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Original article

First molecular identification of *Babesia gibsoni* in dogs from Slovakia, central Europe

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ABSTRACT

Canine babesiosis is a severe and potentially life threatening infection. In Europe, *Babesia canis* is considered to be the most common species responsible for the disease.

We report two cases of babesiosis caused by *Babesia gibsoni*. The polymerase chain reaction, restriction fragment length polymorphism analysis and further sequencing of 18S rRNA gene fragments from blood samples of both dogs revealed the identity of isolates with *B. gibsoni* genotypes from other dogs worldwide.

This species was previously not known to infect dogs in Slovakia. It is resistant to traditional antibabesial therapy. Therefore, correct diagnosis is crucial for the successful treatment, especially in dogs with hemolytic anemia and febrile conditions.

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1. Introduction

Canine vector-borne diseases have become a significant problem in the veterinary medicine and constantly present an increasing challenge for the differential diagnosis and therapy to the vet practitioners (Hurnikova et al., 2013; Majlathova et al., 2011; Miterpakova et al., 2010; Vichova et al., 2014).

Canine babesiosis is one of the most common and worldwide distributed tick-borne diseases (Solano-Gallego et al., 2008). So far, altogether 12 *Babesia* species, which are able to evoke infections in dogs, have been identified (Irwin, 2010). Based on the morphology they are referred to as large and small *Babesia*. In Europe, two small and two large canine piroplasms can be found (Birkenheuer et al., 2004). Nevertheless, an introduction and improvement of molecular methods revealed sporadic evidence of other piroplasms, such as *Theileria equi* and/or *Theileria annulata* which have been identified in several dogs from Croatia (Beck et al., 2009) or Spain (Criado-Fornelio, 2007), respectively.

The spectrum of diseases caused by *Babesia* species is highly diverse and ranges from subclinical mild anemia to a life threatening infection with multiple organ failure and death. Acute phase of infection is typically associated with remittent fever, progressive anemia, thrombocytopenia, haemoglobinuria, marked splenomegaly and hepatomegaly (Birkenheuer et al., 1999). Typical clinical manifestations are lethargy, pale mucous membranes, weakness, jaundice and vomiting. Hyperglobulinemia, hyperbilirubinemia, increased liver enzyme activities, bilirubinuria, haemogobinuria and proteinuria are typical laboratory findings observed during the serum biochemistry and urinalysis, respectively (Boozer and Macintire, 2003). Chronic infections are also common and infected dogs can remain long-term carriers without evident clinical signs.

The course and severity of canine babesiosis are determined by the pathogenicity of the causative agent, level of parasitemia, age and immune status. The main risk factors which should be taken into consideration during the differential diagnosis include travel history or stay in areas with endemic occurrence of competent vector ticks, history of tick bites, recent dog bites and blood transfusions (Birkenheuer et al., 2004). In Europe *Babesia canis* is considered as the most widespread and pathogenic species (Criado-Fornelio et al., 2003b). The distribution of Babesia overlaps with the distribution of its arthropod vectors. The ornate dog tick (*Dermacentor reticulatus*) *is a competent vector of B. canis* (Foldvari et al., 2005; Majlathova et al., 2011). *Babesia gibsoni* is distributed in areas of Asia, Africa, Middle East, Brazil, North America, Australia and Southern Europe (Birkenheuer et al., 1999; Kocan et al., 2001; Onishi

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Table 1
The complete blood count (CBC) and biochemical parameters of "Dog 1". Overview of the parameters from the Day 0 after the initiation of the treatment.

Parameters CBC (reference intervals)	Day 0	Day 25	Day 70	Day 98	Day 122	Day 256	Day 270	Day 291	Day 328	1 year and Day 35	1 year and Day 113
WBC ($5.05–16.76 \times 10^9$/L)	16.05	6.38	**4.25**	5.46	8.16	13.46	8.56	9.75	8.04	6.34	12.33
LYM ($1.05–5.10 \times 10^9$/L)	2.91	1.69	1.08	1.26	1.38	3.14	1.11	1.33	1.47	**1.04**	1.67
MONO ($0.16–1.12 \times 10^9$/L)	**1.46**	0.81	0.51	0.59	0.87	**1.49**	0.51	0.84	**1.18**	0.4	0.90
NEU ($2.95–11.64 \times 10^9$/L)	**11.66**	3.83	**2.4**	3.20	5.44	8.19	6.91	7.44	5.03	4.64	9.02
EOS ($0.06–1.23 \times 10^9$/L)	**0.01**	**0.05**	0.20	0.32	0.35	0.63	**0.02**	0.12	0.33	0.13	0.73
BASO ($0.00–0.10 \times 10^9$/L)	0.01	0.00	0.06	0.09	**0.12**	0.01	0.01	0.02	0.03	**0.13**	0.01
HCT (37.3–61.7%)	**15.7**	**21.4**	**27.5**	**31.5**	**33.2**	**24.7**	**34.6**	**29.9**	**25.6**	**27.4**	37.3
RBC ($5.65–8.87 \times 10^{12}$/L)	**1.69**	**2.28**	**4.17**	**5.47**	6.08	**3.63**	**4.69**	**4.46**	**3.95**	**4.43**	**5.51**
HGB (13.1–20.5 g/dL)	**4.2**	**6.0**	**9.2**	**10.4**	**11.4**	**7.8**	**10.8**	**9.7**	**8.3**	**9.0**	**12.5**
RETIC (10.0–110.0 K/μL)	**469.0**	**379.2**	**171.4**	**116.5**	**127.1**	**359.0**	**271.1**	**178.0**	**185.7**	69.6	**186.8**
MCV (61.6–73.5 fL)	**92.9**	**93.9**	65.9	**57.6**	**54.6**	68.0	**73.8**	67.0	64.8	61.9	67.7
RDW (13.6–21.7%)	**32.0**	17.3	16.5	19.8	**23.1**	**30.9**	21.4	19.7	21.2	19.6	19.8
MCHC (32.0–37.9 g/dL)	**26.8**	**28**	35.5	33.0	34.3	**31.6**	**31.2**	32.4	32.4	32.8	33.5
MCH (21.2–25.9 pg)	24.9	**26.3**	22.1	**19.0**	**18.8**	21.5	23.0	21.7	**21.0**	**20.3**	22.7
PLT (148–484 K/μL)	**71**	**121**	**127**	149	**109**	**24**	**26**	**59**	**91**	**106**	**133**
Biochemical abnormalities*	ALKP 155 ALT 61 **AST 70** ↑ UREA 9.6 CREA 89							**ALB 45** ↑ ALKP 108 ALT 95 UREA 4.6 **CA 3.02** ↑ CREA 118			
PCR	+ referred as *B. canis*										

Numbers in bold represent the deviations (increase/decrease) from the reference interval.
* Reference intervals of biochemical parameters: ALB/albumin (23–40 g/L); ALKP/alkaline phosphatase (23–212 U/L); ALT/alanine aminotransferase (10–100 U/L); UREA (2.5–9.6 mmol/L); CREA/creatinine (44–159 μmol/L); GLOB/globulins (25–45 g/L); GLU/(4.11–7.95 mmol/L); TP/total protein (52–82 g/L).

and Suzuki, 1994; Trapp et al., 2006). *Haemaphysalis longicornis*, *Haemaphysalis bispinosa*, *Dermacentor variabilis* and *Rhipicephalus sanguineus* are believed to be the principal vectors of *B. gibsoni* (Boozer and Macintire, 2003).

In Europe, the epidemiology of *B. gibsoni* is not well known. However, several cases of infection have been confirmed in dogs from Croatia, Germany, Italy, Spain, Hungary and Romania (Beck et al., 2009; Criado-Fornelio et al., 2003a; Hamel et al., 2012; Hartelt et al., 2007). Increased dog traveling, global warming and changes in the tick population distribution may potentially cause the spreading of pathogens, such as *B. gibsoni*, to previously non-endemic areas.

2. Material and methods

On June 18th 2013, a 3 years old mixed pit bull male (Dog 1) was presented to the veterinary clinic in Bratislava, Slovakia, with a history of massive haemoglobinuria and blood transfusion repeated twice during the previous treatment at another clinic.

A few months later, the owner visited the veterinary clinic again, with a 2 years old mixed pit bull terrier (Dog 2) with a 3 days history of lethargy, weakness and repeated blackouts.

The complete blood counts (CBC) of both patients were performed in automatic hematology analyzer IDDEX VetLab®UA™. The thin blood smears prepared from the EDTA (Ethylenediaminetetraacetic acid) treated blood samples taken from the cephalic vein were Giemsa stained and examined microscopically.

Genomic DNA was extracted from 200 μL of EDTA-blood samples, using a commercial DNA extraction kit (NucleoSpin Blood kit, Machery Nagel, Germany). For the molecular detection of *Babesia* spp., PCR amplification of approximately 450 bp long fragment of 18S rRNA gene, spanned by a reverse BJ1 (5′GTCTTGTAATTGGAATGATGG3′) and forward BN2 (5′TAGTTTATGGTTAGGACTACG3′) primer was performed according to Casati et al. (2006). In each PCR reaction, sequenced DNA from *Babesia*-positive dog was used as positive control and nucleases free water was added as the template in negative control. The PCR products were visualized by electrophoresis on 1.5% agarose gels stained with GoodView Nucleic Acid Stain (Beijing SBS Genetech, Beijing, China).

All positive PCR products were purified using a purification kit (Qiagen, Hilden, Germany) and sequenced. Nucleotide sequences were manually edited in MEGA 6 (Tamura et al., 2013) and further compared with GenBank entries by BLAST (Altschul et al., 1997). For the alignment of the homologous nucleotide sequences

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