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Parasites and vector-borne pathogens in client-owned dogs in Albania. Blood pathogens and seroprevalences of parasitic and other infectious agents

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Abstract Knowledge on the epidemiology of parasitic and vector-borne infections is still very limited for Albania, a country located in the Balkan Peninsula in southeast Europe. Recent publications indicated prevalence rates of up to 52 % for vector-borne infections in less-cared dogs in Albania. To provide data on the epidemiological situation in dogs under veterinary care, a total of 602 client-owned dogs presented to four small animal clinics between March 2010 and April 2011 in Tirana, Albania, were screened by examination of Giemsa-stained blood smears, PCR, and serological methods for the presence of arthropod-borne infections, as well as *Neospora caninum* and *Toxoplasma gondii*. Eight different pathogens, namely *Babesia vogeli*, *Hepatozoon canis*, *Leishmania infantum*, *Dirofilaria immitis*, *Anaplasma phagocytophilum*, *Anaplasma platys*, *Ehrlichia canis*, and *Mycoplasma haemocanis*, were detected by direct methods with prevalence rates ranging from 1 to 9 %. Seroprevalence for *Babesia* spp., *L. infantum*, *Anaplasma* spp., and *E. canis* were 6.6, 5.1, 24.1, and 20.8 %, respectively. Dogs >1 year of age were positive for vector-borne infections significantly more often than younger dogs (*p*=0.003). More than half (51.7 %) of the dogs were seroreactive to *T. gondii* and 18.3 % to *N. caninum*. This is the first report on the detection of *A. phagocytophilum*, *A. platys*, *E. canis*, and *M. haemocanis* by PCR as well as the serological confirmation of exposure of dogs to *N. caninum* and *T. gondii* in Albania. The spectrum of pathogens and the seroprevalences for *N. caninum* and *T. gondii* in client-owned dogs from Tirana, Albania, are comparable to that reported in other countries in the Mediterranean Basin. The prevalence rates of vector-borne pathogens are at the lower range of that reported in studies from this geographical region. This is probably due to increased awareness of the owners of pet dogs, including better husbandry conditions and ectoparasiticidal treatment, thus limiting exposure of dogs to vectors.

Keywords Canine vector-borne infections · Albania · *Babesia* · *Leishmania* · *Anaplasma* · *Toxoplasma* · *Neospora*

Introduction

Knowledge on the epidemiology of parasitic infections in companion animals in general and vector-borne infections in particular is still very limited for the formerly remote and politically isolated country of Albania. A more comprehensive picture on parasitic (e.g., gastrointestinal helminths, lung-worms, and ectoparasites) infections in dogs and cats including vector-borne pathogens (e.g., *Babesia canis*, *Leishmania infantum*, *Dirofilaria immitis*, and *Ehrlichia canis*) has been established only very recently (Cicko and Cani 1998; Cicko et al. 1999; Dhamo et al. 2006; Lazri et al. 2008; Hamel et al. 2009; Xhaxhiu et al. 2009, 2011; Rapti and Rehbein 2010; Knaus et al. 2011a, b, 2012, 2014; Silaghi et al. 2012, 2013, 2014; Bizhga et al. 2013; Sommer et al. 2015). Previous studies mostly in less well-cared dogs reported on the presence of *Babesia* spp., *Hepatozoon canis*, filarial infections, as well as

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E. canis in PCR, serological and/or microscopical examinations (Lazri et al. 2008; Hamel et al. 2009). In order to corroborate previously published findings and to provide updated information on parasitic and vector-borne infections in dogs, a survey was conducted from March 2010 to April 2011 to assess the status of client-owned dogs receiving veterinary care presented to four small animal clinics in Tirana, Albania. This paper presents the results of the microscopical, serological, and molecular biological screening of blood smears, serum, and whole blood samples for vector-borne infections, as well as from a serosurvey on *Neospora caninum* and *Toxoplasma gondii*. Results of the examination and identification of parasitic stages in fecal samples and ectoparasites collected from these dogs are reported elsewhere (Shukullari et al. 2015; Shukullari et al. Parasites and vector-borne diseases in client-owned dogs in Albania. Infestation with arthropod ectoparasites, in preperation).

Materials and methods

Sample collection and animal data

At total of 602 dogs presented between March 2010 and April 2011 in Tirana, Albania, to four small animal clinics were sampled with the informed consent of their owners. Whole blood (EDTA anticoagulant) and serum samples were obtained from all animals. Blood smears were prepared, air-dried, and stored at room temperature until further processing. Blood and serum samples were stored at −18 °C. Basic demographic data of the sampled animals with the parameters of interest is presented in Table 1. Additional information retrieved from a questionnaire answered by the owners of each dog is given elsewhere (Shukullari et al. Parasites and vector-borne diseases in client-owned dogs in Albania. Infestation with arthropod ectoparasites, in preperation).

Laboratory diagnostics

Blood smears were Giemsa-stained and examined with a light microscope at 500-fold magnification for pathogens in the blood. Whole blood samples were screened by PCR for DNA of the following pathogens: *Babesia* spp., *L. infantum*, *E. canis*, *Anaplasma phagocytophilum*, *Anaplasma platys*, and *Mycoplasma* spp. (Table 2). DNA extraction was performed from 200 μl blood with the QIAamp DNA MiniKit (Qiagen, Hilden, Germany) following the manufacturer's instructions. Quality and quantity of extracted DNA were checked with the spectrophotometer NanoDrop ND-1000 (Peqlab, Erlangen, Germany). Conventional PCR was used for the detection of *Babesia* spp. and *Mycoplasma* spp. DNA (Watanabe et al. 2003; Casati et al. 2006). Species identification was performed on the species-specific length of the PCR products. Primers and PCR conditions used are

Table 1 Basic data of 602 dogs sampled for testing

Factor	Number	Percent
Breed		
Pure breed dogs	515	85.6
Mixed breed dogs	87	14.4
Age		
≤1 year	138	22.9
>1 year	464	77.1
Sex[a]		
Male	334	55.5
Female	268	44.5
Habitat		
City	297	49.3
Suburban	200	33.2
Rural	105	17.4
Feed		
Cooked/canned/dry	479	79.5
Raw	22	3.7
Both	101	16.8

[a] Includes intact and neutered animals, by sex

summarized in Table 2. The HotMaster Taq DNA Polymerase Kit (5PRIME, Darmstadt, Germany) was used. Real-time PCR was used for the detection of *L. infantum*, *A. phagocytophilum*, *A. platys*, and *E. canis* DNA (Courtney et al. 2004; Mary et al. 2004; Teglas et al. 2005; Silaghi et al. 2011a; Ionita et al. 2013). Real-time PCR was carried out in an AB7500 (Applied Biosystems, Darmstadt, Germany) using the TaqMan® Gene Expression MasterMix (Applied Biosystems, Darmstadt, Germany), according to the manufacturer's instructions and with primers and probes under conditions listed in Table 2. Positive and negative controls were included in each PCR run. An 2 % agarose-gel electrophoresis of amplification products of conventional PCRs was performed, and products were visualized under UV light with GelRed™ (Biotium, Hayward, USA). PCR products of the partial *Babesia* spp. *18srRNA* gene were purified (QIAquick PCR Purification Kit, Qiagen, Hilden, Germany), and sequenced to Eurofins MWG Operon (Martinsried, Germany), and obtained sequences were analyzed as described (Silaghi et al. 2011b).

The DiroChek® Canine/Feline Antigen Test Kit (Synbiotics Corp., San Diego, USA) was used for screening for the circulating antigens of female *D. immitis*. Antibodies to *Babesia* spp., *L. infantum*, *Anaplasma* spp., *E. canis*, *N. caninum* and *T. gondii* were detected using commercial IFA-testkits (MegaCor, Hörbranz, Austria) and an in-house *L. infantum*-IFAT (cf. Mancianti et al. 1995). Antibody titers ≥1:40 to *E. canis*, ≥1:50 to *N. caninum* and *T. gondii*, and ≥1:64 to *Babesia* spp., *L. infantum*, and *Anaplasma* spp. were considered as seropositive. No endpoint titers were assessed in this survey.

Table 2 Summary of PCR and real-time PCR methods for specific pathogen detection used in this study on client-owned dogs from Albania

Target	Primers 5'–3'	Cycle conditions	Positive control	Reference
Conventional PCR methods				
Babesia spp. *18s rRNA* gene	BJ1: GTCTTGTAATTGGAATGATGG BN2: TAGTTTATGGTTAGGACTACG	95 °C, 5 min 40 cycles: 94 °C, 30 s; 54 °C, 30 s; 72 °C, 40 s; 72 °C, 5 min	Genomic *B. divergens* DNA from bovine EDTA-blood 1:100 dilution	Casati et al. 2006
Mycoplasma spp. *16S rRNA* gene	OHOK1_for: ATGCCCCTCTGTGGGGGATAGCCG CaB2_for: CTGGGAAACTAGAGCTTCGCGAGC OOCBr1_rev: ATGGTATTGCTCCATCAG ACTTTCG	94 °C, 2 min 35 cycles: 94 °C, 45 s; 58 °C, 45 s; 72 °C, 45 s; 72 °C, 5 min	pEX-A-*Mycoplasma haemominutum* 1:256,000,000 dilution pEX-A-*Mycoplasma haemofelis* 1:2048000000 dilution1	Watanabe et al. 2003
Real-time PCR methods				
Leishmania infantum Kinetoplast DNA	Lsh-kF: CTTTTCTGGTCCTCCGGGTAGG Lsh-kR: CCACCCGGCCCTATTTTACACCAA Lsh-kp: FAM-TTTTCGCAGAACGCCCCTACCC GC-BHQ1	95 °C, 20 s 40 cycles: 95 °C, 30 s; 60 °C, 30 s	*L. infantum* DNA from cell culture 1:1000 dilution	Mary et al. 2004
Anaplasma platys 16S rRNA gene	EPlat-19f: CGGATTTTTGTCGTAGCTTGCTAT EPlat-117r: CCATTTCTAGTGG CTATCCCATACTACT EPlat-55p-S1:FAM-TGGCAGACGGGTGAGTAAT GCATAGGA-BHQ1	95 °C, 20 s 40 cycles: 95 °C, 30 s; 60 °C, 30 s	Genomic *A. platys* DNA from canine EDTA blood 1:10 dilution	Teglas et al. 2005
Anaplasma phagocytophilum Msp2 gene	ApMSP2f: ATGGAAGGTAGTGTTGGTTATGGTATT ApMSP2r: TTGGTCTTGAAGCGCTCGTA ApMSP2p-FAM: TGGTGCCAGGGTTGAGCTT GAGATTG-TAMRA	50 °C, 5 min 95 °C, 10 min 40 cycles: 95 °C, 15 s; 60 °C, 1 min	*A. phagocytophilum* DNA from dog	Courtney et al. 2004; Silaghi et al. 2011a
Ehrlichia canis p30-10 gene	Ecanisp30-ANYF: TGGATACTACCATGGCGTT ATTGG E.canisp30-ANYR: GAGGAGCATCATTAATACTA CAGGAGTT E.canisp30-ANYM2: FAM-CAGGTATCTTCTCA AATTT-NFQ	50 °C, 2 min 95 °C, 10 min 40 cycles:95 °C, 15 s; 60 °C, 1 min	*E. canis* plasmid-DNA pEC-3.3 1:1000 dilution[a]	Ionita et al. 2013

[a] Eurofins MWG Operon, Ebersberg, Germany

Statistical analysis

The statistical analysis including calculation of the 95 % Clopper-Pearson confidence intervals for prevalence was performed with software package R version 2.13.1 (R Development Core Team 2010). Associations between presence of/ exposure to pathogens and dog management factors or age (≤1 and >1 year) of the animals were also analyzed. Factors with *p* values of less than 0.2 and cofounders (sex, age, season of sampling [winter, spring, summer, fall]), use (pet dog, hunting dog, working dog), habitat [city, suburban, rural], husbandry [indoors, kennel, yard], feed [raw, cooked/dry, both]) were forced into a multiple linear regression model to evaluate the adjusted effects of the associated factors. Level of significance for all analyses was set at $p < 0.05$.

Results

A total of 151 out of 602 dogs (25.1 %) were positive for vector-borne pathogens by direct methods (Giemsa-stained blood smear, PCR) including samples analyzed using the DiroChek®-ELISA and 237 dogs (39.3 %) by indirect methods (IFATs for detection of antibodies against *Babesia* spp., *L. infantum*, *E. canis*, and *Anaplasma* spp.). Furthermore, 311 (51.7 %) samples were seropositive for *T. gondii* and 110 (18.3 %) for *N. caninum*. Combining all IFAT results 390 (64.8 %) of all dogs were seropositive. Seven different vector-borne pathogens, namely *Babesia vogeli*, *Hepatozoon canis*, *L. infantum*, *D. immitis*, *E. canis*, *A. phagocytophilum*, *A. platys*, and *M. haemocanis* were identified by direct methods and in the DiroChek®-ELISA. Six (1.0 %) dogs were demonstrated to be positive for *H. canis* gamonts, three for microfilariae (0.5 %), and one for large *Babesia* (0.2 %) in the Giemsa-stained blood smears. The DiroChek®-ELISA was positive for *D. immitis* antigen in 13 dogs (2.2 %), and one of the seropositive dogs was also microfilaraemic in the Giemsa-stained blood smear. Serum samples were seropositive (IFAT) for *Babesia* spp. (6.6 %), *L. infantum* (5.1 %), *E. canis* (20.8 %), and *Anaplasma* spp. (24.1 %). Results from microscopical, PCR, and serological testing are summarized in Table 3.

Single infections as well as double and triple mixed infections of vector-borne agents were detected in 118 (19.6 %, 95 % CI 16.5–23.0), 30 (5.0 %, 95 % CI 3.4–7.0), and 3 (0.5 %, 95 % CI 0.12–1.6), respectively, of the dogs by direct methods including DiroChek®-ELISA (Table 4). By IFAT (*Babesia* spp., *L. infantum*, *E. canis*, *Anaplasma* spp.), 86 (14.3 %, 95 % CI 11.6–17.3) dogs were seropositive to two pathogens, 18 (3.0 %, 95 % CI 1.8–4.7) to three pathogens, and 4 (0.7 %, 95 % CI 0.2–1.7) animals to four pathogens (Table 5).

Multivariate logistic regression models for risk factors for the presence of or exposure to pathogens showed that increasing age of the dogs was the greatest risk factor (Table 6).

Discussion

The overall spectrum of pathogens detected in the present study is similar to the findings of previous reports on the occurrence of some important vector-transmitted pathogens in ticks and in Albanian dogs (Adhami and Murati 1977; Cicko and Cani 1999; Cicko et al. 1999; Christova et al. 2003; Dhamo et al. 2006; Lazri et al. 2008; Hamel et al. 2009; Rapti and Rehbein 2010; Xhaxhiu et al. 2011; Bizhga et al. 2013). To the authors' knowledge, this study presents the first record of detection of *E. canis*, *A. phagocytophilum*, *A. platys*, and *M. haemocanis* by PCR and of antibodies against *T. gondii* and *N. caninum* in dogs from Albania.

Babesia spp.

Whole blood samples of two dogs harbored DNA of *B. vogeli*. In previous studies, babesias have been detected microscopically in blood smears from dogs in Albania and neighboring Greece (Diakou 2000; Dhamo et al. 2006). *B. vogeli* has been identified by PCR in dogs from Albania as well as in dogs from close-by Croatia and Slovenia for instance (Cacciò et al. 2002; Duh et al. 2004; Beck et al. 2009; Hamel et al. 2009). In contrast to the latter studies, neither *B. canis* nor *B. vulpes* sp. nov (syn. *Theileria annae*; Baneth et al. 2015) and *B. gibsoni* were detected, although these species are present on the Balkan Peninsula (Duh et al. 2004; Beck et al. 2009). The seroprevalence of anti-*Babesia* antibodies in 6.6 % of the client-owned dogs is within the range of approximately 2 to 10 % previously reported in dogs from Albania, Kosovo, and neighboring Greece (Jensen et al. 2003; Lazri et al. 2008; Hamel et al. 2009). Similar to a previous study from Spain, no risk factors for canine *Babesia* infection were identified (Solano-Gallego et al. 2006). In contrast, Adaszek et al. (2011) identified young age and originating in rural areas as risk factors in Poland. Similarly, use as hunting dog was identified as a risk factor in a study from Romania (Imre et al. 2013). However, the *Babesia* species in these studies was *B. canis*.

Leishmania infantum

Canine leishmaniosisis is a widespread infection in the Mediterranean region, and canids play a major role in the epidemiology of this disease as they act as primary reservoir hosts for human disease (Solano-Gallego et al. 2011). The prevalence rates of 5.1 % by serology and 4.7 % by PCR found in this study are lower than those determined in previous

Table 3 Results on the testing of Giemsa-stained blood smears, EDTA-blood (PCR), and serum (IFAT, ELISA) samples of 602 dogs from Albania

Method		Prevalence		
		Total	%	95 % CI[a]
Giemsa-stained blood smear				
	Microfilariae	3	0.5	0.12–1.6
	Hepatozoon spp.	6	1	0.4–2.3
	Babesia spp.	1	0.2	0.008–1.1
PCR				
Conventional	*B. vogeli*[b]	2	0.3	0.06–1.3
	M. haemocanis	53	8.8	6.7–11.4
Real-time	*Leishmania infantum*	28	4.7	3.2–6.7
	A. phagocytophilum	6	1	0.4–2.3
	A. platys	20	3.3	2.1–5.2
	E. canis	57	9.5	7.3–12.2
Serology				
	D. immitis (DiroChek®-ELISA)	13	2.2	1.2–3.8
	Babesia spp.-IFAT	40 (61)[c]	6.6 (10.1)	4.8–9.0 (7.9–12.9)
	L. infantum-IFAT	31 (57)[c]	5.1 (9.5)	3.6–7.3 (7.3–12.2)
	E. canis-IFAT	125	20.8	17.6–24.3
	Anaplasma spp.-IFAT	145	24.1	20.8–27.7
	N. caninum-IFAT	110	18.3	15.3–21.6
	T. gondii-IFAT	311	51.7	47.6–55.8

[a] 95 % confidence interval, Yates continuity correction performed

[b] Species according to sequencing results

[c] Including samples with borderline titers (1:32)

serology-based surveys from Albania (Cicko et al. 1998; Cicko and Cani 1999; Bizgha et al. 2013), neighboring northwestern Greece, and the former Yugoslav Republic of Macedonia (Diakou 2000; Papadopoulou et al. 2005; Athanasiou et al. 2012; Stefanovska et al. 2012) or in dogs imported into Germany from this region (Röhrig et al. 2011). However, serological test methods may underestimate the true number of infected animals as asymptomatic carriers often remain seronegative (Solano-Gallego et al. 2011). Considering risk factors, male dogs were described as being at higher risk of infection (Zaffaroni et al. 1999; Živičnjak et al. 2005; Miranda et al. 2008), which was also the case in the present study. Increasing age and being kept outdoors also have been identified as risks (Zaffaroni et al. 1999; Cardoso et al. 2004; Solano-Gallego et al. 2006; Cortes et al. 2012). This was not observed in the present study.

Hepatozoon canis

Two *Hepatozoon* spp. are recognized in dogs with *H. canis* being the only species present in dogs in Europe, while *H. americanum* only occurs in the Americas (Baneth et al. 2003). Gamonts of *H. canis* were present in six (1 %) Giemsa-stained blood smears. *Hepatozoon canis* has been identified in previous studies in Albania applying more sensitive methods with prevalences of approximately 17 % in

Giemsa-stained buffy smears and 52 % by PCR (Lazri et al. 2008; Hamel et al. 2009). *H. canis* has also been reported in case reports or larger surveys in other countries in southeast Europe, e.g., Croatia (Vojta et al., 2009), Greece (Kontos and Koutinas 1990; Diakou 2000; Jensen et al. 2003), or Bulgaria (Ivanov and Kanakov 2003; Tsachev et al. 2008). No risk factor for *H. canis* was identified in this study, probably due to the low number of positive cases detected by blood-smear examination.

Filarioidea

Several filarial nematodes parasitize canids worldwide with *D. immitis* and *Dirofilaria repens* as the two most common and important species in the Mediterranean region (Simón et al. 2012). Approximately 2 % of the dogs tested positive for *D. immitis* antigen with the DiroChek®-ELISA. Although the Knott's test was not performed, three microfilaremic dogs were detected in Giemsa-stained blood smears, indicating a high microfilaremia. Previous studies in Albania reported detection rates of 1.8 to 13.5 % for *D. immitis* and up to 11.5 % for *D. repens* applying various tests, i.e., detection post-mortem, Knott's test followed by PCR in the case of *D. repens*, or serological methods for the detection of *D. immitis* antigen (Lazri et al. 2008; Hamel

Table 4 Occurrence of mono- and mixed infections as detected by analysis of Giemsa-stained blood smears, PCR, and DiroChek®-ELISA

	Prevalence, total (%)
Single species infection	118 (19.6)
B. vogeli	1 (0.2)
Hepatozoon spp.[a]	3 (0.5)
L. infantum	18 (3.0)
Microfilariae (not identified)[a]	2 (0.3)
D. immitis[b,c]	5 (0.8)
A. phagocytophilum	5 (0.8)
A. platys	14 (2.3)
E. canis	33 (5.5)
M. haemocanis	37 (6.1)
Double infection	30 (5.0)
Hepatozoon spp.[a] + *A. platys*	2 (0.3)
Hepatozoon spp.[a] + *M. haemocanis*	1 (0.2)
B. vogeli[d] + *E. canis*	1 (0.2)
L. infantum + *D. immitis*[b]	1 (0.2)
L. infantum + *M. haemocanis*	1 (0.2)
L. infantum + *A. phagocytophilum*	1 (0.2)
L. infantum + *E. canis*	5 (0.8)
D. immitis + *E. canis*	5 (0.8)
E. canis + *A. platys*	1 (0.2)
E. canis + *M. haemocanis*	9 (1.5)
A. platys + *M. haemocanis*	3 (0.5)
Triple infection	3 (0.5)
L. infantum + *D. immitis*[b] + *E. canis*	1 (0.2)
L. infantum + *E. canis* + *M. haemocanis*	1 (0.2)
D. immitis[b] + *E. canis* + *M. haemocanis*	1 (0.2)
Total	151 (25.1)

[a] Giemsa-stained blood smear

[b] DiroChek®-ELISA

[c] One animal microfilaraemic

[d] Also *Babesia*-positive in Giemsa-stained blood smear

et al. 2009, Rapti and Rehbein 2010; Xhaxhiu et al. 2011). The prevalence rate of 2.2 % in dogs under veterinary care from Albania is at the lower range of 2.8 to 37 % reported in dogs from Greece, the former Yugoslav Republic of Macedonia, Serbia, and Bulgaria (Diakou 2000; Dimitrijević et al. 2007; Kirkova et al. 2008; Stefanovska et al. 2012; Morchón et al. 2012). Similar to results reported by Rapti and Rehbein (2010), increasing age was not identified as a risk factor in the present study. Nevertheless, results from other studies indicate that keeping dogs outdoors, male gender, and higher age are risk factors for *D. immitis* infection (Yildirim et al. 2007; Cardoso et al. 2012; Mircean et al. 2012).

Table 5 Number of dogs tested seropositive for multiple vector-borne pathogens in IFAT (borderline titers (1:32) for *L. infantum*, *Babesia* spp. not considered)

	N
Dogs seropositive for two pathogens	86
Babesia spp. + *L. infantum*	3
Babesia spp. + *E. canis*	3
L. infantum + *Anaplasma* spp.	5
L. infantum + *E. canis*	73
A. phagocytophilum + *E. canis*	2
Dogs seropositive for three pathogens	18
Babesia spp. + *L. infantum* + *A. phagocytophilum*	1
Babesia spp. + *L. infantum* + *E. canis*	10
Babesia spp. + *Anaplasma* spp. + *E. canis*	1
L. infantum + *Anaplasma* spp. + *E. canis*	6
Dogs seropositive for four pathogens	4
Babesia spp. + *L. infantum* + *Anaplasma* spp. + *E. canis*	4
Total	108

Ehrlichia canis

Ehrlichia canis, the agent of canine monocytic ehrlichiosis, is a widespread tick-borne pathogen in the Mediterranean region and southeast Europe, associated with the presence of the vector tick *Rhipicephalus sanguineus* (Dantas-Torres 2010). This is the first record of *E. canis* by PCR in dogs from Albania. The seroprevalence of 20.8 % is within the range of prevalence rates of 17 and 50 % in semi-domesticated dogs from Albania (Lazri et al. 2008; Hamel et al. 2009). Generally, data on *E. canis* is limited for Southeast Europe, with few reports available from neighboring Bulgaria, Greece, the former Yugoslav Republic of Macedonia, and Serbia (Mylonakis et al. 2004a, b; Pavlovic et al. 2012; Tsachev et al. 2008; Stefanovska et al. 2012). Although increasing age was identified as risk factor in this study, age was not identified as a risk factor study from Spain (Solano-Gallego et al. 2006). In contrast, younger dogs were tested positive more often than older dogs in a multicenter study performed in Spain (Miró et al. 2013).

Anaplasma spp.

A. platys causes canine cyclic thrombocytopenia (Harvey et al. 1978). *R. sanguineus* has been incriminated as a vector, although not yet confirmed (Sanogo et al. 2003). *A. platys*-DNA was detected by PCR in 20 (3.3 %) blood samples. To the authors' knowledge, this is the first report of *A. platys* from Albania. There is basically no information available on *A. platys* on the Balkan Peninsula with only one case report describing an infection in a dog imported into Germany from Croatia (Dyachenko et al. 2012) Another single case report is

Table 6 Multivariate analysis of risk factors associated with presence/exposure to pathogens in 602 client-owned dogs from Albania

Factor and group[a]	Odds ratio	95 % CI	p value
Overall presence/exposure (PCR + IFAT; vector-borne)			
Age			
>1 vs ≤1 year	1.8	1.2–2.7	0.003
Habitat			
City	Reference	NA	NA
Suburban	1.5	1–2.1	0.04
Rural	1.1	0.7–1.7	0.8
Overall exposure (IFAT; vector-borne)			
Age			
>1 vs ≤1 year	1.9	1.3–2.9	0.002
Habitat			
City	Reference	NA	NA
Suburban	1.5	1–2.2	0.03
Rural	0.9	0.6–1.5	0.7
Babesia IFAT			
Age			
>1 vs ≤1 year	2.79	1.17–6.66	0.02
Season			
Winter	Reference	NA	NA
Spring	0.41	0.18–0.95	0.04
Summer	1.01	0.46–2.19	0.99
Autumn	1.45	0.70–3.02	0.32
Leishmania IFAT			
Sex			
Male vs female	1.8	1 - 3.2	0.04
Ehrlichia IFAT			
Age			
>1 vs ≤1 year	1.7	1–2.9	0.0
Anaplasma IFAT			
Age			
>1 vs ≤1 year	1.8	1.1–2.9	0.02
IFAT *Neospora*			
Age			
>1 vs ≤1 year	1.8	1–3.1	0.04
IFAT *Toxoplasma*			
Feed			
Raw/raw and cooked vs cooked	1.7	1.1–2.5	0.01

NA not applicable

[a] Season, age, sex, feed, breed, use, husbandry, habitat

available from Romania (Andersson et al. 2013). Reports on *A. platys* are available from several countries in the Mediterranean region including Spain, Portugal, Italy, France, and Greece (Kontos and Koutinas 1990; Kontos et al. 1991; Sainz et al. 1999; Beaufils et al. 2002; Mylonakis et al. 2004b; Cardoso et al. 2010; Otranto et al. 2010).

A total of six (1.0 %) of the sampled dogs were positive for *A. phagocytophilum*-DNA by PCR. *A. phagocytophilum* is one of the most common tick-borne pathogens in Europe, associated with the occurrence of *Ixodes ricinus* ticks (Carrade et al. 2009). This study presents the first record of *A. phagocytophilum* DNA in dogs from Albania.

More than 24 % of the dogs were seropositive to *Anaplasma* spp. in IFAT. A previous small-scale study reported a seroprevalence rate of 40 % although none of the dogs were positive for *A. phagocytophilum* DNA by PCR (Hamel et al. 2009). Lower seroprevalence rates of approximately 8 % have been reported in surveys from the former Yugoslav Republic of Macedonia and Serbia (Pavlović et al. 2012a; Stefanovska et al. 2012). Serum/plasma from *A. platys*-infected dogs may cross-react to *A. phagocytophilum* antigen in IFAT as well as in SNAP 4dx tests (Beaufils et al. 2002; Santos et al. 2009; Gaunt et al. 2010). Thus, antibodies against *Anaplasma* spp. detected in this study and, possibly, in the previous study from Albania (Hamel et al. 2009), may also be attributed to exposure to *A. platys*, which is probably the predominant *Anaplasma* species in Albania when considering incidence rates detected by PCR in the current study. This is supported by the predominance of *R. sanguineus* ticks collected from these dogs (Shukullari et al. 2015). Considering the results of the *Anaplasma*-IFAT, increasing age was identified as a risk factor in the present study, while other authors identified male gender and being kept outdoors as risks (Solano-Gallego et al. 2006; Kybicova et al. 2009), or no risk factors were identified (Jensen et al. 2003).

Mycoplasma haemocanis

PCR-identified DNA of *M. haemocanis* in 8.8 % of the blood samples of the dogs. This represents the first report of this pathogen in dogs from Albania. A comparable prevalence has been reported in a study in privately owned dogs from North Macedonia in Greece, a region neighboring Albania in the East (Tennant et al. 2011). *M. haemocanis* infections have also been detected in Italy, Portugal, Spain, Hungary, and Romania with prevalence rates of more than 40 % reported in dogs from Portugal (Novacco et al. 2010; Hamel et al. 2012). Although dogs are usually asymptomatic carriers of *M. haemocanis*, sporadic cases with severe anemia have been described, primarily in immune-compromised or kenneled dogs (Kemming et al. 2004; Novacco et al. 2010; Willi et al. 2010).

Mixed infections

Mixed infections with as many as three vector-borne pathogens, based on PCR, blood smear evaluation, and detection of *D. immitis* antigen by ELISA, have been observed in this study, involving *Babesia* spp., *H. canis*, *L. infantum*, *E. canis*,

Anaplasma spp., *M. haemocanis*, and/or *D. immitis*. Similar results have been reported in other studies of the Mediterranean region (Kontos and Koutinas 1990; Heyman et al. 2007; Mylonakis et al. 2004a, 2004b; Tsachev et al. 2008; Sasanelli et al. 2009; Cardoso et al. 2010, Otranto et al. 2010; Andersson et al. 2013; De Tommasi et al. 2013). Mixed infections are of considerable importance as they may alter clinical signs as well as disease progression (Sasanelli et al. 2009; De Tommasi et al. 2013).

Neospora caninum

About 18 % of the dogs in this study were seropositive to *N. caninum*. Dogs are definitive hosts of *N. caninum* and play an important role in the horizontal transmission of this protozoan parasite to other animals. It is considered the major parasitic cause for abortion in cattle with estimated losses in cattle production exceeding US$ 1200 million per year worldwide (Reichel et al. 2013). Clinical manifestation in dogs is rare, although transplacentally infected puppies may develop neuromuscular symptoms (cf. Dubey et al. 2007). Seropositivity indicates exposure to this pathogen but not necessarily infection. Identification of *N. caninum* oocysts by coproscopy is hampered by transient excretion and the occurrence of morphologically indistinguishable *Hammondia heydorni* oocysts. Therefore, usually, only a few dogs are identified shedding *N. caninum*-like oocysts, e.g., 4.9 % of 386 dogs surveyed in Romania and only 0.2 % of more than 24,000 dogs screened in Germany (Schares et al. 2005; Mitrea et al. 2012a). To the authors' knowledge, there are no studies on *N. caninum* available from Albania. Seroprevalence rates range from 20 to over 32 % in dogs from Romania and from 5 to 60 % in livestock from Serbia, Croatia, Greece, Bulgaria, and Romania (Beck et al. 2010; Prezelov et al. 2008; Sotiraki et al. 2008; Gavrea et al. 2011, 2012; Mitrea et al. 2012a, b; Gavrilović et al. 2013). As described in other studies, increasing age was associated with infection due to an increasing chance of acquiring infection with age (Dubey et al. 2007; Mitrea et al. 2012b). This is also indicative for infection of the animals post gestation and, therefore, environmental or feed-borne infections are most likely route of transmission in animals in this study.

Toxoplasma gondii

Toxoplasmosis affects various warm-blooded species worldwide as intermediate hosts and felids act as definitive hosts (Dubey 2008). Approximately 52 % of the dogs were seropositive to *T. gondii* in this study. To date, there is only limited data on toxoplasmosis in Albania, and there is no data available in dogs. In neighboring Greece, studies identified antibodies to *T. gondii* in 21 to 34 % of canine blood samples (Chambouris et al. 1989; Diakou 2000). A recent publication reported almost 50 % of 496 pregnant Albanian women were positive for anti-*T. gondii* IgG, and 55 % out of 61 Albanian migrants were positive in Italy (Ventura et al. 2004; Maggi et al. 2009). Prevalence rates in humans in countries of the Balkan Peninsula and neighboring Greece range from 20 to 50 %, regardless of test-specific variations (cf. Bobić et al. 2011). Seroprevalence rates of 28.9 to 84.5 % have been reported in livestock (sheep, goat, cattle, and pigs) from the region (Klun et al. 2006; Prezelov et al. 2008). As confirmed in previous studies (Azevedo et al. 2005; Gennari et al. 2006; Dubey et al. 2007), lifestyle, and husbandry conditions, including access to *T. gondii* cysts, were also the most important factors affecting seropositivity in dogs in this study.

Conclusion

The results of this investigation revealed a wide range of vector-borne pathogens in blood samples from client-owned dogs in Tirana, Albania, including the first reports on *A. platys*, *A. phagocytophilum*, and *M. haemocanis*. The prevalence rates for vector-borne infections in dogs under veterinary care were lower than those in less well-cared dogs from this area. This was probably due to increased owner awareness indicated by better husbandry conditions and ectoparasiticidal treatment (Shukullari et al. Parasites and vector-borne diseases in client-owned dogs in Albania. Infestation with arthropod ectoparasites, in preperation), thus limiting vector exposure. Additionally, serological screening gave first evidence for *N. caninum* and *T. gondii* exposure in dogs from Albania.

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