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Detection of *Babesia canis* subspecies and other arthropod-borne diseases in dogs from Tirana, Albania

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Nachweis von *Babesia canis* Subspezies und anderen Arthropoden-übertragenen Erkrankungen in Hunden aus Tirana, Albanien

Zusammenfassung. Durch Arthropoden übertragene Infektionen haben in der jüngeren Vergangenheit zunehmend an Bedeutung gewonnen, auch bedingt durch vermehrte Reisen in, beziehungsweise Importe von Hunden aus Regionen, in denen die Erreger endemisch sind. Während die epidemiologische Situation im westlichen Mittelmeerraum gut dokumentiert ist, sind aus Osteuropa und dem Balkan vergleichsweise wenige Informationen verfügbar. In der vorliegenden Studie wurden Blutproben von 30 klinisch unauffälligen Hunden aus den Randgebieten von Tirana, Albanien, auf vektor-übertragene Infektionen untersucht. Mittels direkter und/oder indirekter Verfahren wurden die Blutproben auf *Babesia canis*, *Hepatozoon* spp., *Leishmania* spp., *Dirofilaria* spp., *Ehrlichia canis*, *Anaplasma phagocytophilum*, *Bartonella* spp. und *Rickettsia* spp. untersucht. Im Blut von 20 Hunden (=67%) wurden Antikörper bzw. Erreger durch Arthropoden übertragene Infektionen nachgewiesen. Antikörper gegen *B. canis, E. canis* und/oder *A. phagocytophilum* waren im Serum von 19 Hunden (=63%) nachweisbar. Bei 13 Hunden (=43%) erfolgte ein Erregernachweis mittels Blutausstrich, PCR oder ELISA, wobei *B. canis canis*, *B. canis vogeli, Hepatozoon* spp., *D. immitis* und/oder *E. canis* identifiziert wurden. Infektionen mit *Leishmania* spp., *Bartonella* spp. und *Rickettsia* spp. waren nicht nachweisbar.

Summary. The importance of arthropod-borne diseases increased in the recent past in particular due to frequent travel with dogs in or by importing of dogs from regions with endemic occurrence of these diseases. While the epidemiological situation is well known for the western parts of the Mediterranean, only limited data is available for Eastern Europe and the Balkans. Thirty clinically healthy dogs from suburban areas of Tirana, Albania, were tested for *Babesia canis, Hepatozoon* spp., *Leishmania* spp., *Dirofilaria* spp., *Ehrlichia canis*, *Anaplasma phagocytophilum*, *Bartonella* spp. and *Rickettsia* spp. using direct and indirect methods.

Antibodies against and/or pathogens of arthropod-borne diseases were detected in the blood of 20 (67%) dogs. Nineteen dogs (63%) had antibodies against *B. canis*, *E. canis* and/or *A. phagocytophilum. Babesia c. canis, Babesia c. vogeli, Hepatozoon* spp., *D. immitis* and/or *E. canis* were identified by blood smear, PCR or ELISA in 13 (43%) dogs. There was no evidence for *Leishmania* spp., *Bartonella* spp. and *Rickettsia* spp. infections.

Key words: Dog, arthropod-borne disease, parasitic infection, Albania.

Introduction

The diagnosis, treatment and prophylaxis of canine vector-borne disease are of considerable interest in the small animal practice [1, 2]. Pet owners take their companion animals along with them on vacation to the Mediterranean countries or animals are transferred from this region to Western Europe [3]. Apart from countries like Portugal, Spain, Italy or Greece, countries in Eastern Europe including the Balkans came only recently into the focus of animal welfare organisations. Dogs from this origin are at risk of carrying a variety of vector-borne diseases such as babesiosis, hepatozoonosis, leishmaniosis, ehrlichiosis, heartworm infection and cutaneous dirofilariosis. While the epidemiological situation of canine vector-borne diseases is well known for the south-western countries of the Mediterranean, only limited information is available for most parts of Eastern Europe and the Balkans [4, 5].

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Table 1. Methods applied for detection of pathogens or antibodies in 30 dogs from Tirana, Albania

Pathogen	Technique	References
Babesia canis	IFAT (>1:32)[1], blood smear, PCR, sequencing	PCR: [7, 8]
Hepatozoon spp.	Blood smear	
L. infantum	IFAT (>1:32)[1], real-time PCR	real-time PCR: [9]
Dirofilaria spp.	Knott-Test, DiroChek®-ELISA	
E. canis	IFAT (>1:40)[1], blood smear, real-time PCR	real-time PCR: [10]
A. phagocytophilum	IFAT (>1:32)[1], blood smear, real-time PCR	real-time PCR: [11]
Bartonella spp.	PCR	PCR: modified [12]
Rickettsia spp.	PCR	PCR: [13]

[1] Titre considered positive.

In addition to an epidemiological survey from the Kosovo and Albania [6], the purpose of this study was to identify vector-borne infections in dogs from Tirana, Albania and thus to contribute to the knowledge on the epidemiology of those diseases.

Materials and methods

EDTA-blood and serum samples from 30 semi-domesticated dogs from suburban areas from Tirana, Albania, were collected in September 2008. All animals were physically examined prior to blood sampling and considered clinically healthy. The EDTA-blood and serum samples were transferred to the laboratory of the Chair of Comparative Tropical Medicine and Parasitology, Ludwig-Maximilians-University, Munich, Germany, within 24 hours and further processed.

The samples were analyzed by direct and indirect methods for the following pathogens: *Babesia* (*B.*) spp., *Hepatozoon* spp., *Leishmania* (*L.*) *infantum*, *Dirofilaria* (*D.*) spp., *Ehrlichia* (*E.*) *canis*, *Anaplasma* (*A.*) *phagocytophilum*, *Rickettsia* spp. and *Bartonella* spp. (Table 1).

The IFAT test kits approved for the dog were for commercial use (*B. canis, E. canis*: Megacor Diagnostik GmbH, Hörbranz, Austria; *A. phagocytophilum*: Focus Technologies, California, USA); for anti-*L. infantum* antibody detection an in-house IFAT was applied. A Giemsa-stained blood smear from each dog was microscopically examined for pathogens in the blood cells and the Knott-test was used to detect microfilariae in 29 dogs. The DiroChek® Canine/Feline Antigen Test Kit (Synbiotics Corp., San Diego, USA) was employed for the detection of adult heartworm antigen.

DNA was extracted using a commercial kit according to the manufacture's instructions (QIAamp DNA MiniKit, Qiagen, Hilden, Germany). Quality and quantity of the extracted DNA were checked with a spectrophotometer (NanoDrop 1000, Peqlab, Erlangen, Germany).

Real-time PCRs were carried out on a BioRad iCycler iQ (Bio-Rad, Munich, Germany), conventional PCRs on an Applied Biosystems GeneAmp 9700 (Applied Biosystems, Darmstadt, Germany). For PCRs and real-time PCRs the HotStart Taq Polymerase Kit (Qiagen, Hilden, Germany) was used and for the *Rickettsia* spp. PCR, the Expand High-Fidelity Plus Taq Polymerase Kit (Roche, Mannheim, Germany). The products of conventional PCRs were examined under UV-light, after 2% agarose gel electrophoresis and staining with GelRed (Biotium, Hayward, USA). Positive *B. canis* PCR products were purified with QIAquick PCR Purification Kit according to the manufacturer's instruction (Qiagen, Hilden, Germany), the fragments of the *18S rRNA* gene were sequenced (Eurofins, Martinsried, Germany). The results were evaluated with Chromas©Lite (www.technelysium.com.au), sequence homology searches were made by BLASTn analysis of GenBank (www.ncbi.nlm.nih.gov) and multiple alignments performed with ClustalW (www.ebi.ac.uk). The graphical view of the alignment was done with the programme BioEdit (www.mbio.ncsu.edu).

Results

Antibodies and/or pathogens were detected in 20 of 30 dogs. Nineteen dogs (63%) had antibodies against *B. canis*, *E. canis* and/or *A. phagocytophilum*. *Babesia c. canis, Babesia c. vogeli, Hepatozoon* spp., *D. immitis* and/or *E. canis* were identified by blood smear, PCR or ELISA in 13 dogs (43%). There was no evidence for *Leishmania* spp., *Bartonella* spp. and *Rickettsia* spp. infections (Table 2).

No *Babesia* spp. were found in Giemsa stained blood smears but *B. canis* DNA products were recovered in the blood of seven dogs. The seven positive *B. canis* PCR products (Table 2) were sequenced. Alignment of the sequences showed that there were two different sequence types which were 100% identical amongst each other. BLASTn analysis of GenBank revealed that they were >99.9% homologue to previously deposited *18S rRNA* gene sequences of *B. c. vogeli* (accession nos. AM183216 and AY371197) in three cases and with *B. c. canis* (accession nos. EU711060, EU622739 and FJ209024) in four

Table 2. Pathogen and antibody detection in 30 dogs from Tirana, Albania

Pathogen	Method of analysis	
	Serology[1]	Direct pathogen detection
Babesia canis	13% (4/30)	23% (7/30)
Hepatozoon spp.	n.a.[2]	17% (5/30)[3]
L. infantum	0% (0/30)	0% (0/30)
Dirofilaria immitis	n.a.	3% (1/30) and 0% (0/29)[4]
E. canis	50% (15/30)	17% (5/30)
A. phagocytophilum	40% (12/30)	0% (0/30)
Rickettsia spp.	n.a.	0% (0/30)
Bartonella spp.	n.a.	0% (0/30)

[1] in case of positive titres on IFAT.
[2] n.a. not analysed.
[3] Detection of gamonts in Giemsa-stained blood smear.
[4] Knott test was negative for all but one animal.

cases. In the amplified 290/291 base pair sequence, *B. c. vogeli* and *B. c. canis* differ in 19 nucleotide positions from each other. The consensus sequences from this study were deposited in GenBank under accession nos. FJ790245 for *B. canis canis* and FJ790246 for *B. canis vogeli*.

Microfilariae were not detected in any dog with the Knott-test. Circulating antigen of *D. immitis* was demonstrated in the serum of one dog; unfortunately, coagulation of the corresponding EDTA-blood sample did not allow testing that dog for microfilariae with the Knott-test.

No *Ehrlichia*/*Anaplasma* morulae were demonstrated in the Giemsa stained blood smears.

A total of five animals carried mixed infections with detectable pathogens and eight animals were diagnosed with a mono-infection.

Discussion

Reports available from the past mentioned only the presence of leishmaniosis in dogs in Albania in relation to cases in humans [14–17]. Only recently, some vector-transmitted bacterial agents which may also infect dogs were detected in ticks [18] and *B. canis*, *Hepatozoon canis*, *D. immitis* and *D. repens* were recorded in Albanian dogs [6].

Vector-borne diseases are a growing field of veterinary and public interest as some of the pathogens are known to cause zoonotic diseases. The most frequently diagnosed parasitic diseases of dogs imported from the Mediterranean into central European countries are babesiosis, leishmaniosis, ehrlichiosis and dirofilariosis [19].

In Europe, canine babesiosis is caused by two *B. canis* subspecies; *B. c. canis*, transmitted by *Dermacentor* (*D.*) *reticulatus* ticks and *B. c. vogeli* transmitted by *Rhipicephalus* (*R.*) *sanguineus* ticks. In the present study antibodies against *B. canis* were seen in 13% of the dogs; a similar sero-prevalence (9.9%) was reported recently in Albanian dogs [6]. The targeted *B. canis* spp. 18S rRNA gene sequence was detected in 23% of the dogs and sequencing revealed both, the presence of *B. c. canis* and *B. c. vogeli* in this study. Interestingly, *R. sanguineus*, the vector for *B. c. vogeli*, occurs frequently on the Balkans and has been recorded also in dogs from Albania [20–22]. However, *D. reticulatus*, the vector for *B. c. canis*, was not reported in Albanian tick surveys conducted in the past [20–22] but it has been observed recently parasitizing dogs in Bosnia and Herzegovina and Serbia [23, 24]. Reports from Croatia [8] and Slovenia [25] proved prevalence of *B. c. canis* on the Balkans. The implications of these findings should generate further investigations on the epidemiology on canine babesioses and their vectors in Albania.

Canine hepatozoonosis is caused by *H. canis* in Asia, Africa and southern Europe [26]. There are single case reports from Bulgaria [27, 28] and Greece [29, 30]. Compared to work done earlier in Albania, which revealed positive PCR products in 52.8% of the dogs [6], in the present study only 17% of the dogs harboured gamonts in the blood smears.

Leishmania infantum leishmaniosis is considered an endemic zoonosis in many countries of the Mediterranean [31]. There are several reports of visceral leishmaniosis in man from Albania and the growing number of human cases in Tirana hospital indicates a spread for the last decades and a rising risk of infection [6, 14, 16, 17, 32, 33]. Although there was no evidence of *Leishmania* spp. infections in the dogs of this study, previous analysis indicated sero-prevalences of up to 15.8% in dogs from Albania [6, 15, 16]. Therefore, further investigation will be necessary to elucidate a more complete picture of canine *Leishmania* epidemiology in Albania.

Worldwide, *Dirofilaria* spp. transmitted by *Culicidae*, are known to cause diseases affecting the subcutaneous tissue (*D. repens*) or compromising the cardio-vascular system (*D. immitis*). Reports from Greece indicated *D. immitis* antigen in the blood from 4.8 to 13% of the dogs [29, 34]. The results in this study based on the DiroCheck® ELISA indicated a lower rate of *D. immitis* infections in dogs from Tirana area (3%). This supports recent data from different Albanian cities where 5.3% of the dogs were positive for *D. immitis* antigen [6]. Whereas no *D. repens* was found in the present investigation, recently *D. repens* prevalence was reported as of 11.5% [6].

An important bacterial vector-borne agent in dogs is *E. canis*, causing monocytic ehrlichiosis [35]. In accordance to data from Greece [12, 36], *E. canis* was a widespread pathogen in Albanian dogs, too.

Rickettsia spp. and *Bartonella* spp. were not detected in the present study in Albanian dogs but there is evidence for infections with these agents in dogs from Greece [36, 37].

It is concluded, that additional, systematic investigations from different regions of Albania are necessary to establish a more precise picture of the epidemiological situation and seasonal aspects of arthropod-borne infections in dogs, the arthropod vectors and possible zoonotic risks.

Conflict of interest

The authors declare they have no conflict of interest.

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