotine (*Eptesicus serotinus*), noctule (*Nyctalus noctula*) (4) and parti-coloured bat (*Vespertilio murinus*) (5). The common pipistrelles and noctules, in particular, form frequently large, overwintering colonies in human dwellings. Currently, there is a lack of data on the number of bats taking up occupancy in prefab houses in Slovakia.

The aim of this study was to obtain information on the population of bats overwintering in prefabricated apartment houses in Košice. More detailed information about species composition of bat populations and the gender structure of the bat species overwintering in urban ecosystems such as Košice is needed for future epizootiological-ecological studies of the selected species of insectivorous bats.

MATERIAL AND METHODS

Bats were captured directly in the interior of prefabricated buildings and measures were taken to prevent their re-entry into the building interior. The health status of captured bats was evaluated and the healthy bats were released in the Košice area or in Jasovská cave. Noctules and common pipistrelles were marked with chiropterological rings to obtain information on their subsequent migrations and hiding place ecology. If necessary, the bats were fed mealworms, subjected to rehydration therapy and prepared for artificial overwintering in a refrigerator.

RESULTS

During the hibernation period we captured, in prefab houses in different parts of Košice, 79 bats of three species: common pipistrelle (*P. pipistrellus*), noctule (*N. noctula*) and parti-coloured bat (*V. murinus*) (Table 1).

Table 1. Species proportion and gender structure of captured bats

Species	♂	♀	Total	% of the total number of bats
P. pipistrellus	16	28	48	60,7
N. noctula	25	5	30	38,0
V. murinus	1	-	1	1,3
∑			79	100

♂ – males; ♀ – females

On the 17th of January, 2010, we captured a ringed common pipistrelle female on Pražská street in Košice. This bat carried a ring marked SLOVAKIA M0539, which had been affixed on 26th August, 2009, after capturing a number of bats of the same species on the premises of the Eastern Slovakia Oncological Institute in Košice. The ringed bat was released in Moldava above Bodva. The bat migrated in the north – east direction for 23 kilometres before we captured it again in Košice after 144 days.

DISCUSSION

According to the available literature the three species of bats found in our study are the most common species populating prefab houses in Slovakia (5, 4, 1). They enter the buildings through: various ventilation openings in the roof of apartment houses; unsealed holes for antennae cables; elevator shafts; untightnesses in older windows; and cracks between individual panels. Openings in the outer shell of buildings are most advantageously sealed with polyurethane foam and air-shafts should be covered with small wire mesh netting. Any dehydrated noctules found in the buildings were successfully treated by administering Ringer's solution at a dose of 0.5 ml four times daily in the dorsal region according to the recommendations of Jahelková *et al.* (3).

The information obtained by the capture of the ringed common pipistrelle female bat indicates that bats of this species use human dwellings as alternative hiding places during their migration after the end of the reproductive season and also in the period of hibernation. They also appear to be strongly bonded to the location where they spent the summer or where they were born.

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REFERENCES

1. Ceľuch, M., Kaňuch, P., 2002: Bat overwintering in prefabricated apartment blocks at housing development Sekčov in Prešov (In Slovak). *Vespertilio,* 6, 44.

2. Ceľuch, M., Ševčík, M., 2006: How many bats really live in our country? Contemplating the new findings (In Slovak). *Vespertilio,* 9–10, 239.

3. Jahelková, H., Hájková, P., Bláhová, A., 2009: *Manual on Care of Found, Injured and Handicapped Bats* (In Czech). ZO ČSOP 11/30, Nyctalus, Prague, 65 pp.

4. Lehotská, B., Lehotský, R., 2000: Experience with protection of winter colony of noctule (*Nyctalus noctula*) in a prefab house at housing development Dlhé Diely in Bratislava (In Slovak). *Vespertilio,* 4, 105–110.

5. Matis, Š., Dittel, L., 1997: Autumn observation of Vespertilio murinus in some Slovak towns (In Slovakia). *Vespertilio,* 2, 141–142.

*

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EVALUATION OF BEHAVIORAL AND HORMONAL INDICATORS OF WELFARE IN WORKING GERMAN SHEPHERD DOGS

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ABSTRACT

The aim of this study was to validate in practice the use of non-invasive methods which allows one to minimise deviations that might result from invasive sampling. The determination of salivary cortisol by ELISA is advantageous with regard to: a simple collection of samples; stability of the procedure; and relatively ease of analysis. Our study showed that due to high variability, high levels of cortisol cannot be considered an unambiguous indicator of stress, but must be compared with additional parameters to supplement it and increase its validity. A suitable additional parameter is, for example, the behaviour of animals, as behaviour responses frequently correlate with the physiological responses to stress.

Key words: behaviour; salivary cortisol; stress; welfare; working dog

INTRODUCTION

Working dogs must frequently perform under pressure in very stressful environments. Increased physical activity and more frequent exposure to various stressors may have undesirable influence on the physical and psychical health of these animals. This may affect their performance and ability to collaborate reliably in the assigned service work.

Cortisol is considered one of the principal indicators of the stress response in the majority of mammals including dogs (3). It is produced by the adrenal cortex. Up to 95 % of the total cortisol is bound to large proteins (transcortin, albumin) and circulated in the blood. Only small portions of the free cortisol is biologically active. Owing to its low molecular weight and lipophilic nature, free cortisol enters into cells by passive diffusion which enables it to be measured in fractions of body fluids (sweat, tears, saliva, and urine). Cortisol participates in catabolic and anabolic mechanisms in the body and has an immunosuppressive effect. The level of salivary cortisol correlates with its concentration in blood (7). The cortisol half time is 70 to 110 min (4). The basal level of salivary cortisol in dogs is 3–6 nmol.l^{-1} (2).

The aim of this study was to validate in practice the non-invasive way of the collection of samples for determination of cortisol and confirm the necessity of parallel assessments of physiological and behavioural parameters in the evaluation of dog welfare.

MATERIAL AND METHODS

Tests were carried out on 24 dogs (13 females and 11 males) mostly pedigree German Shepherds who were from 0.4 to 8.6 years old. Two were German Shepherd mongrels. All dogs were vaccinated and clinically healthy. The tested dogs were either in training or had already been used as working dogs (watchdogs, police, and sport). Twenty one were housed in pens and 3 in an apartment. Saliva samples were collected between September 2009 and February 2010. All of the dog's owners were instructed how to collect and store the saliva samples. Samples were taken with previously unused sterile swabs. The dogs were not allowed to eat before sampling and their mouth cavity had to be free of mechanical soiling or injury. Samples were taken in six predetermined situations (1 – when entering the pen, 2 – before training, 3 – during training, 4 – after training, 5 – when taken for a walk, not on a leash and no given commands, 6 – when meeting another dog) and were stored at –18 °C.

Salivary cortisol was determined by the commercial human set DIAMETRA Cortisol Saliva DKO 020, using the method of the competitive quantification of antigens. All owners were asked to characterise their dogs in the respective sampling situations and provide their general characteristics, focusing on their behavioural manifestations.

RESULTS AND DISCUSSION

The levels of salivary cortisol were affected by the situation at the time of sampling. The highest levels were associated with situation 4 and the lowest with situation 6. Inappropriately long, physically demanding, unprofessional training under unsuitable conditions can stress the dog and induce health and behavioural changes. The salivary cortisol levels were independence of gender, similar to the findings of other authors (3). Dogs younger than 1 year exhibited high cortisol levels in situations 3 and 4. This may be due to the fact that these young dogs: only started with the training; may have not adapted to it completely; their temperament had not yet formed; and they may have been disturbed by the presence of other dogs at the training site.

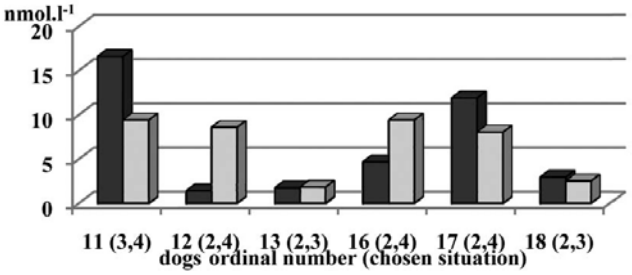

Fig. 1. Mean levels of salivary cortisol in dogs 11, 12, 13, 16, 17 and 18

Salivary cortisol levels are higher in puppies than in adult dogs (1). Dogs older than 5 years showed higher cortisol levels in situation 2. They perhaps know they are going to be trained and their response is reasonable. The way of use of the dogs affected their salivary cortisol. Increased cortisol in police dogs during and after training indicates that their training is very demanding (Table 1). The time of sampling had no influence on salivary cortisol levels. In dogs, cortisol is released in pulses and the normal circadian character of its release has not been confirmed nor disproved (5).

Fig. 1 shows considerable variations in salivary cortisol. The increase in dogs 11 and 17 was ascribed to positive stimuli: meeting other dogs and playing, and in dog 17 pleasure from training even without a reward. On the contrary, cortisol levels in dogs 12 and 16 were affected by manifestations of aggression to other dogs and in dog 16 by fear during training, i.e. negative stimulus. The changes in physiology may result from both pleasant and negative experiences. Low levels of salivary cortisol in dogs 13 and 18 occurred after long-term exposure to chronic stress (situations 2 and 3). They showed frank behavioural disorders (e.g., self-destruction, destroying the pen, and scattering of faeces). Rising cortisol levels may indicate chronic stress although its physiological levels do not confirm its absence (6). Levels of the salivary cortisol in dogs included in our study were lower than those found in some other similar studies (1, 2). Lower levels of salivary cortisol in dogs were reported also by Haubenhofer (3).

Saliva is a suitable diagnostic material for determination of the stress hormone cortisol. In comparison with invasive blood sampling, the non-invasive technique is more advantageous as it is not associated with the extensive excitation of animals during sampling leading to spurious results. However, validity of results can be ensured only by the combination of several supplementary methods so that the respective individual can be evaluated from different points of view.

Table 1. The influence of gender, age and use of dogs on salivary cortisol level (nmol.l^{-1})

Sampling situation	Gender			Age in years				Use of dogs		
	♂ + ♀	♂	♀	up to 1	1–3	3–5	above 5	police	watchdog	sport
1	3.177	2.194	3.458	3.160	1.794	3.266	3.560	2.484	4.186	2.861
2	3.361	3.718	3.189	3.997	2.727	0.966	6.203	2.525	4.953	0.607
3	3.602	1.825	5.887	9.218	2.492	2.174	3.588	7.291	3.292	1.868
4	6.797	7.576	6.421	9.039	6.788	5.713	2.884	9.384	6.091	7.820
5	2.724	2.415	2.901	1.711	2.622	3.901	3.878	2.726	3.535	1.708
6	2.481	1.711	2.577	2.346	1.904	2.208	3.238	3.243	3.054	1.670

REFERENCES

1. Beerda, B., Schilder, M. B., Van Hoff, J. A., De Vries, H. W., Mol, J. A., 1997: Behavioral, saliva cortisol and heart rate responses to different types of stimuli in dogs. *Applied Animal Behaviour Science,* 58, 365–381.

2. Beerda, B., Schilder, M. B., Van Hoff, J. A., De Vries, H. W., Mol, J. A., 2000: Behavioural and hormonal indicators of enduring environmental stress in dogs. *Animal Welfare,* 9, 49–62.

3. Haubenhofer, D. K., Kirchengast, S., 2007: Dog handlers' and dogs' emotional and cortisol secretion response associated with AAT sessions. *Society and Animals.* 15, 127–150.

4. Kirschbaum, C., 1991: *Cortisolmessungen im Speichel: eine Methode der Biologischen Psychologie.* Bern, Hans Huber, 107 pp.

5. Kobelt, A. J., Hemsworth, P. H., Barnett, J. L., Butler, K. L., 2003: Sources of sampling variation in saliva cortisol in dogs. *Res. Vet. Sci..* 75. 157–161.

6. Moberg, G P., 2000: *The Biology of Animal Stress. Basic Principles and Implications for Animal Welfare.* CAB International. 377 p.

7. Vincent, I. C., Michell, A. R., 1992: Comparison of cortisol concentrations in saliva and plasma of dogs. *Res. Vet. Sci.,* 53, 342–345.

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