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ORIGINAL ARTICLE

Babesia canis and tick-borne encephalitis virus (TBEV) co-infection in a sled dog

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Abstract

Introduction and objectives. Sporting dogs, including sled dogs, are particularly prone to tick-borne infection either due to training/racing in forest areas or through visits to endemic areas. The aim was to present tick-borne infections in a 6-dog racing team after a race in Estonia.

Materials and methods. On the 4th day after return to Poland, the first dog presented with babesiosis symptoms and was diagnosed and treated accordingly. Next morning, the dog showed neurological symptoms and was diagnosed with tick-borne encephalitis (TBE). Diagnosis was confirmed by a high level of IgG antibodies (922 IU/ml), detected in serum 3 months later. The second dog presented with babesiosis symptoms on the 7th day after return. *Babesia* DNA was extracted from blood, amplified and sequenced to answer the question of whether the dogs became infected during the race in Estonia or in Poland.

Results and conclusions. Sequencing of a fragment of *Babesia* 18S rDNA revealed that these two isolates were identical to one another and closely related to the *B. canis* sequence originally isolated from the dog and *Dermacentor reticulatus* ticks in Poland. Thus, this is the first confirmed case of *B.canis* and TBEV co-infection and first confirmed case of TBE in a dog in Poland.

Keywords

Babesia canis, tick-borne encephalitis virus, sled dogs, co-infections

INTRODUCTION

Vector-borne infections constitute increasing health problem in dogs worldwide [1, 2, 3, 4]. Sporting dogs, including sled dogs, are particularly prone to tick-borne infection either due to training/ racing in forest areas or because of frequent visits to endemic areas. Canine babesiosis in Europe is caused by different *Babesia* species- *Babesia canis*, *B. vogeli*, *B. microti*-like (*Theileria annae*), *B. caballi* and *B. gibsoni* [5]. To date, only one species has been identified in Poland, either in dogs or in its vector *Dermacentor reticulatus* tick: *Babesia canis* [1, 6, 7]. No data on canine babesiosis in Estonia is available. Babesiosis remains an emerging disease in dogs in Poland. Infections begin with fever, lethargy, anorexia, progressive anaemia and haemoglobinuria. Acute haemolytic anemia, kidney and liver dysfunctions often lead to death, despite applied treatment [6, 8].

To date, no confirmed TBE cases have been reported in dogs in Poland, although Poland is the country where TBE constitutes a significant health risk for humans with 200–400 clinical cases per year [9, 10, 11]. TBE in dogs was first reported in Sweden [12], subsequently, other cases were reported, mostly in Austria, the Czech Republic, Switzerland, Germany and Sweden [13, 14, 15, 16, 17]. The dogs become readily infected with TBEV, seroconvert upon infection, but they are also much more resistant to clinical disease than humans [13, 14]. On the other hand, reported clinical cases of TBE in dogs are often fatal [18], and the recently observed expanding range of TBEV and new foci in Eurasia may also lead to an increased emergence of TBE in dogs elsewhere [13, 15]. The monitoring of TBE cases and seroprevalence in dogs was suggested to provide important epidemiological data for public health concerns.

The main questions arising from the case of disease in traveling dogs are: when and where did the dogs became infected? As no ticks were noticed on the dogs, the answer was not simple. It was also complicated by the facts that the incubation period for TBE in dogs is not accurately known, and Poland and also Estonia are endemic areas for both TBE and babesiosis [11]. However, additionally, Russia, the Baltic countries of Lithuania, Latvia and Estonia, are all areas of the highest risk of TBEV infection in Europe, associated with the highest prevalence of TBEV in ticks, exceeding 20–40% [11]. In both cases – babesiosis and TBE – the incubation period in dogs has not been clearly determined, but may vary between 4 – 21 days [13, 19]. The first onset of symptoms in each case took place 4–7 days after the race. To answer this questions, the genotyping of *Babesia* isolates was performed.

The main aims of the presented study wer were:

1) to present tick-borne infections in a 6-dog team following a race in Estonia;
2) to present a case of *B. canis* and TBEV co-infection in a dog, first confirmed by molecular and serological methods;
3) and finally, to provide description on the first case of TBE in a dog in Poland.

MATERIALS AND METHODS

Timing and description of cases. Between September – November 2010, 4 international sled dog races, contributing to the 'Baltic Cup', took place in Estonia, Latvia, Poland and Lithuania. The first of these races occurred on the weekend of 26–27 September in the vicinity of Parnau,

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Estonia (ca. 800 km from capitol city of Poland, Warsaw). A 6-dog team based at the kennel situated in Kury, near Tluszcz (50 km in NE of Warsaw, Poland), took part in this competition. On Wednesday, 23 September, all the dogs were given treatment against tick infestation consisting of the 'spot on' medicine application, containing permethrin. On Friday, 25 September, the dogs traveled to Estonia, and on Saturday and Sunday participated in the race. All the dogs were in perfect health at this time, winning the race. Following a night in Riga, Latvia, the dogs returned to their kennel in Poland by the evening of Monday, 28 September.

On Thursday evening, one of the dogs, a 6-year-old neutered male 'Drop', a German shorthair pointer cross known as a European Sled dog breed, (ESD), presented with the first symptoms of babesiosis – apathy and loss of appetite. Next morning, 1 October, the dog was taken to a veterinary clinic 'Multiwet' in Warsaw. Babesiosis was diagnosed on the basis of a blood examination (Tab. 1). The dog was given standard treatment for babesiosis (and possible tick-borne bacteria co-infection), including imidocarb (6 mg/kg sc, Imizol, Intervet International BV, Boxmeer, Holland), atropinum sulfuricum (0.04 mg/kg sc, Polfa-Warszawa, Poland), amoxycyclinum (15 mg/kg sc Betamox LA, Norbrook Lab. Ltd. Station Works/Scan Vet, UK) and dexamethasone (0.15 mg/kg im, Dexafort (Intervet International BV, Boxmeer, Holland). The dog then improved for several hours, showing increased activity and enhanced appetite.

However, on the following morning (October 2nd) the dog presented with altered behaviour, i.e hyperactivity and symptoms of blindness – visual disturbance were noticed in both eyes. The dog was taken immediately to the nearest veterinary clinic in Tluszcz, presenting with additional neurological symptoms – altered consciousness and behaviour – loss of contact with/recognition of its owner, colliding with and climbing walls. Ophthalmic examination revealed strongly narrowed, unresponsive pupils in both eyes, the menace response was present. Body temperature was normal (38.3°C).

Following the diagnosis of encephalitis, most likely of tick-borne origin, the dog was administered corticosteroid and antibiotic treatment: dexamethasone 0.3 mg/kg intravenously, Dexazone; amoxycyclinum 15 mg/kg sc Betamox LA. The overall physical condition of the dog was not bad, and no other neurological symptoms were observed (motor failures, vestibular syndrome, etc.) and the dog was able to drink and eat. The prognosis was therefore good.

No other neurological symptoms appeared during the following 24 hours. Next morning (3 October), the dog was taken to the veterinary clinic 'Multiwet' in Warsaw for continuation of the treatment. Ophthalmic examination revealed slowly reacting pupils in both eyes, the menace response was present. Body temperature was normal (37.8°C), but the dog still showed abnormal behaviour and occasional loss of consciousness. A sample of blood was taken into 0.001 M EDTA for PCR diagnosis to enable the strain of *B. canis* causing infection to be identified. Anti-inflammatory treatment was continued with the dog being administered dexamethasone (1 mg/kg intravenously, Dexaven®, Jelfa, Jelenia Góra, Poland).

During the following 10-day period, the dog was treated with decreasing doses of prednisone (1 mg/kg orally every 24 h for 3 days, then 0.5 mg/kg orally every 24 h for 3 days, then 0.3 mg/kg orally every 24 h; Encorton®, Polfa-Pabianice, Poland). Antibiotic therapy was changed to doxycycline (10 mg/kg orally every 12 h) and continued for 2 weeks. The dog improved constantly from day-to-day, albeit slowly.

Table 1. Comparison of blood morphology and biochemical parameters in infected sled dogs.

Parameters	Units	Case 1 'Drop'		Case 2 'Koks'	Reference values	
		01.10.2010	03.10.2010	04.10.2010	%	G/l
Morphology:						
Leukocytes	G/l	3.9↓	20.7↑	5.2↓	na	6.0–12.0
Erythrocytes	T/l	5.22↓	4.94↓	5.49↓	na	5.5–8.0
Haemoglobin	mmol/l	8.38	7.82	7.64	na	7.45–11.17
Haematocrit	l/l	0.37	0.35↓	0.35↓	na	0.37–0.55
MCV	fl	70	71	64	na	60.0–77.0
MCHC	mmol/l	22.9↑	22.2	21.7	na	19.8–22.3
Thrombocytes	G/l	32↓	295	44↓	na	200–580
Leucocytes profile:						
Eosinophils	G/l (%)	0 (0)	0.42 (2)	0 (0)	0.1–6.0	0.0–0.6
Neutrophils						
– bands	G/l (%)	0 (0)	0.83↑ (4↑)	0 (0)	0.0–3.0	0.0–0.3
– segmented	G/l (%)	3.28 (84↑)	12↑ (58)	4.37 (84↑)	60–77	3.0–10.0
Lymphocytes	G/l (%)	0.62 (16)	7.45↑ (36↑)	0.83 (16)	12–30	1.0–4.0
Erythrocytes morphology:		Mild anisocytosis	Normal	Mild anisocytosis		
Parasites *Babesia canis*		Present	Not present	Present		
Biochemical profile:						
AST	(U/l)	136.0↑	Nd	205.0↑	1.0–37.0	
ALT	(U/l)	47.0	Nd	31.0	3.0–50.0	
ALP	(U/l)	78.0	Nd	84	20.0–155.0	
Blood glucose	(mg/dl)	125.0↑	78.0	Nd	70.0–120.0	
Creatinine	(mg/dl)	1.6	Nd	1.4	1.0–1.7	
Blood urea nitrogen	(mg/dl)	106.8↑	Nd	47.0↑	20.0–45.0	
Total serum protein	(g/l)	61.0	Nd	79.0↑	55.0–70.0	

↑– value above normal level; ↓ – value below normal level; MCV- mean corpuscular volume; MCHC – mean corpuscular haemoglobin concentration; AST – aspartate aminotransferase; ALT- alanine aminotransferase; ALP – alkaline phosphatase; Nd – not determined;

na – not applicable.

To confirm the diagnosis of TBE, on 15 January 2011, a blood sample was taken, a serum sample was prepared and sent to IN VITRO LABOR in Vienna, Austria, for determination of TBEV antibody titer.

The dog improved constantly and 1 month later returned to training and racing. However, some neurological sequelae were still present in this dog in July 2011: although very active whether on familiar or unfamiliar ground, the dog was unable to notice and catch small objects thrown to him (i.e. pellets of dog dry food) and lost the ability to jump over fences (owner observations).

Second case. On Sunday evening, 3 October, 2010, a second dog from the team, a 9-year-old intact male 'Koks', a German shorthair pointer/Alaskan husky/ mallinois cross presented with the first symptoms of babesiosis – apathy and loss of appetite. On the morning of Monday, 4 October, the dog was taken to the veterinary clinic 'Multiwet' in Warsaw. Babesiosis was diagnosed on the basis of blood examination (Tab. 1). The dog was given the standard treatment for babesiosis, including imidocarb (6mg/kg subcutaneously, Imizol Intervet International BV, Boxmeer, Holland), amoxycyclinum (15 mg/kg sc Betamox LA Norbrook Lab. Ltd. Station Works/Scan Vet, UK), atropinum sulfuricum (0.04 mg/kg sc, Polfa-Warsaw, Poland) and dexamethasone (0.1 mg/kg im, Dexaven®, Jelfa, Jelenia Góra, Poland). Treatment was successful in this case, and no other symptoms have appeared.

Epidemiological study. In order to enable molecular characterization of the pathogens, blood samples were collected into 0.001M EDTA and frozen at -20°C until DNA extraction. A blood sample from the first dog was taken 2 days after treatment for babesiosis had been started; in the second case, the blood sample was taken before treatment was initiated. DNA extractions were performed using the AxyGen MiniPrep Blood kit (AxyGen, USA).

Amplification of 18S rRNA *Babesia* gene fragment was performed using the previously described PCR protocol [20, 21]. Primers BAB GF2 (5' GYYTTGTAATTGGAATGATGG 3') and BABGR2 (5' CCAAAGACTTTGATTTCTCTC 3') were used to produce a ~550 bp fragment. Sequencing reactions were conducted with the ABI-PRISM 377 automatic DNA sequencer (Applied Biosystem). The resulting sequences were assembled using the programmme ABI™ BigDye™. BLAST comparisons were run against the GenBank database (www.ncbi.nlm.nih.gov/BLAST).

Analysis of DNA sequences and phylogenetic relationships for the 2 current *B. canis* isolates and for a group of isolates from dogs from the same kennel obtained in 2006 [1], were conducted using MEGA version 5.0 [22]. A phylogenetic tree was created using alignments performed with Kimura-2 parameter algorithm as a distance method, and NJ as the tree construction method. For comparison, sequences of *Babesia* species and strains obtained from GenBank (www.ncbi.nlm.nih.gov) were included in the sequence alignment.

RESULTS

Clinical cases. Two dogs of 6-dog team presented with tick-borne diseases (TBD) following a sled dog race in Estonia, showing high risk of TBD infection in this group of working dogs. The results of the blood examination of the dogs are presented in Table 1. Common features of babesiosis were observed in both dogs: anaemia, thrombocytopenia, elevated liver enzymes, elevated level of blood urea (Case 1), anisocytosis (Tab. 1, columns 1 and 3). Significant changes were observed in blood morphology of the first dog in comparison to the blood test 2 days earlier – leukocytosis instead of leukocytopenia; normal numbers of platelets; normal morphology of erythrocytes (Tab. 1, columns 1 and 2). Results of the ELISA test showed IgG antibody titer 922 IU/ml. The threshold value for a positive result with this test was 20 IU/ml. Positive results of PCR reaction confirmed *B. canis* infection in both dogs. Thus, the co-infection of *B. canis* and TBEV was confirmed for the first dog.

Genotyping of *Babesia* from the cases. Sequencing of a *Babesia* 18S rDNA fragments revealed that these two isolates were closely related (99.6% identity) to the *B. canis* genotype 2 (EU622793) originally isolated from a dog with babesiosis in Poland [6] (Fig. 1). Analysis of the 18S rRNA gene demonstrated that these two isolates clustered with our previous 10 canine isolates, and with other *B. canis* strains from either Southern Europe Albania, Italy), Central Europe (Hungary, Netherlands, Slovakia) or North-Eastern Europe (Poland), and Siberia (Fig. 1). The second cluster seen on Figure 1 comprised isolates of *B. canis* genotype 1 [6], but also originated from different parts of Europe. On the basis of this phylogenetic tree, no regional specificity of parasite isolates was observed, therefore origin of the *Babesia* infection in the two presented cases could not be clearly determined. High homology to genotype 2 and an isolate from *D. reticulatus* from the same locality, Kury, (Fig. 1) suggested rather an autochthonous infection. Based on this suggestion, an autochthonous infection with TBEV should be considered also in the dog with the co-infection.

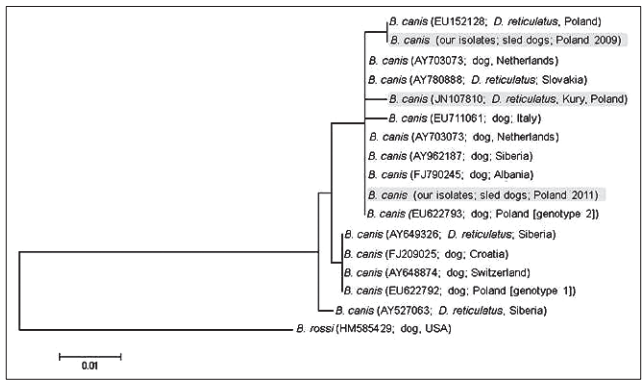

Figure 1. Phylogenetic tree of *Babesia* isolates from the presented study (grey shading) and selected accessions from GenBank, based on *18S rRNA* gene fragment sequences. The phylogenetic tree was created using nucleotide sequence alignments performed using the Kimura-2 parameter algorithm as a distance method and NJ as the tree construction method. 0.01 on the scale bar indicates 1 nucleotide substitution per 100 sites.

DISCUSSION

The main aim of the presented study was to describe the first case of *B. canis* and TBEV co-infection in a dog, confirmed by molecular and serological methods. On the basis of PCR, a high prevalence of *Babesia* DNA in sled dogs has been previously found in Central Poland [1]; however, this is the first description of a clinical case of TBEV infection in a dog that country. Although TBE constitutes a significant health

risk for humans in Poland, no reports on TBE in dogs seem to have been published. The most frequent symptoms of TBE in the reported case were associated with impairment of vision. Only a single case of optic neuritis due to TBEV infection in a 3-year-old female Siberian Husky was described in Vienna, Austria [23]. Also in that case, vision impairment was the main symptom in the first phase of TBE, body temperature was normal (38.4°C) and the IgG antibody titer was high. The authors considered TBE as the cause of optic neuritis because the optic nerve is surrounded by the meninges and subarachnoid space. Therefore, infections of the meninges may gain access to the optic nerve [23].

To date, the reasons for the development of clinical disease are not known. The immunological status of a dog probably plays an important role, as the pathology in the central nervous system is caused primarily by the immune-mediated inflammation at the site. This assumption was supported by the reported increasing rate of clinical cases with increasing levels of IgG titer among seropositive dogs [14, 15]. In the presented case, the determined IgG titer was also high. Pertinent in this context, co-infections with two or three tick-transmitted pathogens were reported to cause more severe courses of diseases in humans [24, 25].

In the presented case, the dog first presented with symptoms of babesiosis and then TBE symptoms; it is therefore likely that the earlier *Babesia* infection predisposed the dog to development of clinical TBE, or that the immunosuppressive steroid treatment applied for babesiosis was the reason for the subsequent development of clinical TBE. Irrespective of the exact reason, the other four cases of presumed TBE were diagnosed in 2010 by a local veterinarian in Tluszcz; two of them developed also in dogs following clinical babesiosis. Of these four cases, two were fatal, despite the administered treatment (one puppy, one 6-year- old female). The presumed TBE cases support the hypothesis of autochthonous origin of TBE in sled dog (Mazowsze is one of known endemic TBE areas in Poland), and the hypothesis of synergistic pathogenic effects of co-infection with *B. canis* and TBEV. Interestingly, the results of haematological tests in canine babesiosis and during the early viremic phase of TBE in humans are very similar: leukocytopenia, thrombocytopenia, elevated liver enzymes [13, 26]. Thus, it seems that the effect of these two pathogens on host organisms is either very similar, or that co-infections are much more common than originally thought. Also, in two out of the eight fatal canine TBE cases reported by Weissenbock and others [18], anorexia and retention of urine were observed, prior to the appearance of neurological symptoms and apathy, all manifestation identical to those often seen in babesiosis.

It is worth noting that the increased number of neutrophils in the second examination of the first dog may have resulted not only from viral infection but also from glucocorticosteroid therapy, as the dog had been treated with dexamethasone.

The main vector of TBEV in Central Europe is *Ixodes ricinus* but the infection rate in ticks is about 1%, very rarely exceeding 5% even in high-risk areas [11, 27]. On the other hand, the main vector of *B. canis* in Central Europe is *Dermacentor reticulatus* tick [19]. Ticks of this species are actually the most common ticks found on dogs in the Warsaw area [28]. It has been recently confirmed that 11% of *D. reticulatus* individuals collected from dogs in Warsaw were positive for *B. canis* [7]. In the reported case of co-infection, this tick species should be considered as the vector of TBEV. This hypothesis is supported by a recent study of the prevalence of TBEV in *I. ricinus* and *D. reticulatus* in another TBE endemic region of Poland – the Lublin region [29]. In this study, TBEV was recorded in 1.6% of *I. ricinus* but in 11% of *D. reticulatus*. Comparable rates of TBEV and *B. canis* infections in *D. reticulatus* support the idea that transmission of co-infections to dogs is by this tick species. As the geographical range of *D. reticulates* continues to expand in Poland and throughout Europe [30, 31, 32, 33], probably due to both climate change and changes in agricultural practices (abandoned fields), these two serious tick-borne diseases may soon pose a real health risk for dogs throughout Europe. As this tick species feeds on cattle, and milk-borne TBE cases are of public health significance, the role of this non-human tick in the transmission of TBE to humans may be significant and more important than hitherto realized.

CONCLUSIONS

The presented study:
1. Provides the first description of a confirmed clinical case of TBE in dogs in Poland;
2. Presents the first case of co-infection with *B.canis* and TBEV in a dog, confirmed by molecular and serological methods;
3. Outlines the possible significance of *D. reticulatus* ticks in the circulation of TBEV in the environment.

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REFERENCES

1. Welc-Faleciak R, Rodo A, Sinski E, Bajer A. *Babesia canis* and other tick-borne infections in dogs in Central Poland. Vet Parasitol. 2009; 166: 191–198.
2. Dantas-Torres F. Canine vector-borne diseases in Brazil. Parasites & Vectors 2008; 1: 25, doi:10.1186/1756–3305–1–25.
3. Matjila PT, Leisewitz AL, Jongejan F, Penzhorn BL. Molecular detection of tick-borne protozoal and ehrlichial infections in domestic dogs in South Africa. Vet Parasitol. 2008; 155: 152–157.
4. Solano-Gallego L, Trotta M, Razia L, Furlanello T, Caldin M. Molecular survey of *Ehrlichia canis* and *Anaplasma phagocytophilum* from blood of dogs in Italy. Ann NY Acad Sci. 2006; 1078: 515–518.
5. Bourdoiseau G. Canine babesiosis in France. Vet Parasitol. 2006; 138: 118–125.
6. Adaszek L, Winiarczyk S. Molecular characterization of *Babesia canis canis* isolates from naturally infected dogs in Poland. Vet Parasitol. 2008; 152: 235–241.
7. Zygner W, Jaros S, Wedrychowicz H. Prevalence of *Babesia canis, Borrelia afzelii,* and *Anaplasma phagocytophilum* infection in hard ticks removed from dogs in Warsaw (central Poland). Vet Parasitol. 2008; 153: 139–142.
8. Adaszek L. Wybrane aspekty epidemiologii babeszjozy, boreliozy i erlichiozy u psów. PhD thesis 2006, Faculty of Veterinary Medicine in Lublin, Poland.
9. Czupryna P, Moniuszko A, Pancewicz SA, Grygorczuk S, Kondrusik M, Zajkowska J. Tick-borne encephalitis in Poland in years 1993–2008- epidemiology and clinical presentation. A retrospective study of 687 patients. Eur J Neurol. 2011; 18: 673–679.
10. Kicman-Gawłowska A, Chrześcijańska I, Stefanoff P. [Meningitis and encephalitis in Poland in 2006]. Przegl Epidemiol. 2008; 62: 253–260 (in Polish).

11. Suss J. Tick-borne encephalitis in Europe and beyond--the epidemiological situation as of 2007. Euro Surveillance 2008; 13: pii: 18916.
12. Lindblad G. A case of tick-borne encephalitis in a dog. *Medlernsblad Sveriges* Veterinarforbund 1960; 12: 416–417.
13. Pfeffer M, Dobler G. Tick-borne encephalitis virus in dogs- is this an issue? Parasites & Vectors 2011; 4: 59.
14. Leschnik MW, Kirtz GC, Thalhammer JG. Tick-borne encephalitis (TBE) in dogs. Int J Med Microbiol. 2002; 291(Suppl. 33): 66–69.
15. Kirtz G. FSME- Infektion in einer osterreichischen Hundepopulation. Diss Med Vet., Vien. 1999.
16. Reiner B, Fischer A. Fruhsommer-Meningoenzephalitis (FSME) beim Hund in Deutschland: zwei Fallberichte. Kleintierpraxis 1998; 43: 255–269.
17. Tipold A, Fatzer R, Holzmann H. Zentraleuropaische Zeckenenzephalitis beim Hund. Kleintierpraxis 1993; 38: 619–628.
18. Weissenbock H, Suchy A, Holzmann H. Tick-borne encephalitis in dogs: neuropathological findings and distribution of antigen. Acta Neuropathol. 1998; 95: 361–366.
19. Zygner W. Choroby pasożytnicze przenoszone przez stawonogi zagrażające psom wyjeżdżającym do europejskich krajów Basenu Morza Śródziemnego i Portugalii. Część II. Babeszjoza, teilerioza i hepatozoonoza. Życie Weteryn. 2006; 81: 595–603 (in Polish).
20. Bonnet S, Jouglin M, Malandrin L, Becker C, Agoulon A, L'Hostis M, Chauvin A. Transstadial and transovarial persistence of *Babesia divergens* DNA in *Ixodes ricinus* ticks fed on infected blood in a new skin-feeding technique. Parasitology 2007a; 134: 197–207.
21. Bonnet S, Jouglin M, L'Hostis M, Chauvin A. *Babesia* sp. EU1 from roe deer and transmission within *Ixodes ricinus*. Emerg Infect Dis. 2007b; 13: 1208–1210.
22. Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. MEGA5: Molecular Evolutionary Genetics Analysis using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. Mol Biol Evol. 2011; 28: 2731–2739.
23. Stadtbaumer K, Leschnik MW, Nell B. Tick-borne encephalitis as a possible cause of optic neuritis in a dog. Vet Ophthalmol. 2004; 7: 271–277.
24. Krause PJ, McKay K, Thompson CA, Sikand VK, Lentz R, Lepore T, Closter L, Christianson D, Telford SR, Persing D, Radolf JD, Spielman A. Deer-Associated Infection Study Group. Disease-specific diagnosis of coinfecting tickborne zoonoses: babesiosis, human granulocytic ehrlichiosis, and Lyme disease. Clin Infect Dis. 2002; 34: 1184–1191.
25. Abrams Y. Complications of coinfection with *Babesia* and Lyme disease after splenectomy. J Am Board Fam Med. 2008; 21: 75–77.
26. Zygner W, Gójska O, Rapacka G, Jaros D, Wędrychowicz H. Hematological changes during the course of canine babesiosis caused by large *Babesia* in domestic dogs in Warsaw (Poland). Vet Parasitol. 2007; 145: 146–151.
27. Suss J, Schrader C, Abel U, Bormane A, Duks A, Kalnina V. Characterization of tick-borne encephalitis (TBE) foci in Germany and Latvia (1997–2000). Int J Med Microbiol. 2002; 291(Suppl. 33): 34–42.
28. Zygner W, Wedrychowicz H. Occurrence of hard ticks in dogs from Warsaw area. Ann Agric Environ Med. 2006; 13: 355–359.
29. Wójcik-Fatla A, Cisak E, Zając V, Zwoliński J, Dutkiewicz J. Prevalence of tick-borne encephalitis virus in *Ixodes ricinus* and *Dermacentor reticulatus* ticks collected from the Lublin region (eastern Poland). Ticks and Tick-borne Dis 2011; 2: 16–19.
30. Dautel H, Dippel C, Oehme R, Hartelt K, Schettler E. Evidence for an increased geographical distribution of *Dermacentor reticulatus* in Germany and detection of *Rickettsia* sp. RpA4. Int J Med Microb. 2006; 296(S1): 149–156.
31. Heile C., Heydorn AO, Schein E. *Dermacentor reticulatus* (Fabricius, 1794)- distribution, biology and vector for *Babesia canis* in Germany. Berlin Münch Tierärzt Wochenschrif 2006; 119: 330–334.
32. Nijhof AM, Bodaan C, Postigo M, Nieuwenhujis H, Opsteegh M, Franssen L, Jebbnik F, Jongejan F. Tick and associated pathogens collected from domestic animals in the Netherlands. Vector Borne Zoonotic Dis. 2007; 7: 584–595.
33. Sreter T, Szell Z, Varga I. Spatial distribution of *Dermacentor reticulatus* and *Ixodes ricinus* in Hungary: evidence for change? Vet Parasitol. 2005; 128: 347–351.