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ORIGINAL PAPER

Epidemiological aspects on vector-borne infections in stray and pet dogs from Romania and Hungary with focus on *Babesia* spp.

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Abstract Canine arthropod-borne infections are of major interest in small animal practice and have been widely investigated in Central and Western Europe. However, only limited epidemiological data are available from South-Eastern European countries, although diseases including babesiosis or dirofilariosis are widely recognised as important canine infections in these countries. A steadily increasing number of dogs imported from South-Eastern Europe into Germany require particular attention by small animal practitioners. In this study, a total of 216 dogs [29 local Romanian pet dogs presented at Salvavet Veterinary Clinic in Bucharest, Romania, and 187 imported stray dogs from Romania (n=109) and Hungary (n=78) into Germany] were screened by molecular biological, serological and haematological methods for canine arthropod-borne infections. Eleven different parasitic and bacterial vector-borne pathogens—*Babesia canis canis*, *Babesia canis vogeli*, *Babesia gibsoni*, *Babesia felis*-like, *Hepatozoon canis*, *Leishmania* spp., *Dirofilaria immitis*, *Dirofilaria repens*, *Acanthocheilonema reconditum*, *Anaplasma phagocytophilum* and *Mycoplasma haemocanis*—were detected. Fifty-six percent of the dogs were positive by direct methods. *B. canis canis* was the most prevalent pathogen in dogs imported to Germany (42.8%) and dogs submitted for clinical consultation in Bucharest (44.8%). Our data strongly suggest the introduction of an adjusted screening panel in dogs from South-East Europe in view of increasing importation of dogs into Germany.

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Introduction

Formerly, state borders and legal travel restrictions confined the movement of dogs within Europe, effectively limiting the introduction of vector-borne infections to non-endemic regions. The founding of the European Union facilitated pet travel and importation of dogs by private persons and animal welfare organisations from all over Europe to Central and Western European countries. Such dogs often originating from endemic areas may act as carriers for different vector-borne infections, e.g. *Babesia* spp., *Hepatozoon canis*, *Leishmania* spp., *Dirofilaria* spp. or *Ehrlichia canis*, into hitherto non-endemic countries.

Such infections necessitate significantly extended differential diagnostic investigations in small animal practice in non-endemic regions in Central and Western Europe (Deplazes et al. 2006). In Germany, *Babesia* spp., *Leishmania* spp., *Dirofilaria immitis* and *E. canis* have repeatedly been recorded in travelling and imported dogs (Dongus and Gothe 1996; Zahler et al. 1997; Glaser and Gothe 1998; Gothe 1998; Gothe and Wegerdt 1991; Menn et al. 2010; Hamel et al. 2011; Röhrig et al. 2011). Only few cases of leishmaniosis, hepatozoonosis or ehrlichiosis, however, have been considered as autochthonous infections based on anamnesis, clinical features and consecutive diagnostics (Gothe 1998; Jensen et al. 2007a; Kellermaier et al. 2007; Gärtner et al. 2008). The transmission of these pathogens in the absence of competent vectors in Germany still remains an open question.

Canine vector-borne infections are recognised as a major health problem in Mediterranean countries. However, despite intensive clinical and epidemiological research, information on the actual distribution and seasonal occurrence of such infections is still lacking for most South-Eastern European countries (Trotz-Williams and Trees 2003). Nonetheless, *Babesia canis* spp. is widely recognised as an important clinical canine disease in Hungary and in Romania (Földvari et al. 2005; Hornok et al. 2006; Ilie et al. 2010; Ionescu et al. 2010). The objective of this study was to obtain more detailed information on the epidemiology and extent of such infections. The added scientific value will contribute to appropriately adjusting routine diagnostic screening of dogs imported from these countries to the current epidemiological situation. Therefore, a total of 216 dogs from Hungary and Romania (29 from a veterinary clinic in Bucharest, Romania and 187 from Romania and Hungary imported to Germany) were tested for the presence of vector-borne infections by direct and indirect diagnostic techniques.

Material and methods

Animal data and clinical assessment

Canine EDTA blood samples obtained during March 2009 to April 2010 were screened for the presence of canine vector-borne infections by direct and indirect methods. One hundred eighty-seven EDTA blood and serum samples of stray dogs from Hungary (n=78) and Romania (n=109) imported into Germany were tested. One hundred seventy-nine samples of clinically apparently healthy animals were supplied by animal welfare organisations and eight by private owners for routine diagnostic to the diagnostic centre at the Chair of Comparative Tropical Medicine and Parasitology. These imported dogs consisted of 117 mixed breed dogs, 22 pure bred dogs and 48 without information on breed. The dogs' age ranged from 4 months to 10 years [mean 2.9 years, standard deviation (SD) 2.2 years]; 64 were male dogs, 68 bitches and for 55 animals no gender was specified.

Additional 29 EDTA blood and serum samples were taken in April 2010 from pet dogs submitted to the Salvavet Veterinary Clinic in Bucharest, Romania. The material was obtained from surplus blood samples taken for routine diagnostic. The age of local Romanian dogs was specified for 28 animals, ranging from 3 months to 13 years (mean 4.8 years, SD 4.0 years). Eight bitches and 21 male dogs were tested. Twenty-eight dogs were from Bucharest and vicinity and one from the Carpathian Mountains region. They were presented at the Salvavet Veterinary Clinic for various reasons, e.g. gastric symptoms (seven), surgery/accidents (six), babesiosis (five), renal problems (three), parasites (two) or other (six). Eight owners reported the use of prophylactic measures against ectoparasites and 12 owners recognised "ectoparasites" on their dogs prior to clinical consultation.

Laboratory testing

Samples were tested with standard techniques established in our laboratory such as blood/buffy coat smear, Knott's test, DiroChek®-ELISA, Indirect Immunofluorescent Antibody Test (IFAT) for anti-*B. canis* spp., anti-*Leishmania* spp. and anti-*E. canis* antibodies and by the conventional and real-time PCRs. Giemsa-stained blood/buffy coat smears were performed for all animals for the detection of pathogens in the blood. For technical reasons, only the blood samples of imported dogs were tested by Knott's test for microfilaria. DiroCheck®-ELISA Canine/Feline Antigen Test Kit (Synbiotics Corp., USA) was applied for screening for the antigen of female adult *D. immitis*.

Molecular methods

DNA was extracted from 200 µl of the EDTA blood samples with the Qiagen DNA MiniKit (Qiagen, Hilden, Germany) according to manufacturer's instructions. Conventional PCRs were carried out for the detection of *B. canis* spp. and *Babesia gibsoni*, *H. canis* and *Mycoplasma* spp. and for discriminating different species of microfilaria in Knott's test-positive samples (Birkenheuer et al. 2003; Inokuma et al. 2002; Watanabe et al. 2003; Rishniw et al. 2006). The reactions were carried out on an Eppendorf Mastercycler gradient (Eppendorf, Hamburg, Germany) or an Applied Biosystems GeneAmp 9700 (Applied Biosystems, Darmstadt, Germany) using the Qiagen HotStarTaq Polymerase Kit (Qiagen, Hilden, Germany) for *Babesia* spp. and the HotMaster Taq Polymerase (5-Prime, Darmstadt, Germany) for *H. canis*, *Mycoplasma* spp. and differentiation of microfilaria. Successful amplification of the gene targets in the conventional PCRs was verified with 2.0% agarose gel electrophoresis after staining with GelRed™ (Biotium, Hayward, USA) and visualized under UV light. For *Babesia* spp., the primers 455-479F and 793-722R were used for the first amplification, and for the heminested reaction, the reverse primer 793-722 R was used with BCC-F for *Babesia canis canis*-specific detection, with BCV-F for *Babesia canis vogeli*-specific detection and with B.gib.ASIA-F for *B. gibsoni*-specific detection. The bands of 185–200 bp in the respective heminested reaction were considered positive. For the first about 30 samples, also the first reaction was run on a gel. For

H. canis, a band of 666 bp was considered positive, for *Mycoplasma* spp., a band of 202 bp indicates *Candidatus* Mycoplasma haematoparvum and of 276 bp, *Mycoplasma haemocanis*, and for the differentiation of microfilaria, 484 bp were considered as *Dirofilaria repens*, 542 bp as *D. immitis* and 578 bp as *Acanthocheilonema reconditum*. The products of the filarial PCR were sequenced with one primer for confirmation.

Amplicons of two *Babesia* spp. and of the filarial PCR were purified with the QIAquick PCR Purification Kit according to manufacturer's instruction (Qiagen) and sequenced at Eurofins MWG Operon (Martinsried, Germany). Sequence homology searches were made by BLASTn analysis of GenBank (www.ncbi.nlm.nih.gov.library.vu.edu.au/BLAST/).

Real-time PCRs were used for the detection of *Leishmania infantum*, *Anaplasma phagocytophilum* and *E. canis* DNA (Mary et al. 2004; Courtney et al. 2004; Silaghi, unpublished data). A CT value smaller than 39 was considered positive for *A. phagocytophilum* and *E. canis*, and smaller than 38 for *L. infantum*.

Serological methods

Antibodies against *B. canis* spp., *E. canis* and *A. phagocytophilum* were tested using commercial IFAT tests (*A. phagocytophilum*, Focus Technologies, Cypress, USA; *E. canis* and *B. canis* ssp., MegaCor, Hörbranz, Austria). An in-house anti-*Leishmania* spp. IFAT was used for the detection of *Leishmania* spp. antibodies (Mancianti et al. 1995). Titres of 1:64 were considered as seroreactive to *B. canis* spp., *Leishmania* spp. and *A. phagocytophilum* and of 1:32 as borderline titres. Titres of 1:40 were seropositve in *E. canis*-IFAT, respectively.

Statistical analysis

To obtain information on the incidence of infections in pet and stray dogs, the dogs were grouped into those presented at the veterinary clinic and imported animals. Contingency table analysis ($p<0.05$) and calculation of confidence intervals was performed with Graph Pad Prism (Graph Pad Software, USA).

Results

The screening for vector-borne infections of 216 canine EDTA blood samples resulted in the detection of 11 different pathogens by molecular methods—*B. canis canis*, *B. canis vogeli*, *B. gibsoni*, *Babesia felis*-like, *Leishmania* spp., *H. canis*, *D. immitis*, *D. repens*, *A. reconditum*, *M. haemocanis* and *A. phagocytophilum*. A total of 56.0% of dogs under investigation were positive for infections by direct and/or 21.3% by indirect methods. *B. canis canis* was by far the most prevalent pathogen (43.1%) while the others were found at lower prevalence rates of <0.1% up to 8.3%. Except for *M. haemocanis*, there was no statistical difference in the presence of pathogens in imported dogs and those from the veterinary clinic ($p<0.05$). *B. felis*-like sequences have been submitted to GenBank: JN543169 and JN543170.

Imported dogs

Of all samples, 55.1% were positive by direct methods and 16.5% were positive for antibodies (Table 1). Microscopic examination of blood/buffy coat smears gave no evidence for vector-borne pathogens. Overall, 97 dogs were positive in the *Babesia* spp.-nested PCR. Two products gave bands in the first reaction, but not in the nested reaction. Sequencing of these products of the first reaction with subsequent BLASTn analysis resulted in two *B. felis*-like (99% similarity to GenBank accession nos. AF244912 and AY452702). The diagnostic procedure was repeated two times in the case of *B. felis*-like for confirmation including bidirectional sequencing and revealed each time the same result. Microfilariae were detected in six samples by Knott's technique. Additional PCR analysis followed by sequencing for species identification revealed one dog from Hungary infected with *D. immitis*, one from Romania with *A. reconditum* and two with *D. repens* (Romania and Hungary). Sequencing further two microfilaraemic samples failed due to low DNA yield. DiroChek®-ELISA was positive in two microfilaraemic samples. Multiple infections were detectable by direct methods in 13 samples: *B. canis canis* and *B. gibsoni* (four times), *B. canis canis* and *B. canis vogeli* (three times), *B. canis canis* and *M. haemocanis* (three times), *H. canis* and *M. haemocanis* (once) and *B. canis canis* and *A. phagocytophilum* (once), one animal was triple positive for *B. canis canis*, *B. canis vogeli* and *M. haemocanis*. Only 8.0% of the imported dogs had antibodies against *B. canis* spp., *Leishmania* spp. (<0.1%) and/or *A. phagocytophilum* (9.4%). Twenty-nine dogs had borderline titres against *Leishmania* spp. (16) or *B. canis* (13). One dog had antibodies against *B. canis* and *Leishmania* spp. and one against *A. phagocytophilum* and *B. canis*.

Dogs submitted to Salvavet Veterinary Clinic in Bucharest

Overall detection rate for pathogens in dogs sampled at the Salvavet Veterinary Clinic was 49.5% by direct methods and 48.3% by indirect methods (Table 1). Six Giemsa-stained blood/buffy coat smears were positive for gamonts of large *Babesia* spp. Eight animals were positive in the *Babesia*-

Table 1 Results of 216 dogs from Romania and Hungary tested for arthropod-borne pathogens by direct and indirect methods

Method	No. positive/no. total (%; CI)							
	Romania				Hungary		Total	
	Dogs from clinic		Imported dogs		Imported dogs			
Indirect methods (IFAT)								
B. canis ssp.	12/29	41.4 (23.5–61.1)	6/109	5.5 (2.1–11.6)	9/78	11.5 (5.4–20.8)	27/216	12.5 (8.4–17.6)
Leishmania spp.	3/29	10.3 (2.2–27.4)	1/109	1.0 (<0.1–5.0)	0/78		4/216	1.9 (0.5–4.7)
A. phagocytophilum	5/29	17.2 (5.9–35.8)	4/92	4.4 (1.2–10.8)	12/78	15.4 (8.2–25.3)	21/199	10.6 (6.7–15.7)
E. canis	1/29	3.5 (0.9–17.8)	0/109		0/78		1/216	0.5 (<0.1–2.6)
Direct methods								
D. immitis-ELISA	1/29	3.5 (0.9–17.8)	0/109		2/78	2.6 (0.3–9.0)	3/216	1.4 (0.3–4.0)
Knott's test	nt		3/109	3.0 (0.6–7.8)	3/78	3.9 (0.8–10.8)	6/187	3.2 (1.2–6.9)
PCR								
B. canis canis	13/29	44.8 (26.5–64.3)	41/109	37.6 (28.5–47.2)	39/78	50.0 (38.5–61.5)	93/216	43.1 (36.4–50.0)
B. canis vogeli	0/29		4/109	3.7 (1.0–9.1)	1/78	1.3 (<0.1–7.0)	5/216	2.3 (0.8–5.3)
B. gibsoni	0/29		3/109	3.0 (0.6–7.8)	1/78	1.3 (<0.1–7.0)	4/216	1.9 (0.5–4.7)
B. felis-like	0/29		2/109	0.9 (<0.1–5.0)	0/78		2/216	0.9 (0.1–3.3)
H. canis	0/29		4/109	3.7 (1.0–9.1)	0/78		4/216	1.9 (0.5–4.7)
D. immitis	nt		0/109		1/78	1.3 (<0.1–7.0)	1/187	<0.1 (<0.1–2.9)
D. repens	nt		1/109	1.0 (<0.1–5.0)	1/78	1.3 (<0.1–7.0)	2/187	1.1 (0.1–3.8)
A. reconditum	nt		1/109	1.0 (<0.1–5.0)	0/78		1/187	<0.1 (<0.1–2.9)
Mycoplasma spp.	1/29	3.5 (0.9–17.8)	16/109	14.7 (8.6–22.7)	1/78	1.3 (<0.1–7.0)	18/216	8.3 (5.0–12.9)
Real-time PCR								
L. infantum	0/29		0/109		2/78	2.6 (0.3–9.0)	2/216	0.9 (0.1–3.3)
A. phagocytophilum	0/29		3/109	3.0 (0.6–7.8)	1/78	1.3 (<0.1–7.0)	4/216	1.9 (0.5–4.7)
E. canis	1/29	3.5 (0.9–17.8)	0/109		0/78		1/216	0.5 (<0.1–2.6)

CI 95% confidence interval, *nt* not tested, *IFAT* Indirect Immunofluorescent Antibody Test, *ELISA* enzyme-linked immunosorbent assay, *PCR* polymerase chain reaction

nested PCR for *B. canis canis* and all eight had detectable anti-*Babesia* spp. antibodies. Multiple infections were not detected by direct methods. Seven (24.0%) animals had antibody titres against two pathogens: three (10.3%) against *Leishmania* spp./*B. canis* spp., three (10.3%) against *A. phagocytophilum*/*B. canis* spp. and one (3.4%) against *E. canis*/*B. canis* spp. Three samples presented borderline titres against *Leishmania* spp. There was no statistical difference between animals treated and not treated against ectoparasites considering presence of infection by direct and/or indirect methods. However, the presence of ectoparasites as reported by pet owners and detection of pathogens by PCR correlated significantly ($p<0.05$).

Discussion

A total of 11 different protozoan, helminthic and bacterial pathogens with predominance of *Babesia* spp. were detected in 187 imported stray dogs from Romania and Hungary and 29 pet dogs from the Bucharest area in Romania.

B. canis canis

More than 40% of all dogs were tested positive for *B. canis canis* by PCR. *B. canis canis* has already been characterized by PCR in dogs from Hungary (Földvari et al. 2005; Furlanello et al. 2005). Large *Babesia* was only detected in Giemsa-stained blood/buffy coat smears from dogs submitted to the Salvavet Veterinary Clinic in Bucharest, Romania, as several of those dogs were presented due to acute signs of clinical disease. Gamonts of large *Babesia* spp. have also been previously detected in blood/buffy coat smears in Romanian dogs (Ilie et al. 2010). The vector tick *Dermacentor reticulatus* is a common tick species in these two countries with considerable vector potential (Teodorescu and Popa 2002; Földvari and Farkas 2005; Hornok 2009; Tudor et al. 2010). *D. reticulatus* has a focal distribution in Germany

and some areas in South-Western parts are considered endemic for *B. canis canis* (Gothe and Wegerdt 1991; Dautel et al. 2006; Heile et al. 2006; Barutzki et al. 2007). Imported dogs infected with *B. canis canis* could potentially act as reservoir hosts when introduced into non-endemic areas of Germany with a presence of the vector tick. Therefore, PCR screening of imported dogs from countries highly endemic for *B. canis canis* and treatment of PCR-positive animals should be recommended to avoid an introduction of the pathogen into non-endemic regions of Germany.

B. canis vogeli

Five dogs from Hungary and Romania were positive for the *B. canis vogeli*-DNA. In Europe, *B. canis vogeli* primarily occurs in the Mediterranean area, coinciding with distribution of its vector tick, *Rhipicephalus sanguineus* (Dantas-Torres 2008). This tick is present in Romania and in Hungary, although the latter finding on a Hungarian farm is supposed to be linked to the introduction of *R. sanguineus* ticks by animal transport from Croatia (Hornok and Farkas 2005; Tudor et al. 2010). While the presence of *R. sanguineus* in Romania supports the occurrence of *B. canis vogeli*, positive dogs from Hungary in this study have most likely attained an infection outside of Hungary prior to transfer to Germany.

Despite comparable detection rates of *B. canis* spp. (~40%) by PCR in stray dogs and dogs presented at the Salvavet Veterinary Clinic in Bucharest, seroprevalence in *B. canis*-IFAT did clearly differ. While only 12% of the imported dogs were seroreactive, more than 41% of the dogs presented at the veterinary clinic had detectable antibodies against *B. canis* spp. Based on these PCR results, we expected a higher seroprevalence in the examined imported dogs. Similar results have been published in dogs from Kosovo and Albania (Lazri et al. 2008; Hamel et al. 2009). The authors assumed that *B. canis canis* infections must have occurred very recently as seroconversion was not detectable (Lazri et al. 2008). A publication from Hungary, although applying a different cut-off for anti-*Babesia* antibodies, also reported a relatively low seroprevalence of anti-*B. canis* spp. antibodies in dogs; PCR was not applied (Hornok et al. 2006). The IFAT result therefore might evolve either from low avidity of antibodies to the applied *B. canis* antigen, or recent infection, although the animals were considered as healthy at the time of testing.

Our results in dogs from the Salvavet Veterinary Clinic clearly indicate a high incidence of infected vector ticks in the Bucharest area. In a recent study, a high infestation rate of dogs with *D. reticulatus* was reported, supporting the serological and molecular biological results on *B. canis canis* (Tudor et al. 2010)

B. gibsoni

Based on morphological criteria, infections attributed to "small" *Babesia* and *B. gibsoni* in dogs have previously been published in various areas of Europe, e.g. Italy, Spain, Hungary and Romania (Casapulla et al. 1998; Suarez et al. 2001; Farkas et al. 2004; Ilie et al. 2010). *B. gibsoni* has only recently been characterized by molecular techniques and may present an underdiagnosed or possibly emerging disease in dogs and in addition also requires a different treatment regime than *B. canis* spp. (Boozer and Macintire 2005; Garcia 2006). Furthermore, other "small" *Babesia* spp., *Theileria annae* or *Babesia microti*-like *Babesiae*, are increasingly diagnosed in blood smears and consecutive PCR examination in dogs from Southern Europe (Camacho et al. 2001; Beck et al. 2009; Dezdek et al. 2010; Simoes et al. 2011). Autochthonous *B. gibsoni* infections in dogs, mainly in American Pitbull Terriers and dogs used in dog fighting, have been confirmed by PCR in several European countries within the last 10 years, e.g. in Croatia, Germany, Italy and Spain (Suarez et al. 2001; Criado-Fornelio et al. 2003a; Garcia 2006; Hartelt et al. 2007; Beck et al. 2009; Trotta et al. 2009). In this study, we present the first molecular identification of *B. gibsoni* in dogs from Hungary and Romania, confirming previous reports on "small" *Babesia* based on morphological criteria (Farkas et al. 2004; Ilie et al. 2010). These findings stress the need for further research on *B. gibsoni* and other "small" *Babesia*: despite their increasing detection rate both in the New and in the Old World, in particular, the transmission and identification of vector tick species still need to be further elucidated (Solano-Gallego and Baneth 2011).

B. felis-like

BLASTn analysis of the *Babesia* spp. sequences derived from two imported Romanian dogs revealed 99% similarity to sequences deposited in GenBank as belonging to the species *B. felis* detected from domestic cats and lions in South Africa (GenBank accession nos. AF244912 and AY452702). The *18S rRNA* gene of piroplasma is highly conserved and small amplicons of this gene might hinder accurate species determination by sequencing due to high similarities amongst many species (Baneth et al. 2004). Therefore, it is difficult to interpret this result accurately and further investigations on the potential occurrence of further *Babesia* species in dogs in South-Eastern European countries will be necessary. *B. felis* has a major health impact in felids, especially in southern Africa, while only a few cases in cats have been reported elsewhere (Penzhorn et al. 2004). There is no information available on the occurrence of *B. felis* in canids. In contrast, there is evidence for the susceptibility

of cats to *B. canis* spp. infections (Criado-Fornelio et al. 2003b; Baneth et al. 2004).

Leishmania spp.

A total of six dogs from Romania and Hungary were positive for anti-*Leishmania* spp. antibodies (four) or for the DNA of *Leishmania* spp. (two). Despite the recent detection of vector-competent phlebotomine sandfly species in Hungary, screening of indigenous dogs and wild canids gave up to date no evidence for *Leishmania* infections (Farkas et al. 2011). Vector-competent *Plebotomus perfiliewi* have been identified in southern Romania but there are no current reports on authochthonous canine or human leishmaniosis available (Danesco 2008; Neghina et al. 2011). Although treatment does not eliminate *Leishmania* spp. in dogs, treatment regimes with allopurinol alone or in combination with meglumine antimony have been proven to successfully inhibit infection of sandflies due to absence or low presence of amastigotes in the canine host (Miró et al. 2011).

H. canis

Four blood samples from Romanian dogs were positive for *H. canis* by PCR and consecutive sequencing of the PCR product. However, gamonts of *Hepatozoon* spp. were not observed in the corresponding blood/buffy coat smears. As the vector tick *R. sanguineus* has been reported in Romania, the endemic occurrence of *H. canis* is highly likely (Tudor et al. 2010). In another study, 144 dogs from Timiş County in western Romania screened for vector-borne pathogens also did not reveal the presence of *H. canis* in blood smears (Ilie et al. 2010). This result might be attributed to the low diagnostic sensitivity of this technique (Karagenc et al. 2006). Imported dogs from Hungary were all tested negative for *H. canis*. Despite the single finding on a farm, the vector tick can up to date be considered as absent in Hungary (Hornok and Farkas 2005). *H. canis* has also been identified in dogs and foxes in other countries, e.g. Albania and Croatia and was supposed to be introduced by wild canids, e.g. foxes or golden jackals (*Canis aureus*), into Slovakia (Majlathova et al. 2007; Lazri et al. 2008; Hamel et al. 2009; Vojta et al. 2009). Golden jackals occur widely in Romania, Serbia and Bulgaria and have expanded their range into Central Europe recently (Nikolova et al. 2001; Blaga et al. 2008; Zachos et al. 2009). Further spreading of vector-borne canine pathogens associated with golden jackals cannot be ruled out in the future.

Filarial infections

A total of seven dogs were positive for filarial infections by Knott's test, DiroChek®-ELISA and PCR. Three different filarial nematode species, *D. immitis*, *D. repens* and *A. reconditum*, were identified from six microfilaraemic dogs in this study. Our results indicate low infection rates when compared to results from Italy with more than 20% of tested dogs positive for filarial infections (Magi et al. 2011). Canine filarial infections occur worldwide in temperate and tropical regions, whereas *D. immitis* and *D. repens* are the predominant species in Europe (Simon et al. 2009). Infections with *D. immitis*, *D. repens* and *A. reconditum* in dogs imported into Germany have been published previously (Glaser and Gothe 1998; Zahler et al. 1997). Single cases of heartworm disease and several cases of canine cutaneous filariosis have been described previously in dogs and a ferret in Hungary and in indigenous Romanian dogs (Olteanu 1996; Coman et al. 2007; Ilie et al. 2009; Jacsó et al. 2009; Tudor et al. 2009; Ciocan et al. 2010a, b; Fernoagă et al. 2010; Fok et al. 2010; Molnar et al. 2010). *A. reconditum* has until now not been reported from dogs from Hungary and Romania, but has been detected in 4 out of 193 dogs in the neighbouring Vojvodina Province in Serbia (Tasić et al. 2008). Due to the long incubation period and the fact that animals in this study have only been tested once, data presented here are preliminary and require further confirmation.

E. canis

Only one dog from Romania was positive for antibodies against *E. canis*. As the vector tick is present in Romania, further research might reveal a wider occurrence of *E. canis* in this country (Tudor et al. 2010). This infection is more common in close-by Balkan countries and prevalence rates of 50% have been detected by IFAT and 17% by PCR in Albania (Hamel et al. 2009).

A. phagocytophilum

A. phagocytophilum is a widespread tick-borne infection with *Ixodes ricinus* as main vector in Europe (Woldehiwet 2010). Several dogs (10.6%) were seroreactive to *A. phagocytophilum* antigen in IFAT while only four dogs were positive by PCR (1.9%). Despite the ubiquitous occurrence of this tick in Europe, prevalence rates in dogs vary considerably between different countries, e.g. studies from Germany report around 6% of the dogs positive by PCR (Jensen et al. 2007b; Kohn et al. 2010) and only up to 1% in Poland (Skotarzack et al. 2004; Welc-Faleciak et al. 2009). Recently, *A. phagocytophilum*-DNA has also been detected by PCR with a minimum prevalence of 1.3% in ticks removed from dogs and foxes in Hungary (Sreter et al. 2004). This prevalence is similar to that (2.9%) reported in *I. ricinus* collected in southern

Germany, although peak prevalence in city parks may reach around 10% (Silaghi et al. 2008)

M. haemocanis

Seventeen dogs from Romania and one from Hungary were tested positive for *M. haemocanis*-DNA by PCR. *M. haemocanis* is transmitted by the brown dog tick *R. sanguineus* and is often found in immunocompromised and splenectomized dogs (Seneviratna et al. 1973; Kemming et al. 2004; Willi et al. 2010). To our knowledge, this is the first report on this agent in dogs from Romania and Hungary. As the brown dog tick is de facto not present in Hungary, the transmission or possible import of this dog into Hungary remains an open question (Hornok and Farkas 2005).

Conclusion

The results of our investigations suggest the need for a routine laboratory screening for vector-borne canine blood infections, particularly in dogs imported from South-Eastern European countries into Germany or other Central and Western European countries. Apart from standard serological methods, more advanced molecular biological techniques should be applied to detect new, hitherto unknown pathogens in these canine populations. Results of such studies should be communicated to veterinarians, dog owners and animal welfare organisations in order to create awareness of impending health risks. Furthermore, prophylactic treatment with recommended parasiticides should be employed on dogs when brought to countries with high incidence of vectors. More research on existing and potential arthropod vectors and their distribution as well as improved identification of arthropod-borne pathogens is deemed necessary in view of increased tourism along with Europe—wide pet trade and imports.

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Conflict of interest The authors declare that they have no conflict of interest.

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