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Toxoplasma gondii in protected wildlife in the Tatra National Park (TANAP), Slovakia

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Abstract

Toxoplasma gondii is an obligatory intracellular protozoan parasite that infects a broad spectrum of warm-blooded vertebrate species. As a part of the food chain, farm animals play a significant role in transmission of *T. gondii* to humans, while rats and mice serve as a main source of infection for free-living animals. The spread of toxoplasmosis in the human population is due to the interchange of the domestic and sylvatic cycles. During 2009–2011, a survey on toxoplasmosis distribution was conducted in wildlife of the Tatra National Park (TANAP) in Slovakia. A total of 60 animals were examined. The presence of *T. gondii* was detected by means of molecular methods based on TGR1E gene analyses. The highest prevalence was recorded in birds (40.0%), followed by carnivores (30.8%) and rodents (18.2%). RFLP analyses of SAG2 locus confirmed in birds the genotype II and III, belonging to the avirulent strain; rodents exclusively had genotype I, characterised as a virulent strain, and in carnivores all three genotypes were detected. These results present the first survey on the parasite's occurrence in several species of free-living animals in the TANAP area. An epidemiological study confirmed the prevalence of 30.0%, implicitly referring to the level of environmental contamination with *T. gondii* oocysts.

Key words

Toxoplasma gondii, free-living animals, TANAP, DNA, genotype

INTRODUCTION

Toxoplasmosis is one of the most widespread parasitic infections, transmissible from animals to humans, with the highest incidence in humans [1]. The etiological agent *Toxoplasma gondii* is a protozoan parasite infecting most domestic and free-living animals [2]. Cosmopolitan distribution of the parasite is not limited by either geographic nor climatic conditions. The only definitive hosts in which the sexual phase of the parasite life cycle is completed are the members of family *Felidae*, most frequently domestic cats (*Felis catus*). All warm-blooded animals (birds and mammals), including humans, can theoretically serve as intermediate hosts. *T. gondi* infection can be transmitted to humans from domestic and farm animals or from contaminated food and the environment.

The infection in healthy individuals often takes an asymptomatic course, or is accompanied by non-specific clinical signs. The parasite affects several organs, primarily the lungs, central nervous system and eyes. Neurological symptoms include disturbances in nerve function, abnormal movements, seizures, depression, and partial or total loss of vision. The respiratory effects include shortness of breath, increased respiratory rate, cough and fever. Other signs involve jaundice, hepatomegaly, ascites, muscle pain, walking difficulties, loss of appetite and weight, and excessive fatigue [3]. Latent toxoplasmosis is often seen in cats, rabbits, sheep and goats, and rarely in horses and cattle.

Several authors ascertained distinct genetic differences among *T. gondii* isolates from different geographic areas of the world [4]. Isolates are characterised as three genotypes: I, II, and III. From a total of 252 investigated sera of chronically-infected pregnant women, [5] the samples from Europe and South America exhibited a homogenous pattern of genotype II, whereas isolates from South America they belonged to genotypes I and III. Distribution of genotypes also differs in various European countries. Several studies indicate that in livestock genotype II predominates [6, 7, 8], while in Portugal all three genotypes were detected, with types II and III considered to be dominant [9, 10].

In Slovakia, there is no official monitoring programme for the diagnosis of toxoplasmosis in livestock or free-living animals. *T. gondii* surveys are conducted solely to assess an upcoming epidemiological situation or at the request of farmers [11].

The data on *T. gondii* distribution in free-living animals in Slovakia are very sporadic and in some species are not known at all. Thus, the aim of the presented study was to determine the prevalence and distribution of *T. gondii* in the favourite tourist destination of the TANAP, and to characterise the obtained *T. gondii* genotypes using nested PCR and RFLP methods of *SAG2* locus, coding parasite surface antigen.

MATERIALS AND METHOD

Samples from 6 bird species, 5 rodent species and 10 species of carnivores (Tab. 1) were obtained from the TANAP territory. Muscle samples were collected from the carcasses at the Research Station and Museum of the TANAP and stored at –20 °C until further use.

T. gondii isolation from the tissues was performed according to Jauregui et al. [12]. Briefly, muscle tissue (20–50 g) was homogenised in saline solution (0.14 M NaCl)

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Table 1. Occurrence of *Toxoplasma gondii* in free living animals from the TANAP (Tatra National Park, Slovakia)

CLASS Order	Species	No. of examined animals	PCR positive samples	Genotype		
				I	II	III
AVES						
Strigiformes	Barn owl (*Tyto alba*)	1	1		1	
Falconiformes	Golden eagle (*Aquila chrysaetos*)	1	0			
	Northern goshawk (*Accipiter gentilis*)	2	1		1	
	Common buzzard (*Buteo buteo*)	1	0			
Passeriformes	Eurasian jay (*Garrulus glandarius*)	2	1			1
	Common magpie (*Pica pica*)	3	1			1
Total	6	10	4	0	2	2
MAMMALIA						
Rodentia	Eurasian red squirrel (*Sciurus vulgaris*)	6	1	1		
	Muskrat (*Ondatra zhibethicus*)	1	0			
	Mound building mouse (*Mus spicilegus*)	2	0			
	Bank vole (*Myodes glareolus*)	1	1	1		
	Brown rat (*Ratus norvegicus*)	1	0			
Total	5	11	2	2	0	0
Carnivora	Racoon dog (*Nyctereutes procyonoides*)	1	0			
	European marten (*Martes martes*)	5	2		1	1
	Beech marten (*Martes foina*)	7	4		2	2
	Eurasian lynx (*Lynx lynx*)	3	1	1		
	European badger (*Meles meles*)	7	1		1	
	European polecat (*Mustela putorius*)	4	1			1
	European otter (*Lutra lutra*)	6	2		1	1
	Wildcat (*Felis silvestris*)	2	1			1
	Gray wolf (*Canis lupus*)	1	0			
	Brown bear (*Ursus arctos*)	3	0			
Total	10	39	12	1	5	6

and digested with the same volume of pepsin-HCl (1.4 mg pepsin and 10 mg of NaCl per ml 0.1 N HCl) for 1 hour. The samples were incubated in a thermostat at 37 °C for 2 hours, stirred continuously, then centrifuged at 1,180 × g for 10 min. Sediment was neutralised using 0.1 M TRIS–HCl (hydroxymethylaminomethane–HCl, pH 8.0) and centrifuged repeatedly. Sediment containing isolated *T. gondii* was digested in 1 ml of digestion solution with 0.1 mg of proteinase K and incubated in a water bath at 55 °C overnight.

T. gondii DNA was isolated using phenol-chloroform-isoamyl alcohol (25:24:1) [13] and dissolved in re-distilled water at laboratory temperature. Isolated DNA was stored until further use at –20 °C.

Isolated DNA was analysed by PCR methods using TGR1E gene and 2 primers: TGR1E-1 5′-ATGGTCCGGCCGGTGTATGATATGCGAT-3′ and TGR1E-2 5′-TCCCTACGTGGTGCCGCATTGCCT-3′[14, 15]. For amplification, 5 µl of the isolated DNA template were used to obtain a fragment of 191 bp, visualised under the UV lamp at 254 nm on 3% agarose gel stained with GoldView™.

Genotyping of *T. gondii* was performed using nested PCR of the SAG2 locus [16], located on chromosome VIII and coding the P22 surface antigen. Amplification was accomplished separately for the 5 and 3 ends of the locus, and the PCR included 2 amplification reactions with 2 primer pairs.

The amplified fragments of the second reaction were used in the RFLP analysis. The 5-end was digested by restriction endonuclease *Sau*3A I (Promega, USA) and 3-end by restriction endonuclease *Hha*I (Promega, USA)[17]. *T. gondii* genotype was determined based on the location of restriction fragments of the 3- and 5-ends, visualised by electrophoresis in 1.2% agarose gel.

Ethics. All tissue samples used in the study were obtained from animals which had died in preserved area of the Tatra National Park, with the agreement of the Ministry of Agriculture of the Republic of Slovakia [11].

RESULTS

The study was conducted during 2009–2011 in protected areas of the TANAP in the High Tatra Mountains, in free-living animals that may have come into contact with humans and become a source of infection.

In total, 10 bird specimens belonging to 3 orders were assayed. Four were *Toxolasma*-positive, representing a prevalence of 40.0%. Genotype II was based on the presence of the SAG2 locus genetically characterised in 2 isolates (Barn owl – *Tyto alba*, Northern goshawk – *Accipiter gentilis*), and 2 isolates were characterised as genotype III (Eurasian jay – *Garrulus glandarius*, Eurasian magpie – *Pica pica*). Both genotypes belong to avirulent strains (Tab. 1).

Molecular analyses were performed on the DNA from 11 rodent samples from the TANAP, establishing 2 *Toxoplasma*-positive isolates (18.2%) from red squirrel (*Sciurus vulgaris*) and bank vole (*Myodes glareolus*). In both, genotype I was confirmed (Tab. 1, 2).

Table 2. Prevalence and genotypes of *Toxoplasma gondii* in free living animals from TANAP detected at *SAG2 locus*

Animals	PCR			Genotypes					
				I		II		III	
	No. of examined animals	No. of positive samples	Positivity (%)	No of positive samples	(%)	No of positive samples	(%)	No of positive samples	(%)
Aves	10	4	40.0	0	–	2	50.0	2	50.0
Rodentia	11	2	18.2	2	100.0	0	–	0	–
Carnivora	39	12	30.8	1	8.3	5	41.7	6	50.0
Total	60	18	30.0	3	16.7	7	38.9	8	44.4

A total of 39 carnivores from the TANAP area, classified into 10 species, were examined for *T. gondii*. 12 individuals were positive (30.8%) (Tab. 2). *T. gondii* was not detected in tissues of the racoon dog (*Nyctereutes procyonoides*), the grey wolf (*Canis lupus*) and brown bear (*Ursus arctos*). Genotype I, regarded as the virulent strain, was diagnosed in 1 isolate from lynx (*Lynx lynx*). Genotypization revealed the presence of genotypes II and the most frequent genotype III in the majority of carnivore samples (Tab. 1).

A total of 60 individuals were examined from the TANAP area. Overall prevalence of toxoplasmosis represents 30.0%.

Genetically, 44% of *T. gondii* isolates were characterised as genotype III, 38.9% were represented by genotype II and 16.7% isolates belonged to genotype I (Tab. 2).

Due to the small number of examined samples belonging to individual species, because in protected areas the hunting of animals is strictly limited, statistical analyses was not carried out.

DISCUSSION

Toxoplasmosis represents a serious public health problem as infected meat and/or meat products from farm and free-living animals can serve as a potential source of infection. The presented study conducted during 2009–2011 focused on an epidemiological survey of *T. gondii* prevalence in protected areas of the TANAP, in free-living animals that may come into contact with humans and become a source of infection.

Many bird species are ground feeders and therefore suitable bio-indicators of environmental contamination with *T. gondii* oocysts [17]. Out of 10 examined specimens from the TANAP, 4 were *Toxoplasma*-positive, representing a prevalence of 40.0%. Genotype II was detected in 2 isolates (Strigiformes and Falconiformes), and 2 isolates were characterised as genotype III (Passeriformes). Both isolates belong to avirulent strains.

The occurrence of the parasite in Slovakia has been confirmed for the first time in 13 bird species [18]. According to Dubey [19], who surveyed its prevalence in free-living birds, the infection is asymptomatic. It was suggested that seropositivity in birds of prey was due to feeding on infected prey [20]. Barn owls (*Tito alba*) usually feed on sparrows and voles, while other members of Strigiformes feed on small mammals. Differences in seroprevalence rates in birds of prey refer to various levels of infection in prey, in particular small mammals. The authors performed serological examination of bird samples from 2 rescue and rehabilitation facilities in France. Despite not detecting any antibodies against *T. gondii* in the Common Kestrel (*Falco tinnunculus*) and Eurasian Sparrowhawk (*Accipiter nisus*), the current analysis using TGR1E gene confirmed the presence of *T. gondii* in Goshawk (*Accipiter gentilis*). In contrast to a high prevalence (79%) detected in the Common Buzzard (*Buteo buteo*), the presented research does not confirm toxoplasmosis in this species. The above-mentioned authors detected (*Tyto alba*) only 11% prevalence in the Barn Owl. The presented analyses revealed the presence of genotype II in 1 individual of this species. This is in compliance with results of Aubert et al. [20] who in birds from the order Strigiformes confirmed the presence of *T. gondii*, characterised as genotyp II.

Small mammals are a significant part the diet of foxes, raptorial birds and many other animal species. They are considered an important reservoir of *T. gondii* for predators. Their role has also been confirmed in the transmission of the parasite to pigs [21]. Using PCR analysis of the B1 gene, vertical transmission of *T. gondii* were confirmed in the House Mouse (*Mus musculus*) and Wood Mouse (*Apodemus sylvaticus)* with experimentally infected oocysts [22].

Molecular analyses were performed on the DNA from 11 rodent samples from the TANAP, establishing 2 *Toxoplasma*-positive isolates (18.2%). The prevalence of 1.0% was detected in free-living rodents from the Czech Republic [23]. The findings of 59% *T. gondii* infected House Mice (*Mus domesticus)* captured in the vicinity of human settlements in the UK is alarming [24]. Data on the genetic structure of *T. gondii* in rodents are rather scarce. *T. gondii* isolates was genetically characterised from 2 free-living rodents captured on an English farm, based on the*SAG2* locus as the genotype II[22]. The presented results from 2 isolates from the Eurasian Red Squirrel (*Sciurus vulgaris*) and Bank Vole (*Myodes glareolus*) were identified as genotype I.

The presence of *T. gondii* in free-living carnivores implicitly refers to environmental contamination with the parasite [25]. The most common source of *Toxoplasma* infection by oocyst infected insects, were domestics cats, rodents, artiodactyles, and subsequently, carnivores [23]. The role of sylvatic carnivores in the natural cycle of *T.gondii* transmission has been generally accepted [26, 27]. Throughout 1995–1997, 865 members of neotropic felines were serologically examined [28], determining the prevalence of 54.6%.

A total of 39 carnivores from the TANAP area, classified into 10 species, were examined for *T. gondii*. 12 individuals were positive (30.8%). *T. gondii* was not detected in Racoon Dog, Grey Wolf and Brown Bear. The occurrence of toxoplasmosis in those species is also rarely mentioned in the literature.

The Lynx (*Lynx lynx*) is considered both the intermediate and definitive host for *T. gondii* [29]. Out of 3 Lynx samples from the TANAP, 1 positive isolate was diagnosed, characterised as genotype I, regarded as the virulent strain. Unlike the presented data, Lynx isolates obtained in California, USA, were typed as genotypes II and III [30]. *T. gondii* isolates from various carnivorous species in the USA [31], confirming the presence of all 3 genotypes. The Canada Lynx (*Lynx canadensis*), the only free-living feline in Alaska, may replace the domestic cat in *T. gondii* transmission in the area [32]. *T. gondii* infection was serologically confirmed in the Eurasian lynx in the Czech and Slovak Republics [33].

Out of 12 samples from Martens, 6 samples were *T. gondii*-positive for genotypes II and III. The presence of *T. gondii* was determined in Martens (*Martes* spp.) using molecular methods – 4.92% prevalence of the parasite [34]. Anti-*Toxoplasm*a antibodies in Martens were detected in zoos, both in the Czech and Slovak Republics [33].

Only 1 of 7 samples from badgers was positive. The DNA was typed as genotype II. *T. gondii* was not detected in badgers in the Czech Republic [23]; however, in the UK [1] and in Spain [35], a seroprevalence of over 70% has been reported in the Eurasian Badger.

There are no available data on *T. gondii* prevalence in the European Polecat and European Otter, whereas a total of 10 samples were obtained from the TANAP in the presented study. Isolates from the Polecat were typed as genotype III, and isolates from the Otter typed as both genotypes II and III.

T. gondii was detected only in 1 sample from the Wild Cat (*Felis silvestris*) and typed as genotype III. A high seroprevalence of toxoplasmosis in Wild Cats was confirmed in the UK [37], Spain [35] and the Czech Republic [36].

Monitoring of toxoplasmosis in free-living animals from the TANAP area revealed a 30.0% prevalence of the parasite, with a predominance of genotype III. A high positivity (16.7%) of the genotype I, belonging to virulent strain, highlights the need for safe practices while handling potentially infected animals and animal products.

CONCLUSION

Toxoplasmosis is a serious zoonotic disease the agent of which permanently circulates in the environment. The presented study shows the results of the first survey on the parasite's occurrence in several species of free-living animals in the TANAP area of the Slovak Republic. An epidemiological study confirmed the prevalence of 30.0%, implicitly referring to the level of environmental contamination with oocysts and high incidence of the parasite in the intermediate and definitive hosts populations. Field workers and hunters should therefore have a good knowledge about basic sanitation and hygiene principles while handling and processing raw meat. Animal scraps and offal should be disposed of properly to eliminate the potential spread of *T. gondii* to birds, rodents, and other free-living animals, and should not be fed to hunting dogs.

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REFERENCES

1. The EFSA Journal. 2007; 583:1–64.
2. Tomavo S. The differential expression of multiple isoenzyme forms during stage conversion of *Toxoplasma gondii*: An adaptive developmental strategy. Int J Parasitol. 2008; 31: 1023–1031.
3. Luptáková L, Valenčáková A, Bálent P. Toxoplasmosis – cover Protozoan infection. Infovet. 2007; 14: 112–117 (in Slovak).
4. DaSilva AV, Mendonca ADO, Pezerico SB, Domingues PF, Langoni H. Genotyping of *Toxoplasma gondii* strains detected in pork sausage. Parasitol Latinoam. 2005; 60: 65–68.
5. Peyron F, Lobry JR, Musset K, Ferrandiz J, Gomez-Marin JE, Petersen E, Meroni V, Rausher B, Mercier C, Picot S, Cesbron-Delauw MF. Serotyping of *Toxoplasma gondii* in chronically infected pregnant women: predominance of type II in Europe and types I and III in Colombia (South America). Microbes Infect. 2006; 8: 9–10.
6. Owen MR, Trees AJ. Genotyping of *Toxoplasma gondii* associated with abortion in sheep. J Parasitol. 1999; 85: 382–384.
7. Dubey JP, Edelhofer R, Marcet P, Vianna MCB, Kwok OCH, Lehmann T. Genetic and biologic characteristics of *Toxoplasma gondii* infections in free-range chickens from Austria. Vet Parasitol. 2005; 133: 299–306.
8. Duométre A, Ajzenberg D, Rozette L, Mercier A, Dardeé ML. *Toxoplasma gondii* infection in sheep from Haute-Vienne, France: seroprevalence and isolate genotyping by microsatellite analysis. Vet Parasitol. 2006; 142: 376–379.
9. Dubey JP, Vianna MCB, Sousa S, Canada N, Meireles S, Correia Da Costa JM, Marcet PL, Lehmann T, Dardé ML, Thulliez P. Characterization of *Toxoplasma gondii* isolates in free-range chickens from Portugal. J Parasitol. 2006; 92: 184–186.
10. DeSousa S, Ajzenberg D, Canada N, Freire L, Da Costa JMC, Dardé ML, Thulliez P, Dubey JP. Biologic and molecular characterization of *Toxoplasma gondii* isolates from pigs from Portugal. Vet Parasitol. 2006; 135: 133–136.
11. Ministry of Agriculture of the Slovak Republic. The report on zoonoses and zoonotic agents in the Slovak Republic. 2009; NLC, Zvolen: 90 (in Slovak).
12. Jauregui LH, Higgins J, Zarlenga D, DubeyJP, Lunney JK. Development of a real-time PCR assay for detection of *Toxoplasma gondii* in pig and mouse tissues. J Clin Microbiol. 2001; 39: 2065–2071.
13. ManiatisT, Fritsch EF, Sambrook J. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory. Cold Spring Harbor Springer, Laboratory Manual. New York, 1982.
14. Lamoril J, Molina JM, Gouvello AD, Garin YJ, Deybach JC, Modai J, Derouin F. Detection by PCR of *Toxoplasma gondii* in blood in the diagnosis of cerebral toxoplasmosis in patients with AIDS. J Clin Pathol. 1996;49**:** 89–92.
15. Čermáková Z, Plíšková L, Prášil P, Ryšková O. Method of polymerase chain reaction in toxoplasmosis diagnosis. Acta Medica (Hradec Kralove) Suppl. 2004; 47(2):71–73 (in Czech).
16. Howe DK, Honoré S, Derouin F, Sibley LD. Determination of genotypes of *Toxoplasma gondii* strains isolated from patients with toxoplasmosis. J Clin Microbiol. 1997; 35: 1411–1414.
17. Waap H, Vilares A, Rebelo E, Gomes S, Ângelo H. Epidemiological and genetic characterization of *Toxoplasma gondii* in urban pigeons from the area of Lisbon (Portugal).Vet Parasitol. 2008; 157: 306–309.
18. Čatár G. Toxoplasmosis in ecological conditions in Slovakia. Biological Works (Bratislava). 1974; 20: 1–138 (in Slovak).
19. Dubey JPA. Review of Toxoplasmosis in wild birds. VetParasitol. 2002; 106: 121–153.
20. Aubert D, Terrier ME, Dumétre A, Barrat J, Villena I. Prevalence of *Toxoplasma gondii* in raptors from France. J Wildl Dis. 2008;44: 172–173.
21. Dubey JP, Weigel RM, Siegel AM, Thulliez P, Kitron UD, Mitchell MA, Mannelli A, Mateus-Pinilla NE, Shen SK, Kwok OCH. Todd KS. Sources and reservoirs of *Toxoplasma gondii* infection on 47 swine farms in Illinois. J Parasitol. 1995; 81: 723–729.
22. Owen MR, Trees AJ. Vertical transmission of *Toxoplasma gondii* from chronically infected house (*Mus musculus*) and field (*Apodemus sylvaticus*) mice determined by polymerase chain reaction. Parasitology 1998; 116: 299–304.
23. Hejlíček K, Literák I, Nezval J. Toxoplasmosis in wild mammals from the Czech Republic. J Wildelife Dis. 1997; 33: 480–485.
24. Marshall PA, Hughes JM, Williams RH, Smith JE, Murphy RG, Hide G. Detection of high levels of congenital transmission of *Toxoplasma gondii* in natural urban populations of *Mus domesticus*. Parasitology 2004; 128: 39–42.
25. Hamilton CM, Gray R, Wright SE, Gangadharan B, Laurenson K, Innes EA. Prevalence of antibodies to *Toxoplasma gondii* and *Neospora caninum* in red foxes (*Vulpes vulpes*) from around the UK. Vet Parasitol. 2005; 130: 169–173.
26. Wolfe A, Hogan S, Maguire D, Fitzpatrick C,Vaughan L, Wall D, Hayden TJ, Mulcahy G. Red foxes (*Vulpes vulpes*) in Ireland as hosts for parasites of potential zoonotic and veterinary significance. Vet Rec.2001; 149: 759–763.
27. Hill D, Dubey JP. *Toxoplasma gondii*: transmission, diagnosis, and prevention. Clin Microbiol Infect. 2002; 8: 634–640.
28. Silva JCR, Ogassawara S, Marvulo MFV, Ferreira-Neto JS, Dubey JP. *Toxoplasma gondii* antibodies in exotic wild felids from Brazilian zoos. J Zoo Wildelife Med. 2011;32: 349–351.
29. Jones JL, Dubey JP. Waterborne toxoplasmosis – Recent developments. Exp Parasitol. 2010; 124: 10–25.
30. Miller MA, Miller WA, Conrad PA, James ER, Melli AC, Leutenegger CM, Dabritz HA, Packham AE, Paradies D, Harris M, Ames J, Jessup DA, Worcester K, Grigg M. Type X *Toxoplasma gondii* in a wild mussel and terrestrial carnivores from coastal California: New linkages between terrestrial mammals, run off and toxoplasmosis of sea otters. Int J Parasitol. 2008; 38: 1319–1328.
31. Dubey JP, Velmurugan GV, Ulrich V, Gill J, Carstensen M, Sundar N, Kwok OCH, Thulliez P, Majumdar D, Su C. Transplacental toxoplasmosis in naturally-infected white-tailed deer: isolation and genetic characterisation of *Toxoplasma gondii* from foetuses of different gestational ages. Int J Parasitol. 2008; 38: 1057–1063.
32. Zarnke RL, Dubey JP, Kwok OCH, Ver Hoef JM. Serologic survey for *Toxoplasma gondii* in selected wildlife speciesf rom Alaska. J Wildlife Dis. 2000; 36: 219–224.
33. Sedlák K, Bártová E. Seroprevalences of antibodies to *Neospora caninum* and *Toxoplasma gondii*i n zoo animals. Vet Parasitol. 2006;136: 223–231.
34. Hůrková L, Modrý D. PCR detection of *Neospora caninum*, *Toxoplasma gondii* and *Encephalitozoon cuniculi* in brains of wildcarnivores. Vet Parasitol. 2006; 137: 150–154.
35. Sobrino R,Cabezón O,Millán J, Pabón M, Arnal MC, Luco DF, Zar C, Dubey JP, Almeria S. Seroprevalence of *Toxoplasma gondii* antibodies in wild carnivores from Spain. Vet Parasitol. 2007; 148: 187–192.
36. Lukešová D, Literák I. Shedding of *Toxoplasma gondii* oocysts by Felidae in zoos in the Czech Republic. Vet Parasitol. 1998; 74: 1–7.
37. McOrist S. Diseases of the European wildcat (*Felis silvestris* Schreber, 1777) in GreatBritain. OIE Revue Scientifique Et Technique. 1992; 11: 1143–1149.