Inaugural-Dissertation zur Erlangung der Doktorwürde
der Tierärztlichen Fakultät der Ludwig-Maximilians-Universität
München

Parasites and Vector-borne Diseases in Client-owned Dogs in Albania

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München 2016

Aus dem Veterinärwissenschaftlichen Department der Tierärztlichen Fakultät

der Ludwig-Maximilians-Universität München

Lehrstuhl für Vergleichende Tropenmedizin und Parasitologie

Arbeit angefertigt unter der Leitung von Univ.-Prof. Dr. Kurt Pfister

Angefertigt am Kathrinenhof Research Center, Merial GmbH, Rohrdorf

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Die vorliegende Arbeit wurde nach §6 Abs. 2 der Promotionsordnung
für die Tierärztliche Fakultät der Ludwig-Maximilians-Universität München
als kumulative Dissertation gestaltet.

Gedruckt mit Genehmigung der Tierärztlichen Fakultät
der Ludwig-Maximilians-Universität München

Dekan:	Univ.-Prof. Dr. Joachim Braun
Berichterstatter:	Univ.-Prof. Dr. Kurt Pfister
Korreferent/en:	Priv.-Doz. Dr. Petra Kölle

Tag der Promotion: 06 Februar 2016

DEDICATION

This thesis is dedicated to the late Prof. Dashamir Xhaxhiu who created the base of this thesis project, who trusted me and encouraged me to this work.

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1. INTRODUCTION and OBJECTIVE

After World War II, Albania was the smallest and became the most isolated country within the former communist bloc of Eastern Europe (principles of 'self-reliance' were sanctioned in 1976) with a highly centralized type of economy. Despite of considerable success with the industrialization of the country in the late 1970s, Albania's economy was still strongly dependent on agriculture. Internal migration was strictly regulated and urbanization was planned and, because of focussing on agriculture, Albania remained predominately rural and maintained the lowest level of urbanization in Europe with approximately two thirds of the total population living in rural areas in 1989. Mainly because of the self-isolation, Albania's economy was brought to a state of total collapse in 1990 and lead subsequently to major changes in the political and economic structures (Gjonça 2001).

The transition from a closed, centralized economy to an open-market, democratic country was accompanied by significant demographic, social and cultural changes including urbanization and modernization (Vullnetari 2012). One aspect associated to these processes is a change in the attitude of humans to dogs and cats such that companion animal ownership is becoming more and more popular in Albania, particularly in cities.

In the past, both dogs and cats did not receive much attention and had no high social support in the country. Based on the importance of agriculture, health care for food animals took an important role while canine and feline diseases were rather undocumented in Albania. This included the knowledge of the parasitological status of dogs and cats. Until 1990, only scarce information was available on parasites of dogs and cats in the country with only one paper from 1960 specifically dedicated to helminth parasites of dogs and cats. From the late 1990s on, a more comprehensive picture on parasitic infections in dogs and cats including vector-borne pathogens has been established through studying mainly less well-cared-for dogs and cats that roam continuously or sporadically. Although surveying of this category of animals provides very important information on the distribution of parasitic infections and their potential public health implications, the dissemination of parasites and vector-borne pathogens in client-owned, veterinary-cared-for dogs have not been studied to date.

Therefore, the aim of this thesis was to determine the prevalence of endo and ectoparasite infections including vector-borne disease agents in dogs presented to four small animal clinics in Tirana in order to provide specific knowledge on the situation in companion animals in Albania.

2. LITERATURE REVIEW

2.1. Brief Introduction to Albania

The Republic of Albania is a country in Southeastern Europe, in the western part of the Balkan peninsula. It shares borders with Montenegro to the northwest, Kosovo to the northeast, Macedonia (FYROM) to the east, and Greece to the south and southeast. Albania is divided into 12 administrative counties. These counties are further divided in 36 districts (Figure 1).

Albania has a coast on the Adriatic Sea to the west and on the Ionian Sea to the southwest. Albania has a surface area of 28,745 square kilometers (MoEFWA 2009; INSTAT 2011). It lies between latitudes 39° and 43° N, and mostly between longitudes 19° and 21° E. Albania's coastline length is 476 km and extends along the Adriatic and Ionian Seas. The country is predominantely mountainous (77%), with 30% lying above 1,000 m and the mean altitude is 708 m (MoEFWA 2009). The lowland zone is the coastal area, while the mountains lie from the northeast of the country to the south-central area (Figure 2).

Based on the preliminary Population and Housing Census 2011 results, the total population of Albania is 2,821,977 (at an average density of 109.3 inhabitants per square kilometer). The population in urban areas is larger than the population in rural areas (53.5% of the population lives in urban areas and 46.5% in rural areas). The western coastal lowland region contains most of the country's arable land and is the most densely populated part of Albania (INSTAT 2011).

The capital city of Albania is Tirana, other principal cities are Durrës, Korçë, Elbasan, Shkodër, Gjirokastër, Vlorë and Kukës. The capital Tirana is the most crowded city in Albania, and according to data from the Population and Housing Census 2011, the population density in the district of Tirana is 454 persons per square kilometer. Tirana County, which can be viewed as a metropolitan area, has a population of 788,330. Several towns and villages within the county have merged with the city, due to urban sprawl, so they can be considered as suburbs of Tirana (INSTAT 2011, 2014).

Figure 1: Administrative divisions (districts) of Albania and neighboring countries

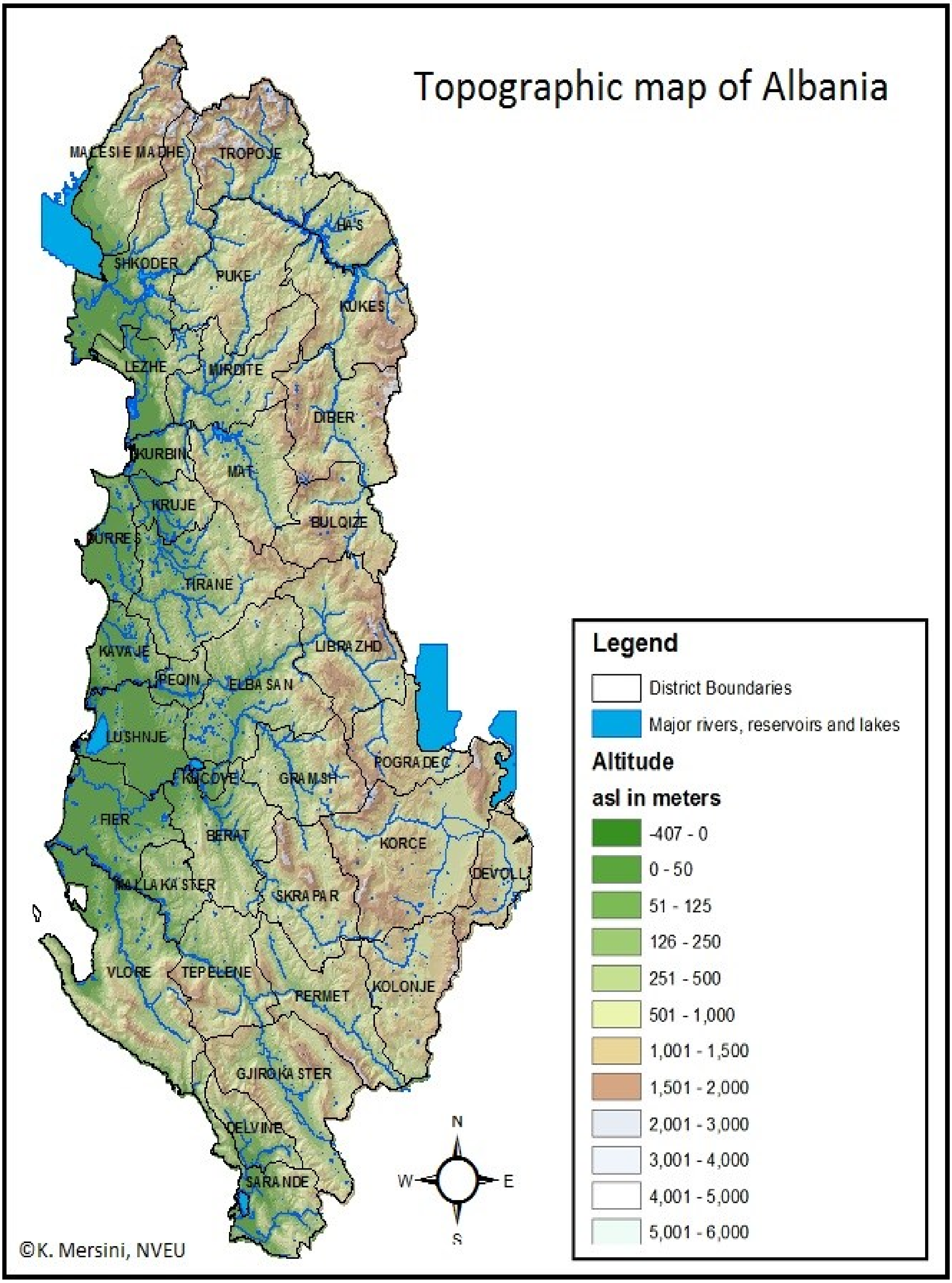

Figure 2: Topography of Albania

Despite being a small country only, Albania has very diverse climatic regions related to the country's topography. The division between the lowland and the highland regions also divides the typical Mediterranean climate of the western coastal parts from the continental climate of the interior (MoEFWA 2009; Climate Change in the West Balkan 2012). In both the lowlands and the interior, the weather varies markedly from north to south. The lowlands have mild winters, averaging about 7 °C and summer temperatures average 24 °C. Lowland's rainfalls average from 1,000 millimeters to more than 1,500 millimeters annually, with the higher levels in the north. Nearly 70% of the rain falls during the colder months (October-March). The continental climate is characterized with average temperatures in winter varying from -2 to 7 °C and snowfall, while summer temperatures average 15 to 20 °C (MoEFWA 2009; ENVSEC 2012).

Tirana (41.33°N, 19.82°E) is located about 32 kilometers east from the Adriatic Sea. Tirana's average altitude is 110 meters above sea level. The city is mostly surrounded by mountains, with Dajti Mountain (1,613 meters) on the east and a slight valley opening on the north-west overlooking the Adriatic Sea in the distance. The Tirana River runs through the city, as does the Lana stream. Tirana has a humid Mediterranean climate with more than 40 millimeters of rainfall in each month, hot and moderately dry/humid summers, and cool and wet winters.

Companion animal ownership, i. e. keeping dogs that usually do not roam without human supervision, is a relatively recent phenomenon in Albania such that veterinary service for companion animals is still under development. However, meanwhile several small animal clinics were established, and veterinarians are becoming specialized to care for dogs and cats. In Tirana, for instance, there are currently approximately 20 small animal clinics and two hospitals for small animals reflecting the demand of owners for appropriate veterinary care for their pets. In the other principal cities, there are usually only two or three smaller clinics for small animals.

2.2. Parasite Infections and Vector-borne Diseases in Dogs in Albania

Until 1990, only few publications provided information on parasites of dogs and cats in the country (Moskvin 1958; Danielova 1960; Prokopič 1960; Rosický et al. 1960; Rosický and Gjini 1960; Luli 1963; Gina 1973/1977; Kero and Gina 1974; Adhami and Murati 1977; Pepa 1987; Miho et al. 1989). With the exception of one publication documenting the helminth parasites isolated at necropsy of 13 dogs and four cats (Prokopič 1960), data on the parasites of dogs and cats were collected rather opportunistically either in the context of general surveys on the parasite fauna of animals in Albania in the 1950s and early 1960s (Moskvin 1958; Danielova 1960; Prokopič 1960; Rosický et al. 1960; Rosický and Gjini 1960; Luli 1963) or in studies on the epidemiology of zoonotic infections (Gina 1973, 1977; Gina and Kastrati 1974; Kero and Gina 1974; Gina et al. 1975; Adhami and Murati 1977; Pepa 1987; Miho et al. 1989).

Reflected through the number and diversity of subjects of papers published from the late 1990s on, various aspects on the occurrence of parasites in dogs (Cicko and Cani 1998; Cicko et al. 1999; Sotira 2000; Cani et al. 2001a; Dhamo and Zanaj 2003; Dhamo et al. 2006; Zanaj et al. 2006; Lazri et al. 2008; Hamel et al. 2009; Xhaxhiu et al. 2009, 2011; Rapti and Rehbein 2010; Refugjati et al. 2012; Bizhga et al. 2013; Bocari et al. 2014; Silaghi et al. 2013; Sommer et al. 2015) and cats (Dhamo and Zanaj 2003; Xhaxhiu et al. 2009; Knaus et al. 2011, 2012, 2014b; Silaghi et al. 2012, 2014) have been studied in Albania in the recent past. Additional information on parasites of cats are presented in publications reporting the evaluation of parasiticides against helminth parasites (Adler et al. 2014; Knaus et al. 2014a, c; Rehbein et al. 2014).

Overall, these publications document a broad spectrum of both endo- and ectoparasites as well as infections with or exposure to other vector-borne pathogens including species which not only may impact the health of the animals but are considered zoonotic with some of them posing serious concerns to the public health.

2.2.1. Principal Endoparasites (Protozoans and Helminths)

2.2.1.1. Protozoan Endoparasites

Protozoan parasites of vertebrates are typically single-celled eukaryotic organisms. Dogs serve as host to many species of several genera of protozoan parasites including flagellates (e. g., *Giardia*), trypanosomids (e. g., *Leishmania* and *Trypanosoma*) and apicomplexan coccidians (e. g., *Cystoisospora, Hammondia, Neospora, Toxoplasma* and *Sarcocystis*), piroplasms (e. g., *Babesia* and *Theileria*) and hepatozoids (e. g., *Hepatozoon*). Of specific interest among these protozoans are species that may infect both dogs and humans (*Giardia* spp. and *L. infantum*).
(Georgi and Georgi 1992; Snowden and Budke 2013)

Giardia canis

Giardia infections are now considered one of the most common enteric parasitic infections for both dogs and cats worldwide, and *Giardia* is one of the most important protozoan pathogens causing diarrheal conditions in humans. But *Giardia* infections can also be asymptomatic in both humans and animals. The fact that *Giardia* can infect both humans and animals has raised concerns about the risk to public health from companion animals. However, this risk is only linked to the presence of the human infective assemblages A and B (corresponding to the species names *G. duodenalis* and *G. enterica*, respectively). Dogs are susceptible to different assemblages/species of *Giardia* which vary in their zoonotic potential; the dog/canid adapted assemblages are C and D (both corresponding to the species name *G. canis*).
(Payne and Artzer 2009; Ballweber et al. 2010; Thompson 2011; Thompson and Monis 2012; Bouzid et al. 2015; Pallant et al. 2015)

In Albania, the occurrence of *Giardia* infections in humans is long known through a study from the late 1950s. In this study, 22.6% of 283 pre-school children tested positive for *Giardia* (Erhardová et al. 1960a). Records of the Institute of Public Health document findings from the 1990s on (Gjoni 2003a). Recently conducted faecal surveys in humans found *Giardia* prevalences ranging from approximately 5% to 44% depending on the sampled population (e. g., diseased vs. healthy individuals) and methodology of analysis (Gjoni 2003a, b, 2015; Berrilli et al. 2006; Spinelli et al. 2006; Mitrushi 2008; Sejdini et al. 2011; Nezaj et al. 2012; Vasjari et al. 2014). Genotyping confirmed the presence of the *Giardia* assemblages A and B (Berrilli et al. 2006).

As regards the knowlegde on *Giardia* infection in dogs in Albania, '*Giardia*' was diagnosed in three of 44 canine faecal samples which were collected in different public places in Tirana (Refugjati et al. 2012). In addition, multilocus sequence typing of 17 isolates extracted from the faeces of client-owned dogs which were examined in this study revealed exclusively the dog/canid adapted assemblages C and D, *Giardia canis* (Sommer et al. 2015).

Leishmania infantum

Leishmaniosis in dogs in Europe is caused by *L. infantum* and is a major, potentially fatal, zoonotic infection. Leishmaniosis is a vector-borne disease and phlebotomine sand flies are the biological vectors. In endemic areas, the same *Leishmania* species infects dogs and other hosts including humans where it causes visceral and cutaneous disease or can be asymptomatic. Dogs showing disease present a wide range of clinical signs (e. g., skin lesions, ocular inflammation, weight loss, lethargy, inappetence, splenomegaly and lymphadenomegaly) and variable degree of severity. The role of the domestic dog as the main vertebrate reservoir of human infection is well established. Human visceral leishmaniosis is endemic to 14 countries in Southeastern and Western Europe. Around 500 new human cases are reported per annum, and almost 75% of these are in Albania, Italy and Spain.
(Baneth et al. 2008; Miro et al. 2008; Ready 2010, 2013; Gramiccia 2011; Alvar et al. 2012; Dantas-Torres et al. 2012; Maroli et al. 2013; Pennisi 2015)

Heuyer and Cornet (1919) reported that leishmaniosis has been diagnosed for the first time on the Balkans 1917 in a Russian soldier in a military hospital in Korçë, Albania. The soldier suffered from cutaneous leishmaniosis and several attempts to treat the condition failed.
According to the Albanian literature, first human cases were diagnosed by local physicians and pathologists in the 1920s (Jorgoni 1953). First reports on the disease in Albanians and in Italians who were assumed to have contracted the infection when visiting Albania were reported in the early 1940s (Angelini 1941; Iannarone 1941; Frashëri 1942). In post-World War II Albania, several reports and papers on the occurrence of the disease were published (Jorgoni 1953; Kërçiku and Dishniku 1954; Todhe 1963; Nini 1977; Adhami et al. 1983; Cerhozi 1986; Adhami and Murati 1986, 1987b; Miho et al. 1989). These papers described the clinical presentation and treatment of the disease but also addressed epidemiological aspects in order to identify and characterize endemic areas. Since the 1990s, human visceral leishmaniosis in Albania received increasing attention resulting in the documentation of solid

case figures and epidemiological data, and in the year of 2000, leishmaniosis was the only parasitic disease which was considered in the list of zoonotic diseases of major concerns in Albania (Sotira 2000). Overall, the data indicate an increase of morbidity compared to the past. In Albania, more than 70% of the human cases are diagnosed in children up to 14 years of age with highest prevalences recorded in people originating from rural areas (Kakarriqi 1997, 2002; Kero and Xinxo 1998; Xinxo and Kero 1998a, b; Pulo et al. 1998; Sotira 2000; Lito et al. 2002; Velo et al. 2003, 2010; Myrseli et al. 2004, 2011, 2014; Cika 2006; Lazri et al. 2008; Petrela et al. 2010; Pipero 2012).

In Albania, where zoonotic leishmaniosis is endemic, canine leishmaniosis was confirmed for the first time in the 1970s through the examination of bone-marrow and other tissues of a symptomatic, four years old dog (Adhami and Murati, 1977). Adhami and Murati (1977) examined 29 dogs from different locations in Albania known for the occurrence of human leishmaniosis and one dog from Ferraj, Tirana district, was tested positive for the infection. The first isolation of *Leishmania* parasites from dogs was reported in 1989 (Miho et al. 1989). Serosurveys indicated average anti-*Leishmania* antibody prevalences of up to 17% in dogs of different categories and geographic origin in Albania (Cicko and Cani 1998; Cicko et al. 1999; Sotira 2000; Cani et al. 2001a; Lazri et al. 2008; Hamel et al. 2009; Bizhga et al. 2013). Characterization of isolates collected from both human and canine cases and sandflies in Albania revealed that all isolates were *L. infantum* belonging to the common zymodeme MON-1 (Cani et al. 2001a, b; Maroli et al. 2012; Gouzelou et al. 2013).

***Cystoisospora* and *Sarcocystis* species**

The most commonly recognized canine coccidians belong to the genera *Cystoisospora* and *Sarcocystis*. *Cystoisospora* spp. and *Sarcocystis* spp. are obligate intracellular parasites which are normally found in the intestinal tract. Their life cycle has an asexual phase and a sexual phase which both may occur in the same host in *Cystoisospora* species whereas in *Sarcocystis* species the two phases occur in different hosts. Generally, the majority of *Cystoisospora* infected dogs remain asymptomatic. Clinical signs are usually recorded in puppies and in situations where animals are congregated, and diarrhea and weight loss is the most common presentation. *Sarcocystis* infection is of no or only little clinical significance in dogs.
(Lindsay et al. 1997; Dubey et al. 2009; Bowman 2014)

In Albania, the first record of canine coccidians dates to the year of 2002 when Dhamo in her unpublished thesis reported the finding of 'oocysts' in 11 of 109 canine faecal samples (10.1%) with seven of the positive samples deriving from 36 ≤6 month old puppies. In addition, Refugjati et al. (2012) found 'oocysts' in one of 44 dog faecal samples which were collected in public places in Tirana. Xhaxhiu et al. (2011) estimated prevalences of 17.1% and 31.5% for *C. canis* and *C. ohioensis/burrowsi*, respectively, in a sample of 111 less well-cared-for dogs from suburban areas of Tirana. In this study, the rate of *Cystoisospora* infection did not differ significantly between ≤6 months (46%) and >6 month-old dogs (35.4%). In the same study, two of the 111 dogs (1.8%) were identified shedding *Sarcocystis* sporocysts.

Hepatozoon canis

All *Hepatozoon* species share a life cycle comprising a sexual phase in a haematophagous invertebrate definitive host and an asexual phase in a vertebrate intermediate host which includes the formation of gamonts in the cytoplasm of white blood cells. Transmission of infection to vertebrates is via ingestion of the definitive (invertebrate) host. In Europe, canine hepatozoonosis is caused by *Hepatozoon canis* and, because of transmitted by the tick *Rhipicephalus sanguineus*, reported mainly from the Mediterranean region. Clinical presentation of *H. canis* infection can vary from apparently healthy to severely affected dogs. (Ivanov and Tsachev 2008; Baneth 2011)

In Albania, *H. canis* infection in dogs was diagnosed by PCR in blood samples (52.8% of 36 stray dogs; Lazri et al. 2008) and by microscopic detection of intracellular gamonts in Giemsa-stained blood smears (17% of 30 dogs; Hamel et al. 2009).

***Babesia* species**

Babesias and the phenotypically similar protozoans of the genus *Theileria* comprise the piroplasms. Piroplasms use mammalian red blood cells in their life cycle. Under natural conditions, these haemoparasites are transmitted by ixodid ticks.

Infection with several *Babesia* species may cause canine babesiosis. Canine babesiosis is a disease with variable pathogenesis and clinical presentation which is influenced by the infecting species as well as the age and immunstatus of the host. Molecular genotyping of canine piroplasms has resulted currently in the recognition of four 'large' and at least four 'small' piroplasms. The originally described 'large' *B. canis* was later classified in three

subspecies (*B. c. canis*, *B. c. vogeli*, *B. c. rossi*) which are now considered as separate species: *B. canis* (sensu strictu), *B. vogeli* and *B. rossi*.
(Irwin 2009; Schoeman 2009; Matijatko et al. 2012; Köster et al. 2015)

As regards the situation of canine babesias in Albania, the occurrence of 'large' babesias (*B. canis*-type) has been confirmed. To the author's knowledge, the first report on canine babesiosis in Albania was published in 2006 and described the microscopical identification of *B. canis* babesias in Giemsa stained blood smears of 23 of 101 dogs from Tirana that were tested in the period from July 2003 to July 2004 (Dhamo et al. 2006). Later studies demonstrated the presence of DNA of both *B. canis* (sensu stricto) and *B. vogeli* in the blood of dogs from Albania and reported a prevalence of anti-*B. canis* antibodies (IFAT) of approximately 10% (Lazri et al. 2008; Hamel et al. 2009). However, it should be considered that the *B. canis* IFAT may also detect anti-*B. vogeli* antibodies via cross-reactivity with the *B. canis* antigen (Verkammen et al. 1995).
In addition to the first record in Albania of canine babesiosis, the study of Dhamo et al. (2006) indicated an inverse association of the prevalence of infection and the age of the dogs, recorded positive cases more frequently in spring compared to summer and autumn and found most cases in dogs with access to the outside. More recently, haematological and clinical findings in dogs from Albania with microscopically confirmed *Babesia* infection were reported (Andoni et al. 2012, 2013).
In endemic areas, there is a strong association of the *Babesia* species that is transmitted and the tick vector present in the environment. *Babesia canis*, the predominant agent of canine babesiosis in temperate Europe, is associated with the occurrence of *Dermacentor reticulatus* ticks whereas *B. vogeli*, the most widespread canine babesia, is transmitted by *R. sanguineus* ticks and occurs mainly in the south of Europe (Mediterranean area) (Matijatko et al. 2012; Halos et al. 2014). Several surveys of the tick fauna in Albania recorded *R. sanguineus*, the vector of *B. vogeli* (Dantas-Torres 2008), collected from dogs, other mammals and from the environment (Rosický et al. 1960; Luli 1963; Gina 1973; Gina et al. 1975/Gina 1977; Xhaxhiu et al. 2009; Silaghi et al. 2013; Knaus et al. 2014). None of these studies identified *D. reticulatus* ticks. However, *D. reticulatus* is listed among the species of animals (including several parasites) recorded in the town of Shkodër, northeast of Albania (Dhora 2006). Unfortunately, this report appears to be uncertain. It does not provide any background information on the collection of the parasite species listed and, furthermore, in the 2010

published 'Register of Species of the Fauna of Albania' of the same author, *D. reticulatus* is not listed among the ticks.

Indication for parasitism of additional protozoans

A recently conducted study on the seroprevalence of infections in cats demonstrated anti-*Neospora caninum* antibodies (IFAT) in approximately 10% of 146 cats from Albania (Silaghi et al. 2014). This finding indicates the presence in Albania of *N. caninum*. *Neospora caninum* is a coccidian parasite primarily associated with dogs and cattle and considered as a major cause for abortation in cattle (Dubey and Schares 2011). However, there is no data available for Albania with respect to bovine neosporosis.

2.2.1.2. Helminth Endoparasites

Helminths ('worms') is a collective term without phylogenetic and taxonomic meaning comprising both non-parasitic and parasitic metazoan organisms. The typical parasitic helminths belong to the phyla Plathyhelminthes ('flatworms': trematodes/flukes, and cestodes/tapeworms), Nematoda ('roundworms') and Acanthocephala ('thorny-headed worms'). Dogs can be parasitized by species of all three taxonomic groups; however, the most important canine helminths are cestodes and nematodes. While several helminths have the potential to induce disease in dogs, of specific relevance are the species that are known agents of zoonotic diseases (e. g., *Echinococcus granulosus* and *Toxocara canis*).
(Georgi and Georgi 1992; Macpherson and Torgerson 2013; Morgan 2013)

For Albania, Moskvin (1958) was the first who published a list of helminth species parasitizing domestic animals (cattle, sheep, goats, horses, pigs, rabbits, chicken, geese, dogs). Helminths were collected from slaughter animals at the Tirana abattoir, animals at the Institute of Agriculture, animals necropsied at the Central Laboratory for Veterinary Bacteriological Diagnostics and in various farms in different districts of Albania. In total 51 species of helminthes were recorded including three species of trematodes, 10 species for cestodes, 37 species of nematodes and one species of acanthocephalans.

2.2.1.2.1. Trematodes (Flukes)

There are several species of trematodes that can be hosted by dogs but there are only few examples causing clinical conditions, usually depending on fluke burden. Many of those parasites have a low host specificity and dogs have to be considered as occasional hosts only.

Infection of dogs depends very much on dietary habits/exposure of the dogs to the intermediate hosts of the flukes. All trematodes that infect domestic dogs and wild canids belong to the class Digenea and require particular molluscs as (first) intermediate hosts. The occurrence of the intermediate hosts is dependent on the temperature and physico-chemical characteristics of local bodies of water and/or soil. Therefore, cases of autochthonous fluke infection tend to be common in certain localities and rare or absent in others. Although many trematode species have been recorded in both humans and dogs, dogs are not considered as important reservoirs for human infection.
(Georgi and Georgi 1992; Gabrielli 2013)

In Albania, there is only one paper which reports the finding of digenean trematodes in dogs which originated from Çukë and Saranda, south Albania (Prokopič 1960). The five trematode species are parasites of the small intestine: the heterophids <u>*Rossicotrema donicum*</u> (syn. *Apophallus donicus*) and <u>*Parascotyle longa*</u> (syn. *Ascotyle longa*, *Phagicola longa*) (order Opisthorchiida, family Heterophyidae), the diplostomid <u>*Alaria alata*</u> (order Strigeida, family Diplostomidae) and the echinostomatid <u>*Isthmiophora melis*</u> (syn. *Euparyphium melis*) (order Echinostomida, family Echinostomatidae), or parasites of the liver: <u>*Opisthorchis felineus*</u> (order Opisthorchiida, family Opisthorchiidae). They are transmitted to dogs either by direct ingestion of amphibians (*A. alata*, *I. melis*) or fish (*R. donicum*, *P. longa*, *O. felineus*) or paratenic hosts carrying the infectious larval stages.

2.2.1.2.2. Cestodes (Tapeworms)

Tapeworms are hermaphroditic flatworms with indirect life cycles which reside as adults in the intestinal tract of the dogs serving as final hosts. Tapeworm infection is contracted in through ingestion of a vertebrate or invertebrate intermediate host containing the infectious larval stage (metacestode). Cestodes are common parasites of dogs worldwide involving various species. The most widespread species belong to the order Cyclophyllidea comprising mainly taeniid (e. g., *Taenia*, *Echinococcus*) and dipylidiid (*Dipylidium*) cestodes while pseudophyllidean tapeworms are less prevalent. Adult tapeworms in the small intestine of dogs are usually well tolerated, producing little or no clinical signs. However, some species are important agents of zoonotic diseases, e. g., *E. granulosus* causing cystic echinococcosis (hydatidosis).
(Georgi and Georgi 1992; Macpherson and Torgerson 2013)

In total five species of cestodes were recorded in domestic dogs from Albania through necropsy (Prokopič 1960; Xhaxhiu et al. 2011) or based on identification of purged specimens (Pepa 1987): the taeniids *Taenia hydatigena* (Prokopič 1960; Xhaxhiu et al. 2011), *T. multiceps* (Pepa 1987), *T. pisiformis* (Prokopič 1960; Xhaxhiu et al. 2011) and *E. granulosus* (Pepa 1987; Xhaxhiu et al. 2011), and the dipylidiid *Dipylidium caninum* (Prokopič 1960; Xhaxhiu et al. 2011). In addition, examination of faeces samples from stray dogs or samples collected from public places in Tirana revealed the presence of taeniid and *Dipylidium* stages (Zanaj et al. 2006; Refugjati et al. 2012).

As indicated through infection rates of over 60%, *D. caninum* was the most common cestode species recovered from less well cared-for dogs in Albania (Prokopič 1960; Xhaxhiu et al. 2011). This category of dogs carry considerable burdens of fleas (Xhaxhiu et al. 2009; Silaghi et al. 2013) which serve as principal intermediate hosts for *D. caninum*. DNA of *D. caninum* has been recently detected in *Ctenocephalides canis* fleas collected from dogs in Albania (Beugnet et al. 2014).

Taenia hydatigena and *T. multiceps* are typical canine-ungulate cestodes, while the intermediate hosts of *T. pisiformis* are chiefly lagomorphs. In Albania, metacestodes of *T. hydatigena* (Cysticercus tenuicollis) and *T. multiceps* (Coenurus cerebralis) have been recorded in cattle, sheep, goats and swine (Moskvin 1958; Erhardová et al. 1960b; Meshi and Veliu 1973) or sheep (Moskvin 1958), respectively.

The key role played by dogs in the transmission of cystic echinococcosis was discussed already by Prokopič (1960) but no studies on the prevalence of canine *E. granulosus* in Albania have been reported. Molecular analyses of *E. granulosus* specimens from the dogs confirmed the presence of the G1 genotype (*E. granulosus* s. s.) which is commonly found on the Balkan peninsula (cf. Xhaxhiu et al. 2011). In the intermediate ungulate hosts, prevalence of cystic echinococcosis according to abattoir surveys ranged from 5% to 75% in Albania with sheep and cattle usually presenting higher rates of infection than goats and swine (Moskvin 1958; Erhardova et al. 1960b; Dizdari 1971; Meshi and Veliu 1973; Melonashi 1975; Papajani 1980; Pandeli 1983; Pepa 1986; Zanaj 1997; Duro et al. 2011).

Cystic echinococcosis is one of the most widespread parasitic zoonoses in the Mediterranean region including Albania and constitutes a serious public health problem. For the period of 1959 to 1983 echinococcosis was diagnosed in 1141 patients including cases with hydatids located in the kidneys and brain (Anastasi et al. 1987; Bakalli et al. 1987). A retrospective survey on surgical cases and autopsy findings estimated the incidence of human cystic echinococcosis in Albania as 2.05 per 100,000 inhabitants for the years 1958 to 1987 (Zanaj and Elezi 1998). Recent studies based on hospital records and serosurveys confirmed that human cystic echinococcosis is still widely endemic in Albania with a considerable variation of incidence between the different districts (Gjoni et al. 2007, 2014; Caushi et al. 2013; Pilaca et al. 2014).

2.2.1.2.3. Nematodes (Roundworms)

The vast majority of parasitic nematodes have a cylindrical form and thus organs are filamentous. There are separate sexes and female nematodes are usually larger than the males. The life cycle comprises the egg, five larval stages and the adults. The life cycle may be direct (with development of the larval stages either in the environment or in the definitive host) or indirect involving an intermediate host. There are two classes of nematodes, the Secernentea and the Adenophorea, and species belonging to both classes are parasites of dogs. Some canine nematodes (e. g., *Toxocara canis*) are important zoonotic parasites.
(Georgi and Georgi 1992; Morgan 2013)

Ascarididae, Ancylostomatidae, Trichuridae, Spirocercidae (gastrointestinal nematodes)

The nematode fauna of dogs from Albania was studied through necropsy by Moskvin (1958) and Prokopič (1960) in the 1950s and 50 years later by Xhaxhiu et al. (2011) who examined an unknown number of dogs or 13 and 111 dogs, respectively. Moskvin (1958) just listed the species found, Prokopič (1960) added information on the frequency of the occurrence of the parasites, and data on both prevalence and parasite counts as well as the association to host and management factors were provided by Xhaxhiu et al. (2011).

The following species of nematodes were identified: ascarids, *Toxascaris leonina* and *Toxocara canis* (Moskvin 1958; Prokopič 1960; Xhaxhiu et al. 2011); hookworms, *Ancylostoma caninum* (Xhaxhiu et al. 2011) and *Uncinaria stenocephala* (Moskvin 1958; Prokopič 1960; Xhaxhiu et al. 2011) and whipworms, *Trichuris vulpis* (Prokopič 1960; Xhaxhiu et al. 2011) from the intestine; *Spirocerca lupi* spirurids were recovered from the stomach (Prokopič 1960).

By examination of the gastrointestinal tracts of 111 approximately two months to eight years old dogs from suburban areas of Tirana from 2004 to 2009, Xhaxhiu et al. (2011) recorded *T. canis*, *T. leonina*, *A. caninum*, *U. stenocephala* and *T. vulpis* in 75.7%, 0.9%, 64.9%, 13.5% and 21.6% of the dogs, respectively. Corresponding parasite counts were 1 to 54, 4, 1 to 460, 1 to 213 and 1 to 53. As discussed exhaustively by Xhaxhiu et al. (2011), these species are common parasites of dogs worldwide and have been recorded from several countries of the Balkan peninsula.

Further to the necropsy studies, results of three recently conducted faecal surveys were reported (Dhamo 2002/Dhamo and Zanaj 2003; Zanaj et al. 2006; Bocari et al. 2014). Alltogther, these authors examined faecal samples of 363 dogs of different categories (mainly stray dogs) from the Tirana region and reported the finding of eggs of *Toxocara*, *Toxascaris*, *Trichuris* and hookworms. *Toxocara* eggs were recorded most often (43.3%), and puppies (≤6 months old) shed *Toxocara* eggs much more frequently than older dogs (75.6% vs. 28.6%).

In addition, the finding of ascarid and hookworm eggs was reported in canine faecal samples which were collected in different public places in Tirana (Refugjati et al. 2012).

Filaroides martis **(respiratory nematodes)**

Canine lungworm infection did not attract much attention in the past until recently when several surveys suggested a spreading of some of those parasites in the dog population in Europe. The spectrum of respiratory nematodes of dogs comprises metastrongyloid nematodes of the genera *Crenosoma*, *Angiostrongylus*, *Filaroides* and *Oslerus*, and capillariids of the genus *Capillaria* (syn. *Eucoleus* in parte). All these nematodes parasitize in the airways of the respiratory tract of dogs but *Angiostrongylus vasorum* reside in the pulmonary arteries and right ventricle of the heart (similar to *Dirofilaria immitis* which will be reviewed later).

(Georgi and Georgi 1992; Conboy 2009; Traversa et al. 2010; Barutzki 2013; Di Cesare and Traversa 2013; Elsheika et al. 2014)

For Albania, there is only one record of canine lungworm infections: Prokopič (1960) reported the finding of *Filaroides martis* in two dogs from Saranda and Shkodër. The speciation of *F. martis* appears questionable as this species is a parasite of mustelids and has not been reported from mustelids from the Balkan peninsula as discussed previously (cf. Xhaxhiu et al. 2011). However, another filaroid lungworm, *O. osleri* was identified as parasite of wild canids in Bosnia & Herzegovina (Delić et al. 1966).

Dirofilaria **species**

Two species of the genus *Dirofilaria* are known as agents of the most relevant filarial diseases in dogs: *D. immitis*, the causative agent of heartworm disease with the adult nematodes

residing in the cardiopulmonary system, and *D. repens*, causing subcutaneous dirofilariosis. Both infections are considered as spreading emerging parasitic zoonoses in Europe and are endemic in the Balkan peninsula. *Dirofilaria* spp. have an indirect life cycle with vertebrates as definitive and culicid mosquitoes as intermediate hosts. Although both species demonstrate a low host specificity with respect to the definitive hosts, they are best adapted to canids, especially dogs. In humans, clinically relevant infections are caused mainly by *D. repens*.
(Genchi et al. 2011; Simón et al. 2012; Tasić-Otašević et al. 2015).

Canine cardiopulmonary dirofilariosis was diagnosed for the first time in Albania in the mid 1990s when 13.5% of 260 dogs from eight coastal lowland districts including Tirana were tested seropositive for circulating *D. immitis* antigen (Rapti and Rehbein 2010). These findings were confirmed through studies involving 151 and 30 dogs in the years 2007 and 2008 that established seroprevalences of 7% and 3%, respectively (Lazri et al., 2008; Hamel et al., 2009). In addition, adult *D. immitis* were recovered from the heart of one dog at necropsy (Xhaxhiu et al. 2011).
In contrast to the infection with *D. immitis*, canine *D. repens* infections are often asymptomatic and thus not noticed. For Albania, there is only one paper which reported the detection of *D. repens* DNA in the blood of 4 of 36 dogs tested by PCR (Lazri et al. 2008).
As discussed later (cf. 2.2.2.4.), several of the incriminated vectors of dirofilarial nematodes are common representatives of the culicid mosquito fauna of Albania.

Dioctophyma renale

Dioctophyma renale, the giant kidney worm, is the largest canine nematode. Its life cycle is linked to intermediate (aquatic annelids) and/or paratenic hosts (fish, frogs) found in freshwater habitats. Infection in domestic dogs is very rare.
(Georgi and Georgi 1992)

In Albania, *D. renale* has been recorded by Prokopič (1960) in three dogs from Çukë in south Albania where dogs were demonstrated also to be infected with five species of trematodes.

Indication of parasitism of filarioid nematodes other than *Dirofilaria* species

PCR analysis of *Ctenocephalides canis* fleas collected from dogs in Tirana for vector-borne pathogens detected the DNA of *Acanthocheilonema* (*Dipetalonema*) *reconditum* (Silaghi et al. 2013).

In Europe, *A. reconditum* is one of the common canine filarioid parasites with microfilariae in the blood (Genchi et al. 2011). It is a low pathogenic parasite of the subcutaneous tissues of the dog, and fleas serve as intermediate hosts (Georgi and Georgi 1992). Thus, the detection of *A. reconditum* DNA in the fleas suggests the presence of this parasite in dogs in Albania. This is further supported by the frequent diagnosis of *A. reconditum* microfilariae in the blood of dogs from the neighbouring Greece (Vakalis and Himonas 1997; Founta et al. 1999; Diakou 2000).

The identification of a single *Onchocerca* specimen removed from a persisting subconjunctival nodule in a young Albanian man in 1999 (Pampiglione et al. 2001) has been suspected to be caused by *O. lupi* (e. g., Sréter et al. 2002; Otranto et al. 2011) and thus may be indicative for the presence of this parasite in dogs in Albania. *Onchocerca lupi* is primarily associated with canids and parasitizes in the connective tissue of the eyes. Canine infections have been reported from various European countries including several cases recorded in Greece (Otranto et al. 2013) which is bordering Albania in the south and southeast.

2.2.1.2.4. Acanthocephalans (Thorny-headed Worms)

Acanthocephalans are very rare parasites of domestic dogs. They have an indirect life-cycle which requires an arthropod as intermediate host and may involve paratenic vertebrate hosts. (Georgi and Georgi 1992)

One immature *Centrorhynchus buteonis* specimen was recovered from a young dog from Tirana (Xhaxhiu et al. 2011). *Centrorhynchus* species are parasites mainly of raptorial birds and a few species are known from mammals. Most of the centrorhynchid species recorded from mammals concerned single immature specimens like this in the dog from Tirana. It was therefore believed that the dog is only an accidental host for *C. buteonis* (Xhaxhiu et al. 2011).

2.2.2. Principal Ectoparasites (Arthropods)

External or ecto parasites include a wide range of parasitic arthropods which belong to the class Arachnida with the sub-class Acari (ticks and mites) and to the class Insecta. The most relevant ectoparasites of dogs globally are ixodid ticks (Ixodidae) and fleas (Siphonaptera). In addition, there are many other species of insects (e. g., lice, biting flies, flying insects, dipteran myiasis larvae) and mites (e. g., mange mites, ear mites, hair follicle mites, fur mites, chiggers) which may infest dogs. Infestation with these arthropods can cause not only discomfort (e. g., pruritus, itching) but is capable to impair the health and well-being of dogs through causing nuisance, feeding blood, inducing dermatitis and hypersensitivity, transmitting pathogens or damaging the tissues during larval development. Apart from directly affecting the dog, some of the ectoparasites can also infest humans and are thus considered zoonotic agents. Ticks and mosquitoes are notorious as vectors of disease agents and may transmit pathogens like viruses, bacteria, protozoans and/or filarial nematodes, but fleas are known to serve as vectors of bacteriae and helminth parasites.
(Georgi and Georgi 1992)

2.2.2.1. Ixodid Ticks

Beside fleas, hard ticks (Ixodidae) constitute the most important ectoparasites of dogs worldwide being adapted to various environmental conditions. Engorgement on dogs is usually painless and the amount of blood drawn small but tick attachments may result in local skin reaction. However, the real significance of ixodid ticks infesting dogs is the broad range of pathogens that ticks can transmit. These pathogens include viral, bacterial, protozoan and nematode disease agents. In addition, some species of ticks are able to induce paralysis or toxicosis. The vast majority of ixodid ticks have a three-host life cycle, i. e., each of the three motile stages (larva, nymph, adult) feeds on a different host.
(Georgi and Georgi 1992; Jongejan and Uilenberg 2004)

In 1947, Enigk was the first to document records of ticks from Albania when he published the results of the identification of ticks collected from horses in different locations in Albania in the years 1943 and 1944: *Haemaphysalis punctata*, *Hyalomma anatolicum*, *Ixodes ricinus*, *Rhipicephalus bursa*, *R. sanguineus*, *R.* (*Boophilus*) *calcaratus*. Thereafter, the tick fauna of Albania was studied through surveys reported in the 1960s and 1970s and a few later studies, and total 14 species of ixodid ticks were recorded including four species parasitizing dogs: *Haemaphysalis concinna*, *H. punctata*, *I. ricinus* and *R. sanguineus* (Table 1).

Although infestation with ticks of dogs did not receive special attention in Albania in the past, *R. sanguineus*, known as 'brown dog tick', can be considered as the most often found species of ticks attaching to dogs in higher number than any other tick. *Rhipicephalus sanguines* was collected mainly from dogs in the coastal lowlands of Albania including cities like Tirana (Rosický et al. 1960; Luli 1963; Xhaxhiu et al. 2009; Silaghi et al. 2013). One has to bear in mind that systematic studies on ectoparasite infestation of dogs were conducted in the recent past only and considered dogs from the Tirana area exclusively (Xhaxhiu et al. 2009; Silaghi et al. 2013). These studies provided also data on the tick load and the seasonality of infestation of dogs with *R. sanguineus*. Infested dogs, which were recorded in all seasons but winter, harboured up to 331 ticks but two thirds of the dogs carried a low level of infestation of up to three ticks. *Ixodes ricinus*, which appears to be widespread in Albania including hilly areas of up to <1,000 m above sea level (Luli 1963; Gina et al. 1986; Çekani 2001), was recorded several times on dogs, usually during the colder months, but load was much lower compared to *R. sanguineus*. Only single *Haemaphysalis* spp. ticks were recorded by one author (Gina et al. 1975; Gina 1977).

As aforementioned in relation to its role as vector for *B. canis* sensu stricto, the record of the tick *D. reticulatus* from the town of Shkodër (Dhora 2006) is apparently uncertain (cf. 2.2.1.1.). However, it should be noted that there is a recent report of *D. reticulatus*-infested dogs from one neighbouring country of Albania, Macedonia (FYROM) (Pavlović et al. 2014).

Table 1: Ticks and their host associations recorded in Albania

Hosts/ Environment	*Dermacentor marginatus*	*Ixodes gibbosus* (syn. *I. candavius*)	*Ixodes ricinus*	*Haemaphysalis inermis*	*Haemaphysalis punctata*	*Haemaphysalis sulcata*	*Hyalomma aegyptium*	*Hyalomma anatolicum*	*Hyalomma detrium*	*Hyalomma marginatum*	*Rhipicephalus* (*R.*) *bursa*	*Rhipicephalus* (*R.*) *sanguineus* (s. l.)	*Rhipicephalus* (*R.*) *turanicus*	*Rhipicephalus* (*Boophilus*) *calcaratus*
Dog[1]			x	x	x							x		
Cat[2]												x		
Cattle[3]	x		x	x	x	x		x	x	x	x	x	x	x
Water buffalo[4]			x					x			x		x	
Sheep[5]	x		x	x	x	x	x	x	x	x	x	x	x	x
Goat[6]	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Horse[7]			x		x			x	x	x	x	x	x	x
Donkey[8]				x						x			x	
Rodents[9]			x	x	x			x					x	
Hedgehog[10]													x	
Reptiles[11]			x		x		x	x		x	x		x	x
Wild birds[12]			x					x						
Vegetation[13]	x		x	x	x	x	x							

[1] Rosický et al. (1960), Luli (1963), Gina (1973), Gina and Kastrati (1974), Gina et al. (1975)/Gina (1977), Xhaxhiu et al. (2009), Silaghi et al. (2013)
[2] Knaus et al. (2014), Silaghi et al. (2014)
[3] Rosický et al. (1960), Luli (1963), Gina (1973, 1983), Gina and Kastrati (1974), Gina et al. (1975), Christova et al. (2003)
[4] Rosický et al. (1960), Luli (1963), Gina et al. (1975)
[5] Rosický et al. (1960), Luli (1963), Gina (1973, 1983), Gina and Kastrati (1974), Gina et al. (1975), Zanaj et al. (2002)
[6] Černý and Rosický (1960), Rosický et al. (1960), Luli (1963), Gina (1973, 1983), Gina and Kastrati (1974), Gina et al. (1975), Koleci et al. (2011, 2013)
[7] Enigk (1947), Luli (1963), Gina and Kastrati (1974), Gina et al. (1975)
[8] Gina (1973)
[9] Rosický et al. (1960), Gina (1973), Gina and Kastrati (1974), Gina et al. (1975) [*Apodemus* spp., *Rattus* spp., *Sorex* sp.]
[10] Rosický et al. (1960)
[11] Rosický et al. (1960), Gina (1973), Gina and Kastrati (1974), Gina et al. (1975) [*Testudo* spp., *Lacerta* spp.]
[12] Rosický et al. (1960) [*Motacilla flava*]
[13] Rosický et al. (1960), Luli (1963), Gina (1973), Çekani (2001), Lika and Bërxholi (2004)

2.2.2.2. Astigmatic and Prostigmatic Mites

Most relevant ectoparasitic mites of dogs belong to the orders Astigmata (Sarcoptiformes; genera *Otodectes* and *Sarcoptes*) and Prostigmata (Trombidiformes; genera *Demodex*, *Cheyletiella* and *Neotrombicula*). Infestation with mites is transmitted mainly by direct physical contact. Notably, infestation with these mites does not only have the potential to induce clinical disease in dogs, human beings may contract infestation with *Otodectes*, *Sarcoptes* and *Cheyletiella* mites from dogs.
(Georgi and Georgi 1992; Halliwell 2013)

Dogs from Albania were identified to host three species of mites: *Sarcoptes scabiei* var. *canis*, *Otodectes cynotis* and *Demodex canis*. Prevalence of infestation in 181 less well-cared-for dogs from suburban Tirana was 4.4% for *S. scabiei* var. *canis* and 0.6% for *D. canis* while *O. cynotis* ear mites were recovered from the ear swabs taken from two of 30 dogs (Xhaxhiu et al. 2009).

2.2.2.3. Fleas

Fleas (Siphonaptera) are wingless, laterally flattened, blood-feeding insect ectoparasites of mammals and birds. Fleas generally can parasitize a range of host species and thus are able to transmit disease agents. Together with ticks, fleas are the most often recorded ectoparasites of dogs worldwide. Infestation with fleas (pulicosis) usually causes discomfort of variable degree (e. g., irritation and pruritus) to their hosts (animals and their owners) through the bites but may also cause allergic dermatitis (hypersensitivity) and anemia. Beside their role as obligate ectoparasites, fleas have an important vectorial capacity for zoonotic bacterial agents and serve as intermediate hosts of some helminth parasites of dogs. In addition, flea infestation of pets can reach sanitary relevance if flea numbers reach pest dimension. Fifteen species of fleas were recorded on domestic dogs worldwide. However, only three species are usually of significance: the ‘cat flea’, *Ctenocephalides felis*, being the most prevalent species worldwide; the ‘dog flea’, *C. canis*, and the ‘human flea’, *Pulex irritans*.
(Georgi and Georgi 1992; Bitam et al. 2010; Dobler and Pfeffer 2011)

Ctenocephalides canis, *C. felis* and *Pulex irritans* have been recorded on dogs in Albania already in the 1950s and 1960s (Rosicky and Gjino 1960; Kero and Gina 1974). All three species of flea were found in two recently conducted studies which examined the infestation with fleas of dogs from suburban areas in Tirana systematically through whole body combing (Xhaxhiu et al. 2009; Silaghi et al. 2013). Xhaxhiu et al. (2009) demonstrated infestation with fleas in more than 75% of 181 dogs examined. Infestation with *C. canis* was most prevalent (75.7%; *P. irritans*, 8.3%; *C. felis*, 5.0%). Considering the studies of both Xhaxhiu et al. (2009) and Silaghi et al. (2013), fleas were found infesting dogs year-round, and *C. canis*, *C. felis* and *P. irritans* made 97.7%, 1.5% and 0.8%, respectively, of the total 2383 fleas collected from 205 dogs. Individual flea load ranged up to 192 for *C. canis* while no more than three specimens per dog of *C. felis* and *P. irritans* were found, always in mixed infestation with *C. canis*.

As reviewed elsewhere (cf. 2.2.1.2.2., 2.2.1.2.3. and 2.2.5.), DNA of several bacterial agents and helminth parasites was detected in *C. canis* and *C. felis* fleas which were collected from dogs in Albania.

2.2.2.4. Insect Ectoparasites other than Fleas

Only recently for the first time in Albania, infestation with the mallophagan louse, *Trichodectes canis*, has been reported in 12 of 181 dogs (6.6%) examined for ectoparasite infestation (Xhaxhiu et al. 2009).

Dogs are hosts to species of both major groups of lice (Phthiraptera): the mallophagans or chewing lice, which ingest epidermals scales and hairs, and the anoplurans or sucking lice, which are obligatory blood feeders. Lice are permanent ectoparasites. Presence of clinical signs of infestation is dependent of the louse burden. Heavy infestations are only seen in poorly nourished puppies or debilitated older dogs.

(Georgi and Georgi 1992)

During the expedition of the Czechoslovak Academy of Sciences to Albania, one ‘louse fly’, *Hippobosca equina* (Hippoboscidae), was collected from a dog in Shkodër (Danielova 1960).

Hippoboscids are blood-feeding dipterous insects which parasitize birds and mammals. For Albania, the occurrence of four species of hippoboscids has been documented: *H. equina*, *Lipoptena cervi*, *Melophagus ovinus* and *Ornithoica turdi* (Danielova 1960; Leclerq 1963). *Hippobosca equina* is primarily a parasite of equines and bovines but was considered as accidental parasite of dogs (Maa 1969).

There is one hippoboscid fly, which is a true parasite of carnivores including dogs: *H. longipennis*. Interestingly, this species has records from several countries of southern and southeastern Europe too (Bulgaria, Greece, Hungary, Italy) (Maa 1969).

Indication of parasitism by mosquitoes

Although not studied in Albania for their relationship to dogs (there are no capture studies available), the endemicity in dogs of leishmaniosis and dirofilarial infections (cf. 2.2.1.1. and 2.2.1.2.3.) indicates that phlebotomine sandflies (Diptera, Nematocera, Psychodidae) and culicid mosquitoes (Diptera, Nematocera, Culicidae) are seeking dogs for feeding blood (to take up macrophages parasitized by amastigotes or microfilariae, respectively, and to transmit promastigotes or infective third-stage larvae, respectively).

Several studies have shown that four of the 20 phlebotomine species that are implicated in the transmission of *L. infantum* (Maroli et al. 2013) are abundant in Albania: *Phlebotomus neglectus*, *P. papatasi*, *P. perfiliewi* and *P. tobbi* with *P. neglectus* being the most widespread species (Adhami 1960, 1986, 1991, 2000; Adhami et al. 1988; Velo et al. 2003, 2005, 2010; Bongiorno et al. 2011; Papa et al. 2010; Lika 2011; Maroli et al. 2012). In addition, testing pools of sandflies collected in Albania with nested PCR revealed genomic *Leishmania* DNA in *P. neglectus* and *P. tobbi* (Bongiorno et al. 2011; Maroli et al. 2012).

Similarly, numerous studies documented the fauna of mosquitoes of the family Culicidae in Albania (Bates 1937, 1941; Lewis 1939; Marcuzzi 1943; Danielova and Adhami 1960; Adhami 1974, 1997; Adhami and Murati 1975, 1987a; Adhami and Reiter 1998; Velo et al. 2009; Rogozi et al. 2012; Rogozi 2013; Shkurti 2013) and revealed the presence of several species which have been described as competent or potential vectors for *Dirofilaria* spp. including the tiger mosquito, *Aedes albopictus* (Cancrini and Gabrielli 2007; Morchón et al. 2012).

2.2.3. Indication of Pentastomid Infection

Canids are definitive hosts of *Linguatula serrata* which live as adult in the nasopharyngeal region (nasal and paranasal cavities); herbivorous mammals serve as intermediate hosts in the life cycle of these pentastomids. The infection in dogs and wild canids is known from several countries on the Balkans in the neighbourhood of Albania, e. g., Bosnia & Hercegovina, Bulgaria, Greece, Romania and Serbia (Haralabidis et al. 1988; Nesterov et al. 1991; Jažić et al. 1992-1995; Gherman et al. 2002; Negrea 2005; Kirkova et al. 2013a; Csordás et al. 2014) but there is, to the knowledge of the author, no record from dogs, other canids or potential intermediate hosts in Albania. However, a single *L. serrata* larva (nymph) has been recently isolated from the lungs of one of 73 cats from Tirana (Knaus et al. 2012). This finding indicates that *L. serrata* is likely to be present in canids in Albania because the life cycle of this parasite requires canids to be maintained.

Pentastomids are worm-like endoparasites ('tongue worms') which are phylogenetically related to arthropods. However, their taxonomic position is unclear and a matter of ongoing discussion. Adults of most species reside in the upper respiratory tract of terrestrial vertebrates (mainly reptiles but also mammals, birds and amphibians). Most species have an indirect, two-host life cycle.

(Böckeler et al. 2010; Christoffersen and De Assis 2015)

2.2.4. Summary of Parasites Identified in Dogs from Albania

Tables 2, 3 and 4 summarize the parasites identified in dogs from Albania in the past.

Table 2: Species of protozoan endoparasites recorded in dogs in Albania

Phylum	Class	Order	Family	Species[1]
Metamonada	Trepomonadea	Diplomonadida	Hexamitidae	*Giardia canis* (*G. duodenalis* assemblages C and D)
Euglenozoa	Kinetoplastea	Trypanosomatida	Trypanosomatidae	*Leishmania infantum* (zymodeme MON-1)
Alveolata	Coccidea	Eimeriida	Eimeriidae	*Cystoisospora canis* *Cystoisospora ohioensis/burrowsi*
			Sarcocystidae	*Sarcocystis* spp.
		Adeleida	Hepatozoidae	*Hepatozoon canis*
	Haematozoea	Piroplasmida	Babesiidae	*Babesia canis* *Babesia vogeli*

[1] Records documented by Cani et al. (2001a, b), Lazri et al. (2008), Hamel et al. (2009), Xhaxhiu et al. (2011), Gouzelou et al. (2013) and/or Sommer et al. (2015)

Table 3: Species of helminth endoparasites recorded in dogs from Albania

Phylum	Class	Order	Family	Species[1]
Plathelminthes	Digenea	Echinostomida	Echinostomatidae	*Isthmiophora melis*
		Opisthorchiida	Opisthorchiidae	*Opisthorchis felineus*
			Heterophyidae	*Parascotyle longa* *Rossicotrema donicum*
		Strigeida	Diplostomidae	*Alaria alata*
	Cestodea	Cyclophyllidea	Dipylidiidae	*Dipylidium caninum*
			Taeniidae	*Taenia hydatigena* *Taenia pisiformis* *Taenia multiceps* *Echinococcus granulosus* (genotype G1)
Nematoda	Secernentea	Strongylida	Ancylostomatidae	*Ancylostoma caninum* *Uncinaria stenocephala*
			Filaroididae	*Filaroides martis* (misdiagnosed?)
		Ascaridida	Ascarididae	*Toxascaris leonina* *Toxocara canis*
		Spirurida	Spirocercidae	*Spirocerca lupi*
			Onchocercidae	*Dirofilaria immitis* *Dirofilaria repens*
	Adenophorea	Enoplida	Diotophymatidae	*Dioctophyme renale*
			Trichuridae	*Trichuris vulpis*
Acanthocephala		Polymorphida	Centrorhynchidae	*Centrorhynchus buteonis*

[1]Records documented by Moskvin (1958), Prokopič (1960), Pepa (1987) and/or Xhaxhiu et al. (2011)

Table 4: Species of ectoparasites recorded in dogs in Albania

Phylum	Class	Order	Family	Species[1]
Arthropoda	Arachnida (sub-class: Acari)	Ixodida (Metastigmata)	Ixodidae	*Haemaphysalis inermis* *Haemaphysalis punctata* *Ixodes ricinus* *Rhipicephalus sanguineus*
		Prostigmata (Trombidiformes)	Demodicidae	*Demodex canis*
		Astigmata (Sarcoptiformes)	Psoroptidae Sarcoptidae	*Otodectes cynotis* *Sarcoptes scabiei*
	Insecta	Phthiraptera (Ischnocera)	Bovicolidae	*Trichodectes canis*
		Diptera	Hippoboscidae	*Hippobosca equina* (accidental)
		Siphonaptera	Pulicidae	*Ctenocephalides canis* *Ctenocephalides felis* *Pulex irritans*

[1] Records documented by Danielova (1960), Rosický and Gjini (1960), Rosický et al. (1960), Luli (1963), Gina (1973), Gina and Kastrati (1974), Kero and Gina (1974), Gina et al. (1975)/Gina (1977), Xhaxhiu et al. (2009) and/or Silaghi et al. (2013)

2.2.5. Vector-borne Pathogens other than Parasites

In principal, a wide range of pathogens, comprising viruses, bacteria including rickettsiae, protozoans and helminths are transmitted by a variety of arthropods and may cause so-called vector-borne diseases. Ticks, fleas and flying insects, e. g., phlebotomine sandflies and mosquitoes, may serve as vectors for these pathogens which eventually may be zoonotic and thus represent a public health concern.

Apart from the before reviewed vector-borne parasites (cf. 2.2.1.1. and 2.2.1.2.3.), there is evidence that dogs in Albania are at risk of infection with *Ehrlichia canis* and *Anaplasma* spp. (Rickettsiales: Anaplasmataceae).

Bizhga et al. (2006) reported on three dogs diagnosed with ehrlichiosis by the examination of Giemsa-stained blood smears while Hamel et al. (2009) confirmed the presence of *E. canis* in the blood of dogs by PCR and found antibodies (IFAT) in the serum of 15 of 30 dogs.

Using a commercial, dog-approved IFAT test kit for the diagnosis of *A. phagocythophilum* antibodies, Hamel et al. (2009) found a seroreactivity of 40% when testing samples of 30 less well-cared-for dogs. However, it should be considered that serum of dogs infected with *A. platys* may cross-react with the *A. phagocytophilum* antigen in the IFAT (Beaufils et al. 2002). Canine *A. platys* infections were diagnosed in several countries of the Mediterranean basin including Greece and Croatia being geographically closest to Albania (Mylonakis et al. 2004; Dyachenko et al. 2012).

The occurrence of both *E. canis* and *A. platys* is associated to the presence of their vector, the tick *R. sanguineus* (Otranto and Dantas-Torres 2010). Silaghi et al. (2013) found DNA of the two agents in *R. sanguineus* ticks collected from dogs in Tirana and earlier already, Christova et al. (2003) had reported the detection of *E. canis* DNA in one *R. sanguineus* tick collected from cattle. *Anaplasma phagocythophilum*, one of the most common tick-borne agents in whole Europe and the agent of canine granulocytic anaplasmosis, is transmitted by *Ixodes* species (Stuen et al. 2013).

Indication of additional non-parasite vector-borne pathogens

Screening ectoparasites collected from less well-cared-for dogs in Tirana for selected vector-borne agents by PCR revealed the presence of several bacterial pathogens (Silaghi et al. 2013). Apart from *A. platys* and *E. canis* (see before), *Rickettsia conorii* and *Rickettsia* spp. were detected in *R. sanguineus* ticks, haemotropic mycoplasmas were identified in *C. canis* fleas, and *Rickettsia felis* was found in *C. felis* fleas.

Rickettsia felis, which may cause spotted fever rickettsiosis in humans, has been detected in *C. felis* fleas of cats from Tirana previously (Silaghi et al. 2012). The detection of DNA of *R. conorii* and *Rickettsia* spp. in the *R. sanguineus* ticks suggests the presence of these pathogens in the dogs sampled and thus implicates a risk of infection for humans.

Although arthropod-borne rickettsioses are considered as one of the most important causes of systemic febrile illness in humans globally (Bitam et al. 2010; Parola et al. 2013), the situation regarding rickettsial diseases in Albania is largely unknown. However, examination of human sera collected in the late 1950s revealed antibodies to the antigen of spotted fever rickettsiae (Brezina et al. 1961) and over the years of 1960 to 2001, 'rickettsioses' were documented with approximately 33 cases per year by the Institute of Public Health (Kakarriqi 2002). Recently, in one of 34 patients with suspected Crimean Congo Haemorrhagic Fever, 'rickettsiosis' was diagnosed by serological test (Papa et al. 2008). In Albania, *R. conorii* and *R. helvetica* have been isolated previously from *H. marginatum*, *R. bursa* and *R. sanguineus* ticks which were collected from cattle (Christova et al. 2003).

The finding of *Mycoplasma haemocanis* and *Candidatus* M. turicensis is an indication for the occurrence of these haemotropic mycoplasmas in dogs in Albania. Canine *M. haemocanis* infections have been reported from the neighbouring Greece (Tennant et al. 2011), and the incriminated vector, *R. sanguineus* (Senevira et al. 1973), is widely distributed in the region.

3. MATERIAL and METHODS

3.1. General Information on Material Collection and Examination

Samples of the study population were collected continuously from March 2010 to April 2011 inclusive. Dog owners were invited to participate with their dogs in the study when visiting four small animal clinics in Tirana, Albania. Faeces, ear swabs, ectoparasites collected by body search and full body combing, and cephalic vein blood samples were obtained from 602 dogs with the informed consent of their owners. A questionnaire was completed for each dog at the time of the visit in the clinics. Apart from the date of sampling, this included information on the dog's breed, age, gender, dog's purpose (main use), habitat and environment, presence of other dogs or cats in household, type of food, history of the use of anthelmintics and of measures for ectoparasite control (see Appendix). Although several dogs were presented repeatedly during the study period, for the purpose of the study the findings of the first visit were considered only.

The methodology of examination of faeces and blood and serum samples is described in the Material and Methods sections of the two publications which report the results of this study (see 4.1.1. and 4.1.2.) while the methodology of the examination for ectoparasite infestation is detailed below.

Examination for ectoparasite infestation

All dogs were examined for ectoparasite infestation by a full body search, and the whole body was combed with a stainless steel fine-toothed flea comb (Zakson et al. 1995). Ticks were manually removed and collected together with any fleas and lice in the comb. The ticks, fleas and lice removed from the dogs were stored in 70% ethanol until they were identified. In eight dogs, lesions suspicious of mite infestation (characterized by scaling, scores, dermal encrustations and hair loss) were observed during the body search and scrapings were taken from the altered skin. In addition, deep ear swab specimens were obtained from both ears from all dogs.

Ticks, fleas and lice were identified with a dissection microscope. Skin scrapings and ear swabs were placed in 10% caustic potash and gently heated to macerate scales, crusts and hair or aural material. Thereafter, the material was centrifuged, and the digest's sediment was examined with a compound microscope under 100X and 400X magnification. For speciation of ectoparasites, existing descriptions and/or keys were used (Jancke 1938; Babos 1964; Lohse et al. 2002; Estrada-Peña et al. 2004). Brown dog ticks of the *R. sanguineus* group or complex (Dantas-Torres et al. 2013; Gray et al. 2013) were recorded as *R. sanguineus* s. l.

3.2. Description of the Study Population (Demographic Data)

The dogs sampled were from 498 private owners (526 dogs), four dog breeders (41 dogs, mainly English Setters and German Shepherd dogs) and one police dog training school (35 German Shepherd dogs). From the dogs included in the study, 516 were from Tirana and surrounding district and 86 dogs from other cities (Durrës, Lushnjë, Shkodër, Vlorë, Librazhd and Fier).

A wide range of breeds and ages was sampled. Pure breed dogs (approximately 85%), dogs >1 year old (approximately 77%), pet dogs (approximately 71%) and dogs living in the city (almost 50%) were the most common categories of dogs. For the whole study period, the number of dogs sampled per month ranged from 31 to 49. Dogs of both age categories (dogs ≤1 year/dogs >1 year) were similarly represented in the four seasons of examination: winter, 24/96 dogs; spring, 57/163 dogs; summer, 34/99 dogs; autumn, 23/106 dogs ($p=0.2498$).

Approximately half of the dogs (306, 50.8%) were presented to the clinics for routine measures (e. g., general health check, vaccination, routine worming/ectoparasite control, examination to fulfil regulations governing the travel of pets/custom clearance); other reasons for admission to the clinics were grouped into the categories surgery (e. g., injuries, trauma, neoplasia) - 107 dogs (17.8%), dermatological conditions - 55 dogs (9.1%), general abnormal condition - 44 dogs (7.3%), gastrointestinal conditions including vomiting - 39 dogs (6.5%), cardiopulmonary conditions - 37 dogs (6.1%), urinary conditions - 14 dogs (2.3%). Information of the dogs sampled and their characteristics is summarized in Table 5.

Table 5: Information of the dogs sampled and their characteristics

Factor	Group and sample size
Breed	Pure breed dogs, 515 (3 Akita, 3 Beagle, 1 Bobtail, 1 Bolognese, 4 Boxer, 1 Chow-Chow, 6 Cocker Spaniel, 1 Collie, 3 Dalmatian, 3 German Shorthaired Pointer, 8 Dobermann Pinscher, 61 German Shepherd, 7 hounds, 2 Illyrian Sheepdog, 1 Jack Russell Terrier, 9 Labrador Retriever, 2 Maltese, 5 Metis, 1 Miniature Spitz, 82 Pekingese, 45 Pit Bull, 3 poodles, 49 Rottweiler, 1 Samoyed, 53 Setter dog, 2 Shar Pei, 12 Shih Tzu, 17 Siberian Husky, 45 Spitz-type, 2 St. Bernard, 81 terriers, 1 Yorkshire Terrier).
	Mixed breed dogs, 87.
Age	Mean age 3.48 years (standard deviation 2.87; range, approximately one-and-a-half months to 20 years).
	≤1 year, 138 dogs; >1 year, 464 dogs.
Gender	Male (entire and neutered), 334; female (entire and neutered), 268.
Dog's purpose	Pet dog, 425; hunting dog, 52; working [guard, police] dogs, 125.
Dog's habitat	City, 297 dogs; suburban, 200 dogs; rural, 105 dogs.
Dog's environment	Mainly indoors, 36 dogs; indoors with regular outside walking, 222 dogs; yard, 103 dogs; kennel/run, 241 dogs.
Other pets in household	'Yes', 256 dogs; 'No', 346 dogs.
Type of food (feeding habit)	Cooked/canned/dry, 479 dogs; raw meat, 22 dogs; both, 101 dogs.
History of anthelmintic use	Use of anthelmintics, if any: 'Yes', 426 dogs; 'No', 176 dogs.
	If 'Yes': 1-2 times per year, 239 dogs; >2 times per year, 187 dogs.
History of ectoparasiticide use	Use of ectoparasiticides, if any: 'Yes', 360 dogs; 'No', 242 dogs.
	If 'Yes': 1-2 times per year, 197 dogs; >2 times per year, 163 dogs.
Season of examination	Winter (December-February), 120 dogs; spring (March-May), 220 dogs; summer (June to August), 133 dogs; autumn (September-November), 129 dogs.

3.3. Data Analysis

A commercially available statistical software package was used for all analyses (R, version 2.13.1; R Development Core Team, 2010). The prevalence was calculated with the 95% Clopper-Pearson confidence intervals. Associations between positivity for parasitism and variables representing reasons for admission to the clinics, host demographic and management factors and season of examination were assessed univariately using contingency tables and the chi squared test or the Fisher's exact test, as appropriate. Variables with a p-value of less than 0.2 for the association with the outcome variable in the single variable analysis and potential factors (breed type, gender, age, dog's purpose, dog's habitat, dog's environment, presence of other pets, type of food, anthelmintic treatment/ectoparasiticide use, season of examination) were included into a multivariate logistic regression model to evaluate the adjusted effects of the associated factors. Level of significance for all analyses was set at $p<0.05$.

4. RESULTS

Results are presented and discussed in Publications 1 (published online, see 4.1.1.) and 2 (accepted, see 4.1.2.) in Parasitology Research (Impact Factor 2014, 2.098), and in Chapter 4.2.

4.1. Publications

4.1.1. Publication 1: Parasites and vector-borne diseases in client-owned dogs in Albania. Intestinal and pulmonary endoparasite infections

Shukullari E, Hamel D, Rapti D, Pfister K, Visser M, Winter R, Rehbein S

Parasites and vector-borne diseases in client-owned dogs in Albania. Intestinal and pulmonary endoparasite infections.
Parasitology Research (DOI 10.1007/s00436-015-4704-8)

Submitted:	30 July 2015
Accepted:	25 August 2015
Published online:	08 September 2015

Parasitol Res
DOI 10.1007/s00436-015-4704-8

ORIGINAL PAPER

Parasites and vector-borne diseases in client-owned dogs in Albania. Intestinal and pulmonary endoparasite infections

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Received: 30 July 2015 / Accepted: 25 August 2015

Abstract From March 2010 to April 2011 inclusive, feces from 602 client-owned dogs visiting four small animal clinics in Tirana, Albania, were examined using standard coproscopical techniques including *Giardia* coproantigen ELISA and immunofluorescent staining of *Giardia* cysts. Overall, samples of 245 dogs (40.7 %, 95 % CI 36.6–45.6) tested positive for at least one type of fecal endoparasite (protozoan and/or helminth and/or pentastomid) stage, of which 180 (29.9 %, 95 % CI 26.3–33.7) and 129 (21.9 %, 95 % CI 18.2–24.9) tested positive for protozoan or nematode endoparasites, respectively. Fecal forms of at least 14 endoparasites were identified. The most frequently identified stages were those of *Giardia* (26.4 %), *Trichuris* (9.5 %), *Toxocara* (8.0 %), hookworms (7.1 %), *Cystoisospora ohioensis* (4.3 %), and *Cystoisospora canis* (3 %). For the first time for dogs in Albania, fecal examination indicated the occurrence of *Hammondia*/*Neospora*-like (0.2 %), *Angiostrongylus* lungworm (0.3 %), capillariid (2.8 %), and *Linguatula* (0.2 %) infections. Single and multiple infections with up to seven parasites concurrently were found in 152 (25.2 %, 95 % CI 21.8–28.9) and 93 dogs (15.4 %, 95 % CI 12.7–18.6), respectively. On univariate analysis, the dog's age, the dog's purpose (pet, hunting dog, working dog), the dog's habitat (city, suburban, rural), and environment (mainly indoors, indoors with regular outside walking, yard, kennel/run), presence/absence of other dogs and/or cats, history of anthelmintic use, and season of examination were identified as significant ($p<0.05$) factors predisposing dogs to various types of endoparasitism while the variables breed (pure breed dogs vs. mixed-breed dogs), gender, and type of food were not significant predictors. Multivariate logistic regression analysis for factors associated with overall endoparasitism revealed that dogs >1 year of age (odds ratio [OR]=0.64), dogs dewormed at least once per year (OR=0.35), and dogs tested during spring, summer, and autumn (OR=0.51, 0.15, and 0.20, respectively) had a significantly lower risk compared with ≤1 year old dogs, dogs not dewormed, or dogs tested during winter. The odds of a dog to be diagnosed positive for endoparasites was 1.56 times higher for dogs living together with other pets than that for a dog without other dogs or cats.

Keywords Dog · Intestinal nematodes · Cestodes · Intestinal protozoans · *Angiostrongylus* · Risk factors

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Introduction

Located in the Balkan Peninsula in southeastern Europe, Albania has experienced major changes in the political and economic structures since the early 1990s. Significant demographic, social, and cultural changes including urbanization and modernization have occurred in recent times, and companion animal ownership is becoming more popular in Albania, particularly in cities. As pets are a relatively recent phenomenon, veterinary service for companion animals is still developing with several small animal clinics established, and veterinarians starting to specialize in the care of dogs and cats. In the capital Tirana, for instance, there are currently approximately 20 small animal clinics and two hospitals for small animals.

Several aspects on the occurrence of parasites in dogs and cats in Albania have been studied in the recent past (Cicko and Cani 1998; Cicko et al. 1999; Sotira 2000; Dhamo and Zanaj 2003; Dhamo et al. 2006; Zanaj et al. 2006; Lazri et al. 2008; Hamel et al. 2009, Xhaxhiu et al. 2009, 2011; Rapti and Rehbein 2010; Knaus et al. 2011, 2012, 2014; Refugjati et al. 2012; Silaghi et al. 2012, 2013, 2014; Bizhga et al. 2013; Bocari et al. 2014). The results of those studies revealed the presence of a broad spectrum of both endo- and ectoparasites as well as other vector-borne pathogens including species which not only may impact the health of the animals but are considered zoonotic with some of them posing serious concerns to the public health, e.g., leishmaniosis and cystic echinococcosis. These two diseases, which both have diverse clinical manifestations, are responsible for a degree of morbidity of the population in Albania (e.g., Gjoni et al. 2007; Pipero 2012).

Despite the increase in Albania during the past years of the number of dogs kept as companion animals, there are no data reporting the situation regarding parasite infections and vector-borne diseases in this category of animals. Therefore, we prospectively conducted a survey in the years 2010 and 2011 to assess the status of client-owned, veterinary cared-for dogs in Albania with respect to the presence of or exposure to parasite infections and selected arthropod-borne pathogens. History and demographic risk factors which may play a role in the epidemiology of these infections in the dogs were also assessed, in the interests of the improvement of animal care, parasite control, and public health.

This paper presents data on the prevalence of canine parasites of the intestinal and respiratory tracts detected by examination of fecal samples while the results of the examination of the dogs for ectoparasite infestation and blood pathogens and seroprevalence of selected parasitic and infectious diseases are reported separately (Hamel et al. 2015; Shukullari et al. 2015).

Material and methods

Sample collection

The study lasted from March 2010 to April 2011 inclusive. Dog owners were invited to participate with their dogs in the study when visiting four small animal clinics in Tirana, Albania. Feces, ear swabs, ectoparasites collected by body search and full body combing, and cephalic vein blood samples were obtained from 602 dogs with the informed consent of their owners. Although several dogs were presented repeatedly during the study period, for the purpose of the study the findings of the first visit were considered only. The dogs were from 498 individual private owners (526 dogs), 4 dog breeders (41 dogs, mainly English Setters and German Shepherd dogs), and 1 police dog training school (35 German Shepherd dogs).

Approximately half of the dogs (306, 50.8 %) were presented to the clinics for routine measures (e.g., general health check, vaccination, routine worming/ectoparasite control, examination to fulfill regulations governing the travel of pets/custom clearance); other reasons for admission to the clinics were grouped into the categories surgery (e.g., injuries, trauma, neoplasia)—107 dogs (17.8 %), dermatological conditions—55 dogs (9.1 %), general abnormal condition—44 dogs (7.3 %), gastrointestinal conditions including vomiting—39 dogs (6.5 %), cardiopulmonary conditions—37 dogs (6.1 %), and urinary conditions—14 dogs (2.3 %). A simple questionnaire was completed for each dog at the time of the visit in the clinics. Apart from the date of sampling, this included information on the dog's breed, age, gender, dog's purpose (main use), habitat and environment, presence of other dogs or cats in household, type of food, history of the use of anthelmintics, and of measures for ectoparasite control.

Examination of feces

The fresh fecal samples were broken down and examined first grossly for the presence of expelled nematodes and cestode segments. Then, all samples were tested for helminth eggs and protozoan oocysts and/or sporocysts using a standardized combined sedimentation/centrifugal floatation technique which is described briefly here. Two grams of feces was suspended thoroughly in a small amount of water in a mortar, and the fecal suspension was then poured through a fine tea strainer and the strained fluid caught in a conical measuring cylinder. Additional water was poured through the strainer to fill the conical cylinder up to 500 mL. The debris left on the strainer was discarded, and the suspension was allowed to stand for 30 min. The supernatant was then removed with a water-jet aspirator pump such that 10 mL suspension including the sediment remained. Following agitation, 1 mL of the sediment-suspension was aspirated and transferred to a 10-mL centrifuge tube. Zinc sulfate solution with a specific gravity adjusted to 1.3 was added to 0.5 cm from the top of the tube such that the sediment-suspension was mixed well with floatation fluid. The tube was then centrifuged at 300 G for 5 min. Using a wire loop with 5 mm in diameter, three drops of the floated material was taken from the surface film of the tube and transferred to a glass slide. The preparation was covered with one coverslip per drop. One slide with three drops per samples was examined with a compound microscope under ×100 and ×400 magnification to provide total fecal parasite stage counts per sample. In an attempt to estimate the density of infection, total fecal parasite stage counts were stratified as "very few," "few," "moderate," or "numerous" corresponding to 1–15, 16–30, 31–60, or more than 60 parasite stages per three drops. In addition, 5 g of feces of each sample was subjected to the Baermann technique for the detection of lungworm larvae.

Identification of fecal stages was based on morphology of the fecal forms alone; thus, identification in most instances was not down to species. All *Cystoisospora* oocysts smaller than those of *Cystoisospora canis* were considered as *Cystoisospora ohioensis* oocysts because oocysts of *Cy. ohioensis*, *Cystoisospora burrowsi*, and *Cystoisospora neorivolta* cannot be distinguished based on morphological characteristics (Lindsay et al. 1997). In addition, because of the similarity of the oocysts of *Hammondia heydorni* and *Neospora caninum* (Dubey et al. 2002), oocysts exhibiting this morphology were documented as *Hammondia/Neospora*-like oocysts. No attempt was made to identify *Giardia* cysts by the combined sedimentation/floatation technique as *Giardia* infections were diagnosed by antigen ELISA and immunofluorescence staining. For testing for *Giardia* infection, an aliquot of feces was stored frozen at approximately −20 °C until analysis. Samples were screened with a commercial ELISA kit for the presence of the *Giardia*-specific antigen GSA65 (PROSPECT® *Giardia* MicroPlate Assay; Oxoid Ltd, Basingstoke Hants, UK), and samples that tested positive by the ELISA were analyzed subsequently for the presence of *Giardia* cysts by immunofluorescence staining using FITC-conjugated *Giardia*-specific monoclonal antibodies (MERIFLUOR® *Cryptosporidium/Giardia* Test Kit; Meridian Bioscience, Inc.; Cincinnati, USA) as described previously (Capári et al. 2013).

Statistical analysis

Statistical analyses were performed using software package R, version 2.13.1 (R Development Core Team 2010). The prevalence was calculated with the 95 % Clopper-Pearson confidence intervals (95 % CI). Associations between positivity for parasitism and variables representing reasons for admission to the clinics, host demographic and management factors, and season of examination were assessed univariately using contingency tables and the chi squared test or the Fisher's exact test, as appropriate. Variables with a p value of less than 0.2 for the association with the outcome variable in the single variable analysis and potential factors (breed [pure breed vs. mixed breed], gender, age, dog's purpose, dog's habitat, dog's environment, presence of other pets, type of food, anthelmintic treatment, season of examination) were included into a multivariate logistic regression model to evaluate the adjusted effects of the associated factors. Level of significance for all analyses was set at $p<0.05$.

Results

Study population and characteristics

Samples of the study population were collected continuously from March 2010 to April 2011 inclusive. A wide range of breeds and ages was sampled. Pure breed dogs (approximately 85 %), dogs >1 year old (approximately 77 %), pet dogs (approximately 71 %), and dogs living in the city (almost 50 %) were the most common categories of dogs (Table 1). For the whole study period, the number of dogs sampled per month ranged from 31 to 49. Dogs of both age categories (dogs ≤1 year/dogs >1 year) were similarly represented in the four seasons of examination: winter, 24/96 dogs; spring, 57/163 dogs; summer, 34/99 dogs; autumn, 23/106 dogs (p= 0.2498).

The query regarding the use of anthelmintics identified exclusively broad spectrum oral wormers containing combinations of nematocidal and cestocidal compounds, i.e., niclosamide + levamisole, niclosamide + oxybendazole, febantel + pyrantel + praziquantel, febendazol + pyrantel + praziquantel, pyrantel + praziquantel.

Prevalence of endoparasite infections, occurrence of multiple parasitism, intensity of shedding of fecal stages

Total prevalence and range of counts of stages of endoparasites detected by coproscopical examination and the rate of *Giardia* cysts shedding dogs are summarized in Table 2. Of the 602 samples examined, 245 (40.7 %, 95 % CI 36.6–45.6) tested positive for at least one type of fecal endoparasite (protozoan and/or helminth and/or pentastomid) stage with 180 (29.9 %, 95 % CI 26.3–33.7) and 129 (21.9 %, 95 % CI 18.2–24.9) testing positive for protozoan and nematode endoparasites, respectively. Fecal forms of at least 14 endoparasites were identified by immunofluorescent staining (*Giardia* cysts), floatation of protozoan stages (*Cystoisospora* and *Hammondia/Neospora*-like oocysts, *Sarcocystis* sporocysts), eggs of nematodes (*Toxocara*, *Toxascaris*, hookworm, *Trichuris*, capillariid), trematodes (*Dicrocoelium*), taeniid cestodes and the pentastomid (*Linguatula*) (Fig. 1), and the extraction of *Angiostrongylus* lungworm larvae by the Baermann-Wetzel migration technique. Overall, *Giardia* infection was the most evident parasitism in the dogs, followed by intestinal nematode parasitism (*Trichuris*, 9.5 %; *Toxocara*, 8.0 %; hookworm, 7.1 %). For diagnosis of *Giardia* infection, screening with the antigen ELISA revealed *Giardia* antigen in 214 of the 602 fecal samples (35.5 %), and *Giardia* cysts were detected in 152 of the 214 *Giardia* antigen-positive samples (74.3 %).

Among the 245 of the 602 dogs with positive results for endoparasites, identification of stages of just one endoparasite indicated the presence of single parasite infections in most of the dogs (n=152; 25.2 %, 95 % CI 21.8–28.9), whereas the results for 93 dogs (15.4 %, 95 % CI 12.7–18.6) were

Table 1 Information of the dogs sampled and their characteristics

Factor	Group and sample size
Breed	Pure breed dogs, 515 (3 Akita, 3 Beagle, 1 Bobtail, 1 Bolognese, 4 Boxer, 1 Chow-Chow, 6 Cocker Spaniel, 1 Collie, 3 Dalmatian, 3 German Shorthaired Pointer, 8 Dobermann Pinscher, 61 German Shepherd, 7 hounds, 2 Illyrian Sheepdog, 1 Jack Russell Terrier, 9 Labrador Retriever, 2 Maltese, 5 Metis, 1 Miniature Spitz, 82 Pekingese, 45 Pit Bull, 3 poodles, 49 Rottweiler, 1 Samoyed, 53 Setter dog, 2 Shar Pei, 12 Shih Tzu, 17 Siberian Husky, 45 Spitz-type, 2 St. Bernard, 81 terriers, 1 Yorkshire Terrier)
	Mixed-breed dogs, 87
Age	Mean age, 3.48 years (standard deviation, 2.87; range, approximately 1.5 months to 20 years)
	≤1 year, 138 dogs; >1 year, 464 dogs
Gender	Male (entire and neutered), 334; female (entire and neutered), 268
Dog's purpose	Pet dog, 425; hunting dog, 52; working [guard, police] dogs, 125
Dog's habitat	City, 297 dogs; suburban, 200 dogs; rural, 105 dogs
Dog's environment	Mainly indoors, 36 dogs; indoors with regular outside walking, 222 dogs; yard, 103 dogs; kennel/run, 241 dogs
Other pets in household	"Yes," 256 dogs; "No," 346 dogs
Type of food (feeding habit)	Cooked/canned/dry, 479 dogs; raw meat, 22 dogs; both, 101 dogs
History of anthelmintic use	Use of anthelmintics, if any: "Yes," 426 dogs; "No," 176 dogs
	If "Yes": 1–2 times per year, 239 dogs; >2 times per year, 187 dogs
History of ectoparasiticide use	Use of ectoparasiticides, if any: "Yes," 360 dogs; "No," 242 dogs
	If "Yes": 1–2 times per year, 197 dogs; >2 times per year, 163 dogs
Season of examination	Winter (December–February), 120 dogs; spring (March–May), 220 dogs; summer (June to August), 133 dogs; autumn (September–November), 129 dogs

indicative for multiple infections with two to seven parasites concurrently (Table 3).

Stratifying the counts of the fecal stages into four categories as a measure of the density of infection revealed that the vast majority of dogs testing positive for protozoan and/or helminth infections was shedding very few fecal parasite stages (Table 4).

Testing of prevalence of any endoparasitism vs. grouped reasons for admission to the clinics or primary diagnoses (routine measures, gastrointestinal conditions including vomiting,

Table 2 Prevalence and number of parasite stages in the feces of 602 client-owned dogs from Albania as determined by conventional coproscopic examination or immunofluorescent staining (*Giardia* cysts only), respectively

Parasite stage	Prevalence			Range of counts of parasite stages[b]
	Total	%	95 % CI[a]	
Giardia cysts	159	26.4	31.4–40.6	NA[c]
Cystoisospora canis oocysts	18	3.0	1.8–4.7	1–651
C. ohioensis oocysts	26	4.3	2.9–6.4	1–61
Sarcocystis sporocysts	2	0.3	0.06–1.3	5–12
Hammondia/Neospora-like oocysts	1	0.2	0.01–1.1	(2)
Toxocara eggs	48	8.0	6.0–10.5	1–276
Toxascaris eggs	5	0.8	0.3–2.0	1–30
Hookworm eggs	43	7.1	5.3–9.6	1–378
Trichuris eggs	57	9.5	7.3–12.2	1–447
Capillariid eggs	17	2.8	1.7–4.6	1–24
Angiostrongylus larvae	2	0.3	0.06–1.3	1–7[d]
Taeniid eggs	2	0.3	0.06–1.3	1–2
Dicrocoelium eggs (pseudoparasitism?)	1	0.2	0.01–1.1	(5)
Linguatula eggs	1	0.2	0.01–1.1	(2)

NA not applicable

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

[b] Total number of fecal stages in three drops taken from the surface film of the floatation tube

[c] Larvae per 5 g of feces

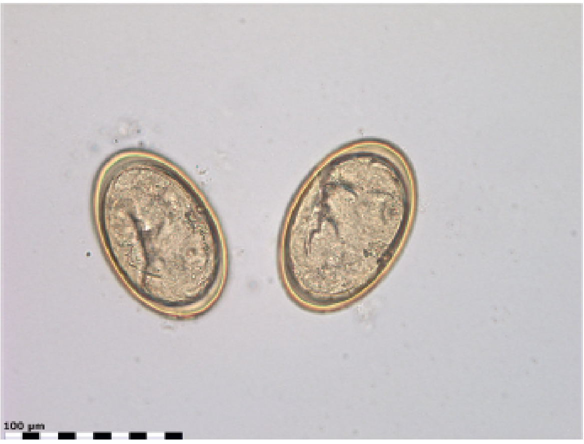

Fig. 1 *Linguatula* eggs

other [surgery, dermatological conditions including ectoparasitism, cardiopulmonary and urinary conditions, general abnormal condition]) did not reveal any significant association.

Assessment of anthelmintic treatment and ectoparasiticide use revealed that dog owners who dewormed their dogs claimed to treat the dogs with ectoparasiticides significantly ($p<0.0001$) more often (316/426, 74.2 %) than owners who did not use anthelmintics (44/176, 25.0 %).

Risk factors

Univariate analysis demonstrated that significant ($p<0.05$) factors of endoparasitism were the dog's age, the dog's purpose, the dog's habitat, the dog's environment, the presence/absence of other dogs and/or cats, the history of anthelmintic use, and the season of examination.

Compared to dogs >1 year of age, younger dogs tested significantly more often positive for overall endoparasites (48.6 vs. 38.1 %), overall protozoans (41.5 vs. 26.5 %), *Giardia* (38.7 vs. 19.4 %), *Cystoisospora* (7.2 vs. 1.7 %), and *Toxocara* (12.3 vs. 6.7 %) but less often positive for *Trichuris* (5.1 vs. 10.8 %). Pet dogs tested less frequently positive than hunting dogs and working dogs for overall endoparasites (34.1, 65.4, 52.0 %), overall protozoans (26.1, 53.8, 32.8 %), overall nematodes (17.2, 36.5, 28.0 %), *Giardia* (23.6, 49.7, 28.2 %), hookworm (5.6, 15.4, 8.8 %), and capillariid (0.9, 3.8, 8.8 %).

Dogs originating from the city tested less frequently positive than dogs from suburban and rural areas for overall endoparasites (34.0, 39.5, 52.0 %), overall protozoans (25.3 vs. 26.5 %, 49.5 %), overall nematodes (17.5, 21.0, 31.4 %), *Giardia* (22.3, 23.7, 38.8 %), and capillariid (1.0, 5.0, 3.8 %). Dogs kept mainly indoors or indoors with regular outside walking tested less frequently positive than dogs kept in yards or were kennelled for overall endoparasites (33.3 vs. 45.9 %), *Giardia* (25.2 vs. 37.4 %), hookworm (3.9 vs. 9.6 %), and capillariid (1.2 vs. 4.1 %), but more often positive for *Cystoisospora* (4.7 vs. 2.2 %).

Dogs living with other pets tested significantly more positive than dogs kept without other pets for overall endoparasites (51.9 vs. 32.1 %), overall protozoans (41.0 vs. 21.7 %), overall nematodes (26.6 vs. 17.1 %), capillariid (0.9 vs. 5.5 %), and *Giardia* (38.1 vs. 18.4 %). Analysis of general anthelmintic use (Yes/No) vs. prevalence of endoparasitism showed that dogs of owners who used wormers tested significantly less frequently positive than dogs whose owners did not administer anthelmintics for overall endoparasites (37.1 vs. 48.9 %) and overall nematodes (18.4 vs. 27.8 %). However, among the subpopulation of dogs which were administered anthelmintics, there was no association of the prevalence of any type of endoparasitism to the annual frequency of deworming. Dogs examined during the winter tested more frequently positive than dogs tested in spring, summer, and autumn for overall endoparasites (60.8, 43.8, 25.6, 31.8 %), overall protozoans (45.8, 29.1, 22.6, 24.0 %), overall nematodes (35.0, 26.4, 3.8, 17.1 %), *Giardia* (42.2, 28.8, 20.1, 22.9 %), *Cystoisospora* (9.2, 1.8, 1.5, 0.8 %), *Toxocara* (17.5, 6.4, 2.3, 7.8 %), hookworm (17.5, 5.9, 1.5, 5.4 %), and *Trichuris* (16.7, 13.2, 0, 6.2 %).

There was no association of any reason for admission of the dogs to the clinics and endoparasitism, and the factors breed (pure breed dogs vs. mixed-breed dogs), gender, and feeding habits were not found to be risk factors for endoparasitism for the examined population of dogs in the univariate analysis.

The final multivariate logistic regression models for risk factors associated with overall endoparasitism, overall protozoan parasitism, and overall nematode parasitism developed on the basis of the results of the fecal examination showed that age of the dogs, presence of other pets, general anthelmintic use, dog's habitat, and season of examination were the strongest predictors (Table 5).

Discussion

Endoparasite spectrum

In the feces of the client-owned dogs presented in four small animal clinics in Tirana, stages of at least 14 endoparasites were identified, including at least five protozoan species, eight species of helminths, and one pentastomid. Most of them are considered as common canine endoparasites with worldwide and frequent occurrence (*Cystoisospora*, *Sarcocystis*, *Giardia*, *Toxocara*, *Toxascaris*, hookworms, *Trichuris*, taeniid cestodes), while others are less often diagnosed, are considered rare, or have a restricted geographic occurrence only (*Hammondia*/*Neospora*, capillarid nematodes, *Angiostrongylus*, *Dicrocoelium*, *Linguatula*). Canine

Table 3 Occurrence of single and mixed endoparasite infections in 602 client-owned dogs

	Prevalence, total (%)
Single endoparasite infections	152 (25.2 %)
Giardia[a]	91 (15.1 %)
Cystoisospora canis	4 (0.7 %)
C. ohioensis	9 (1.5 %)
Sarcocystis	1 (0.2 %)
Hookworm	6 (1.0 %)
Toxocara	11 (1.8 %)
Trichuris	18 (3.0 %)
Capillariid	10 (1.7 %)
Angiostrongylus	1 (0.2 %)
Dicrocoelium (pseudoparasitism?)	1 (0.2 %)
Mixed endoparasite infections	93 (15.4 %)
Giardia + *C. canis*	3 (0.5 %)
Giardia + *C. ohioensis*	3 (0.5 %)
Giardia + hookworm	3 (0.5 %)
Giardia + *Toxocara*	23 (3.8 %)
Giardia + *Trichuris*	8 (1.3 %)
Giardia + capillariid	4 (0.7 %)
Giardia + *Angiostrongylus*	1 (0.2 %)
C. ohioensis + *Toxocara*	3 (0.5 %)
C. ohioensis + *Trichuris*	1 (0.2 %)
Hammondia/Neospora-like + hookworm	1 (0.2 %)
Hookworm + *Trichuris*	11 (1.8 %)
Hookworm + capillariid	2 (0.3 %)
Toxocara + *Trichuris*	2 (0.3 %)
Giardia + *C. canis* + *C. ohioensis*	4 (0.7 %)
Giardia + *C. canis* + *Toxocara*	2 (0.3 %)
Giardia + *C. ohioensis* + capillariid	1 (0.2 %)
Giardia + *Sarcocystis* + hookworm	1 (0.2 %)
Giardia + hookworm + *Toxocara*	1 (0.2 %)
Giardia + hookworm + *Trichuris*	5 (0.8 %)
Giardia + hookworm + taeniid	1 (0.2 %)
Giardia + *Trichuris* + taeniid	1 (0.2 %)
C. ohioensis + hookworm + *Trichuris*	1 (0.2 %)
Hookworm + *Toxocara* + *Trichuris*	2 (0.3 %)
Hookworm + *Trichuris* + *Linguatula*	1 (0.2 %)
C. canis + *C. ohioensis* + hookworm + *Toxocara*	1 (0.2 %)
Giardia + hookworm + *Toxocara* + *Trichuris*	2 (0.3 %)
Giardia + hookworm + *Toxascaris* + *Trichuris*	1 (0.2 %)
Giardia + *C. canis* + hookworm + *Toxoascaris* + *Trichuris*	1 (0.2 %)
Giardia + *C. canis* + *C. ohioensis* + hookworm + *Toxascaris* +*Trichuris*	2 (0.3 %)
Giardia + *C. canis* + *C. ohioensis* + hookworm + *Toxascaris* +*Toxocara* + *Trichuris*	1 (0.2 %)

[a] *Giardia* cyst positive

parasitism of *Cystoisospora* and *Sarcocystis* species, ascarids (*Toxocara canis*, *Toxascaris leonina*), hookworms (*Ancylostoma caninum*, *Uncinaria stenocephala*), *Trichuris vulpis*, and taeniid cestodes (*Taenia hydatigena*, *Taenia multiceps*, *Taenia pisiformis*, *Echinococcus granulosus*) had been recorded in prior necropsy and combined necropsy/coproscopy studies in Albania, and all those species have been recorded also in dogs in other countries on the Balkans as discussed exhaustively before (cf. Xhaxhiu et al. 2011).

For the first time for Albania, this study identified one of 602 dogs (0.2 %) shedding *Hammondia/Neospora*-like oocysts. The prevalence is similar to that obtained in studies conducted in Germany (Schares et al. 2005; Globokar Vrhovec 2013) but markedly lower than the rates of 2 and 5 % found in surveys in Romania (Amfim et al. 2011; Mitrea et al. 2012). However, as indicated through the detection of serum antibodies, almost one fifth of the dogs examined in the present study had exposure to *N. caninum* (Hamel et al. 2015).

As reported recently with reference to this survey (Sommer et al. 2015), canine *Giardia* infections were diagnosed for the first time in Albania in the population of dogs examined in this study and accounted for the most commonly identified enteric pathogen. As observed in several surveys in well-cared-for dogs in developed countries (Pallant et al. 2015), *Giardia* was the most commonly diagnosed intestinal parasite in this study. With approximately 35 % coproantigen-positivity and 25 % cyst-positivity, the present study found one of the highest *Giardia* prevalence rates reported in dogs from Europe (Bouzid et al. 2015). However, similar prevalence rates were found in dogs from other countries in Southeastern Europe (Nikolić et al. 2008; Sommer et al. 2015) and Southern Italy (Napoli et al. 2012), and almost 30 % of a sample of 58 cats from the Tirana area tested positive for *Giardia* coproantigen (Knaus et al. 2014). Multilocus sequenzing of 17 *Giardia* cyst-positive samples from the dogs examined in this study identified exclusively the non-zoonotic, host-adapted species *Giardia canis* (assemblages C and D) (Sommer et al. 2015).

Shedding of capillariid eggs in the feces of dogs from Albania has not been described before (Xhaxhiu et al. 2011), but almost one third of 252 cats from the Tirana area were reported recently excreting capillariid eggs (Knaus et al. 2014). As indicated by the univariate analysis, dogs originating from suburban and rural areas and, correlating with that, hunting and working dogs were more often shedding capillariid eggs. For example, one fifth of the 35 dogs from the police dog training school located in suburban Tirana tested positive for capillariid eggs. Fecal capillariid eggs may originate from adult nematodes residing in the respiratory tract (*Capillaria aerophila*, *Capillaria boehmi*), or the eggs may have passed through the alimentary tract following ingestion of contaminated food or after coprophagy (Georgi and Georgi

Table 4 Fecal parasite stages' counts in 602 client-owned dogs from Albania stratified according to infection density categories

Parasite stage	Number of "positive" samples	Counts per infection density category[a], total (%)			
		"Very few"	"Few"	"Moderate"	"Numerous"
Cystoisospora oocysts	36	31 (86.1 %)	2 (5.6 %)	1 (2.7 %)	2 (5.6 %)
Sarcocystis sporocysts	2	2 (100 %)			
Hammondia/*Neospora*-like oocysts	1	1 (100 %)			
Toxocara eggs	48	30 (62.5 %)	6 (12.5 %)	6 (12.5 %)	6 (12.5 %)
Toxascaris eggs	5	2 (40 %)	3 (60 %)		
Hookworm eggs	43	26 (60.5 %)	6 (13.9 %)	5 (11.7 %)	6 (13.9 %)
Trichuris eggs	57	41 (71.9 %)	5 (8.8 %)	5 (8.8 %)	6 (10.5 %)
Capillariid eggs	17	14 (82.4 %)	3 (17.4 %)		
Taeniid eggs	2	2 (100 %)			
Linguatula eggs	1	1 (100 %)			

[a] Total number of fecal stages in three drops taken from the surface film of the floatation tube: "very few," 1–15; "few," 16–30; "moderate," 31–60; "numerous," >60

1992). There was no detailed microscopic examination of the morphology of the eggs to distinguish the eggs of *Capillaria aerophila* or *Capillaria boehmi* from those of other capillariids which is, in principle, possible based on size and egg shell wall surface pattern (Conboy et al. 2013). However, the finding in the feces of the dogs in Albania of capillariid eggs is consistent with the increase of reports recording this type of eggs in canine feces in Europe which also refer to cases of *Capillaria boehmi* nasal infections (Traversa et al. 2010; Di Cesare et al. 2011, 2012; Magi et al. 2012; Macchioni et al. 2013). On the Balkans, *Capillaria aerophila* infections in dogs were reported previously from Croatia, Bosnia and Herzegovina, and Greece (cf. Xhaxhiu et al. 2011). However, as indicated by a prevalence of greater than 84 % for both *Capillaria aerophila* and *apillaria. boehmi* in red foxes from Serbia (Laloşevic et al. 2012), wildlife may represent an important reservoir of these parasites in the region. Further to the first indication of capillariid lungworms in dogs in Albania, two dogs were diagnosed shedding larvae of *Angiostrongylus vasorum*, which is parasitic in dogs and several wild canids. On the Balkans, this cardiopulmonary nematode has been recorded in dogs—based on the identification of first stage larvae from fecal examination—repeatedly in Greece (Diakou 1995; Founta et al. 2000; Papazahariadou et al. 2007) and recently in a dog in Serbia (Simin et al. 2014). Interestingly, none of the dogs that tested positive for capillariid or *A. vasorum* were presented to the clinics for respiratory conditions or were exhibiting cardio-respiratory and/or other clinical syndromes associated with angiostrongylosis-like coagulopathies when examined. These findings confirm observations of other authors who either reported asymptomatic infections or did not mention clinical signs in positively tested dogs (e.g., Papazahariadou et al. 2007; Al-Sabi et al. 2013; Hurniková et al. 2013; Simin et al. 2014) and thus emphasize the consideration by veterinarians of (cardio)pulmonary nematode infections in dogs living in endemic regions even in dogs with few or no respiratory signs (Di Cesare et al. 2014; Elsheikha et al. 2014).

Both the coprophagic behavior of dogs as well as the consumption of parasitized organs may result in the occurrence in the feces of dogs of stages of parasites which normally do not parasitize dogs. Most findings of *Dicrocoelium* eggs in canine feces likely represent "pseudoparasitism" following ingestion of infected sheep liver or consumption of feces of infected sheep. This aspect may be considered for the dog passing *Dicrocoelium* eggs in its feces as dicrocoeliosis is highly prevalent in ruminants in Albania (Elezi et al. 2003; Kondi and Zanaj 2004; Bizhga et al. 2006; Gjoni et al. 2012). However, it should be mentioned that dogs can serve as final hosts of *Dicrocoelium dendriticum* (Kamenskij 1901; Demidova 1937; Nesvadba 2006).

The finding of *Linguatula* eggs in the feces of one working dog from a rural habitat reported fed, in part, with raw meat is indicative for the occurrence of the "tongue worm," *Linguatula serrata*, in dogs in Albania. Thus, in addition to the indication of the presence of capillariid and metastrongyloid nematodes, *L. serrata* pentastomids, which live as adults in the nasal passages of canids, may parasitize in the respiratory organs of dogs in Albania. To the author's knowledge, neither the occurrence of *L. serrata* in dogs nor that of the encysted larvae in intermediate hosts has been reported before in Albania; however, nymphs of *L. serrata* have been recovered recently from the lungs of cats from Albania (Knaus et al. 2012). While *L. serrata* is prevalent in both dogs and wild canids in southeastern Europe (Bosnia and Hercegovina, Bulgaria, Greece, Romania, Serbia) and Italy (Haralabidis et al. 1988; Nesterov et al. 1991; Jažić et al. 1992–1995; Gherman et al. 2002; Paoletti et al. 2003; Negrea 2005;

Table 5 Multivariate analysis for risk factors associated with overall endoparasitism, overall protozoans, and overall nematodes in 602 client-owned dogs from Albania

Factor and group	Odds ratio	95 % CI	*p* value
Overall endoparasitism			
Age			
≤1 year	Reference	NA	NA
>1 year	0.64	0.41–0.99	<0.05
Presence of other pets			
No	Reference	NA	NA
Yes	1.56	0.89–2.74	<0.01
Anthelmintic use			
No	Reference	NA	NA
Yes	0.35	0.20–0.64	<0.001
Season of examination			
Winter	Reference	NA	NA
Spring	0.51	0.31–0.83	<0.01
Summer	0.15	0.08–0.27	<0.001
Autumn	0.20	0.11–0.37	<0.001
Overall protozoan parasitism			
Age			
≤1 year	Reference	NA	NA
>1 year	0.49	0.25–0.98	<0.01
Dog's habitat			
City	Reference	NA	NA
Suburban	0.92	0.58–1.45	Not significant
Rural	1.83	1.05–3.19	<0.05
Presence of other pets			
No	Reference	NA	NA
Yes	3.71	2.33–5.91	<0.001
Season of examination			
Winter	Reference	NA	NA
Spring	0.46	0.27–0.77	<0.01
Summer	0.32	0.17–0.58	<0.001
Autumn	0.32	0.17–0.58	<0.001
Overall nematode parasitism			
Presence of other pets			
No	Reference	NA	NA
Yes	2.01	1.25–3.24	<0.01
Anthelmintic use			
No	Reference	NA	NA
Yes	0.35	0.17–0.76	<0.01
Season of examination			
Winter	Reference	NA	NA
Spring	0.61	0.37–1.01	<0.05
Summer	0.06	0.02–0.15	<0.001
Autumn	0.25	0.13–0.48	<0.001

NA not applicable

Kirkova et al. 2013; Csordás et al. 2014), infection of domestic dogs is nowadays apparently non-existent in most of Europe but infected dogs are imported occasionally from endemic areas (Globokar Vrhovec et al. 2005; Gjerde 2013; Holter 2013).

Prevalence of endoparasitism

This study establishes for the first time data for intestinal and cardiopulmonary parasitism in client-owned, veterinary cared-for dogs from Albania. With almost 30 and 22 % of the dogs testing positive for protozoan infections (mainly *Giardia*) and nematode parasitism, respectively, results of this survey indicate that substantial levels of endoparasite infection exist in this category of dogs in the country. Given that prevalence data established with a single fecal examination usually underestimate true prevalence, data of the present study should be considered the minimum level of parasitic nematode infection (e.g., Lillis 1967; Al-Sabi et al. 2013). However, prevalence rates of endoparasitism in the dogs examined were substantially lower than those reported from previous studies conducted in Albania which encountered dogs with limited or no veterinary care (Dhamo and Zanaj 2003; Zanaj et al.. 2006; Xhaxhiu et al. 2011; Bocari et al. 2014). Taking *Toxocara* ascarid infection as example because of its particular implication for public health, a coproscopic prevalence of 8.0 % was established in the veterinary cared-for dogs examined in this study while the other studies reported 19.2 to 60 % of the fecal samples demonstrating *Toxocara* eggs (Dhamo and Zanaj 2003; Zanaj et al. 2006; Bocari et al. 2014). Similar conclusions can be drawn for *Toxascaris*, hookworm, *Trichuris*, and *Dipylidium* parasitism in terms of results of examination of feces and are supported by parasite counts established in dogs from suburban areas of Tirana (Xhaxhiu et al. 2011). There was no evidence for *Dipylidium* infection in the examined dogs despite that *Dipylidium caninum* constituted the most prevalent cestode found in dogs from Albania in a prior study (65.8 %, Xhaxhiu et al. 2011) and, as indicated through the recovery of fleas from 6.6 % of the 602 dogs (Shukullari et al. 2015), an adequate opportunity exists for the transmission of this parasite. However, fecal diagnosis of this cestode is not reliable because proglottid shedding is erratic and *Dipylidium* egg capsules are detected very rarely at microscopic examination of fecal samples at routine examination, because they are less often released from expelled segments than the eggs from the proglottids of taeniids (Lillis 1967; Martínez-Carrasco et al. 2007; Barutzki and Schaper 2011).

Although results of different studies cannot necessarily be compared and extrapolated in a simple way given the specific procedural methods, overall prevalence and prevalence rates for most individual types of endoparasitism identified in the current study were considerably higher than the results found in several recent fecal surveys conducted in veterinary (well) cared-for dogs in central and Western Europe, North America, Japan, or Australia (e.g., Claerebout et al. 2009; Batchelor et al. 2008; Palmer et al. 2008; Little et al. 2009; Mohamed et al. 2009; Barutzki and Schaper 2011; Itoh et al. 2011; Joffe et al. 2011; Globokar Vrhovec 2013). These animals benefit in their environment from a high level of general and veterinary care over several decades. Nevertheless, these surveys have shown that intestinal parasitism is a common feature even in those dogs, associated with prevalence rates ranging from 1.8 to 5.6 % for *Cystoisospora*, from 4.0 to 22.8 % for *Giardia*, from 0.4 to 6.1 % for *Toxocara*, from 0.2 to 0.6 % for *Toxascaris*, from 0.3 to 4.5 % for hookworm, and from 0.6 to 1.6 % for *Trichuris*. In common with the results of the aforementioned surveys, overall protozoan infections (mainly *Giardia*) were more often identified in the present study than overall nematode infections. Predictably, studies examining intestinal parasitism in the same category of dogs in the closer geographic region, i.e., in Greece and Serbia, determined similar prevalence rates of most of the nematode endoparasite infections identified in the present survey from Albania (Lefkaditis 2003; Lefkaditis et al. 2005, 2009; Papazahariadou et al. 2007; Nikolić et al. 2008). Prevalence rates indicated in those studies ranged from 9.2 to 35.5 % for *Toxocara*, from 0.7 to 1.3 % for *Toxascaris*, from 2.8 to 15.8 % for hookworm, and from 2.2 to 14.4 % for *Trichuris*. Furthermore, comparable prevalence ranges were reported from Southern Italy for dogs sampled in veterinary clinics or following examination of feces of pet dogs submitted to a university laboratory (Lia et al. 2002; Napoli et al. 2012): 10.8 to 18.7 % for ascarids, 3.3 to 7.5 % for hookworm, and 3.6 to 6.1 % for *Trichuris*.

Risk factors associated with endoparasitism

Risk factors repeatedly reported to be associated with prevalence of endoparasitism in dogs include age, gender, dog's purpose, habitat and environment, use of anthelmintics, and geographic location (e.g., Visco et al. 1977; Martini et al. 1992; Coggins 1998; Fok et al. 2001; Dubná et al. 2007; Palmer et al. 2008; Gates and Nolan 2009; Little et al. 2009; Mohamed et al. 2009; Barutzki and Schaper 2011; Globokar Vrhovec 2013; Zanzani et al. 2014). While gender was not identified as risk factor for parasitism in the population studied, other factors were, with age of the dogs, presence of other dogs/cats, lack of anthelmintic use, dog's habitat, and season of examination, identified as strongest predictors. The strong association found between young age and endoparasitism, largely associated with the predominance of *Cystoisospora*, *Giardia* and *Toxocara* infections, re-emphasizes the importance of the focus of owners and veterinarians on young dogs. Interestingly, season during which fecal examination was performed was found to be a strong risk factor for both protozoan and nematode endoparasitism for the population of dogs examined. Dogs that live in close contact to other pets (multiple pet households) had a higher risk of transmission, likely associated to a more frequent exposure to feces of other animals that are carriers of a parasite. Grouping of dogs on the basis of season tested demonstrated significant variations in the odds of infection with several parasites. The risk to be tested positive for endoparasite infection for the dogs examined in the current study was highest in the winter season which was basically associated with the most frequently diagnosed infections of *Giardia*, *Cystoisospora*, *Toxocara*, hookworm, and/or *Trichuris* during winter. Compared to the epidemiology of parasites in grazing animals, epidemiology of intestinal protozoan and nematode parasitism in dogs with respect to a seasonal pattern in prevalence under temperate climatic conditions is less well documented and findings, including results of the present study, are partly controversial for unclear reasons. However, consistent with the results found in the current study, analysis of canine fecal surveys conducted in Germany, Italy, Argentina, and the USA indicated higher rates of *Giardia* and/or *Toxocara* among dogs tested during winter months (Bianciardi et al. 2004; Andresiuk et al. 2007; Mohamed et al. 2009; Barutzki and Schaper 2011; Globokar Vrhovec 2013); for *Trichuris*, this indication was less well supported (Andresiuk et al. 2007; Mohamed et al. 2009). In contrast, while consistent with the other parasites identified in the dogs from Albania with highest rates of infection recorded in the dogs tested during winter, two large surveys conducted in Germany found most dogs testing positive for *Cystoisospora* during the summer months (Barutzki and Schaper 2011; Globokar Vrhovec 2013). With respect to hookworm, two species, *A. caninum* and *U. stenocephala*, are abundant in Albania with *U. stenocephala* being more prevalent than *A. caninum* (64.9 and 13.5 %, respectively; Xhaxhiu et al. 2011). While no seasonality of hookworm infections was found in surveys from Germany (Barutzki and Schaper 2011; Globokar Vrhovec 2013), where *U. stenocephala* is apparently the only ancylostomatid species present in domestic dogs (Barutzki and Schaper 2011), several studies from the USA, where *A. caninum* predominates, indicated peak prevalence rates during the warmer months of the year (Kirkpatrick 1988; Coggins 1998; Mohamed et al. 2009).

Conclusion

Testing of canine fecal samples submitted to laboratories or samples collected in general veterinary practice or hospital settings helps contribute to our knowledge on the prevalence of various parasitic infections in companion animals. It thus provides valuable information to veterinarians, health care professionals, and dog owners in the area in which the study was performed. This survey is the first which evaluated the endoparasite status of client-owned, veterinary cared-for dogs from Albania, and more canine samples were examined in this study than any other prevalence study previously conducted in Albania. The data presented here demonstrate a wide variety of endoparasites and relatively high prevalence rates indicating that veterinarians and dog owners need to increase their efforts in periodic examination for and routine control of endoparasites including improvement of dog husbandry practices including treatment. This will reduce the parasitism in the dogs and thus lower the potential for transmission of zoonotic agents to humans.

Conflict of interest The authors declare that there no conflicting interests that may have biased the work reported in this paper.

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4.1.2. Publication 2: Parasites and vector-borne diseases in client-owned dogs in Albania. Blood pathogens and seroprevalences of parasitic and other infectious agents

Hamel D, Shukullari E, Rapti D, Silaghi C, Pfister K, Rehbein S

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

Parasitology Research (DOI 10.1007/s00436-015-4765-8)

Submitted: 07 August 2015

Accepted: 28 September 2015

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 for 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*, *A. platys*, *Ehrlichia canis*, and *Mycoplasma haemocanis*, were detected by direct methods with prevalence rates ranging from 1% to 9%. Seroprevalence for *Babesia* spp., *Leishmania 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, lungworms, ectoparasites) infections in dogs and cats including vector-borne pathogens (e. g., *Babesia canis*, *Leishmania infantum*, *Dirofilaria immitis*, *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-for dogs reported on the presence of *Babesia* spp., *Hepatozoon canis*, filarial infections as well as *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. 2015a, b in press).

Materials and Methods

<u>Sample collection and animal data</u>

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. 2015a).

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*, *A. 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 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®GENEEXPRESSION 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, Quiagen, 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 titres ≥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 end-point titers were assessed in this survey.

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 year 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 *B. vogeli*, *Hepatozoon canis*, *Leishmania* 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%), 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 four (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). *Babesia 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 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 *D. 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 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).

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.

Anaplasma platys causes canine cyclic thrombocytopenia (Harvey et al. 1978). *Rhipicephalus 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 basically is 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 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 6 (1.0%) of the sampled dogs were positive for *A. phagocytophilum*-DNA by PCR. *Anaplasma 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., 2015b, in press). 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). *Mycoplasma 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$ 1,200 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; Prelezov 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., 2015a), thus limiting vector exposure. Additionally, serological screening gave first evidence for *N. caninum* and *T. gondii* exposure in dogs from Albania.

Acknowledgements: The authors gratefully acknowledge the technical assistance of the laboratory staff of the Diagnostic Unit of the Institute of Comparative Tropical Medicine and Parasitology. The authors also acknowledge the support of Sanisys SA, Switzerland, for statistical analysis.

Conflict of interest: The authors declare that they do not have a conflict of interest.

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Table 1: Basic data of 602 dogs sampled for testing

Factor	N	%
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[1]		
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

[1] Includes intact and neutered animals, by sex

Table 2: Summary of PCR and real-time PCR methods for specific pathogen detection used in this study

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 30sec, 54°C 30 sec, 72°C 40 sec, 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: ATGGTATTGCTCCATCAGACTTTCG	94 °C 2 min 35 cycles: 94°C 45sec, 58°C 45sec, 72°C 45 sec, 72°C 5 min	pEX-A-*Mycoplasma haemominutum* 1:256.000.000 dilution pEX-A-*Mycoplasma haemofelis* 1:2048000000 dilution[1]	Watanabe et al., 2003
Real-time PCR methods				
Leishmania infantum **Kinetoplast-DNA**	Lsh-kF: CTTTTCTGGTCCTCCGGGTAGG Lsh-kR: CCACCCGGCCCTATTTTACACCAA Lsh-kp: FAM-TTTTCGCAGAACGCCCCTACCCGC-BHQ1	95°C 20 sec 40 cycles: 95°C 30 sec, 60°C 30 sec	*L. infantum* DNA from cell culture 1:1000 dilution	Mary et al., 2004
Anaplasma platys ***16S rRNA* gene**	EPlat-19f : CGGATTTTTGTCGTAGCTTGCTAT EPlat-117r: CCATTTCTAGTGGCTATCCCATACTACT EPlat-55p-S1:FAM-TGGCAGACGGGTGAGTAATGCATAGGA-BHQ1	95°C 20 sec 40 cycles: 95°C 30 sec, 60°C 30 sec	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: TGGTGCCAGGGTTGAGCTTGAGATTG-TAMRA	50°C 5 min 95°C 10 min 40 cycles: 95°C 15sec, 60°C 1 min	*A. phagocytophilum* DNA from dog	Courtney et al., 2004; Silaghi et al., 2011a
Ehrlichia canis ***p30-10*-gene**	Ecanisp30-ANYF: TGGATACTACCATGGCGTTATTGG E.canisp30-ANYR: GAGGAGCATCATTAATACTACAGGAGTT E.canisp30-ANYM2: FAM-CAGGTATCTTCTCAAATTT-NFQ	50°C 2min 95°C 10 min 40 cycles:95°C 15 sec, 60°C 1 min	*E. canis* plasmid-DNA pEC-3.3 1:1000 dilution[1]	Ionita et al., 2013

[1]Eurofins MWG Operon, Ebersberg, Germany

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		N	Prevalence %	95%CI[1]
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*[2]	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)[3]	6.6 (10.1)	4.8 - 9.0 (7.9 - 12.9)
	L. infantum-IFAT	31 (57)[3]	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

[1] 95% Confidence interval, Yates continuity correction performed
[2] Species according to sequencing results
[3] Including samples with borderline titers (1:32)

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.[1]	3 (0.5)
L. infantum	18 (3.0)
Microfilariae (not identified)[1]	2 (0.3)
D. immitis[2,3]	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.[1] + *A. platys*	2 (0.3)
Hepatozoon spp.[1] + *M. haemocanis*	1 (0.2)
B. vogeli[4] + *E. canis*	1 (0.2)
L. infantum + *D. immitis*[2]	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*[2] + *E. canis*	1 (0.2)
L. infantum + *E. canis* + *M. haemocanis*	1 (0.2)
D. immitis[2] + *E. canis* + *M. haemocanis*	1 (0.2)
Total	151 (25.1)

[1] Giemsa-stained blood smear
[2] DiroChek®-ELISA
[3] One animal microfilaraemic
[4] Including *Babesia*-positive in Giemsa stained blood smear

Table 5: Number of dogs tested seropositive for multiple vector-borne pathogens in IFAT (borderline titres [*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

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

Factor and group[1]	Odds Ratio	95%CI	P value
Overall Presence/Exposure (PCR + IFAT; vector-borne)			
Age			
>1 year *vs* ≤1 year	1.8	1.2-2.7	0.003
Habitat			
City	Reference	NA[2]	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 year *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 year *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 year *vs* ≤1 year	1.7	1-2.9	0.0
Anaplasma IFAT			
Age			
>1 year *vs* ≤1 year	1.8	1.1-2.9	0.02
IFAT *Neospora*			
Age			
>1 year *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

[1] Season, age, sex, feed, breed, use, husbandry, habitat
[2] NA = not applicable

4.2. Infestation with ectoparasites in clientowned dogs in Albania

Prevalence of infestation, occurrence of multiple parasitism, intensity of infestation

Total prevalence and counts of ectoparasites (ticks and fleas only) obtained by full body search and total body comb, and examination of ear swaps and scrapings taken from skin lesions suspicious of mite infestation are summarized in Table 6. Overall, 93 dogs (15.4%, 95%CI 12.6 - 18.6) had evidence for ectoparasite infestation, with ticks, fleas and ear mites representing the most common types of ectoparasites. In total nine species of ectoparasites were identified comprising two species of hard ticks (*I. ricinus*, *R. sanguineus* s. l.), three species each of mites (*D. canis, O. cynotis*, *S. scabiei* var. *canis*) and fleas (*C. canis*, *C. felis*, *P. irritans*) and one species of mallophagan lice (*Trichodectes canis*).

Table 6: Prevalence and intensity of ectoparasite infestation on 602 client-owned dogs from Albania with either single or multiple-species infestation

	Total	Prevalence %	95% CI[1]	Intensity, AM[2] (range)
Ixodes ricinus	5	0.8	0.3 - 2.0	1.6 (1 - 4)
Rhipicephalus sanguineus s. l.	49	8.1	6.1 - 10.7	9.2 (1 - 61)
Total ticks	52	8.6	6.5 - 11.2	8.9 (1 - 61)
Otodectes cynotis	18	3.0	1.8 - 4.7	--
Sarcoptes scabiei var. *canis*	4	0.7	0.2 - 1.8	--
Demodex canis	1	0.2	0.01 - 1.1	--
Ctenocephalides canis	29	4.8	3.3 - 6.9	4.5 (1 - 22)
C. felis	18	3.0	1.8 - 4.7	20.1 (1 - 163)
Pulex irritans	1	0.2	0.01 - 1.1	1 (1)
Total fleas	40	6.6	4.6 - 8.8	12.3 (1 - 163)
Trichodectes canis	1	0.2	0.01 - 1.1	--
Total ectoparasites	93	15.4	12.6 - 18.6	--

[1] 95% confidence interval, Yates continuity correction performed
[2] Infested animals, AM = arithmetic mean

Single and multiple infestations with up to four species of ectoparasites concurrently were recorded in 67 (11.1%, 95%CI 8.7 - 13.9) and 26 dogs (4.3%, 95%CI 2.8 - 6.3), respectively (Table 7). *Rhipicephalus sanguineus* s. l. ticks plus *C. canis* or *C. felis* fleas were the most frequently found combinations.

Table 7: Occurrence of single- and multiple ectoparasite infections in 602 client-owned dogs from Albania

	Prevalence, total (%)
Single ectoparasite infections	67 (11.1)
Ixodes ricinus	2 (0.3)
Rhipicephalus sanguineus s. l.	28 (4.7)
Otodectes cynotis	18 (3.0)
Sarcoptes scabiei var. *canis*	3 (0.5)
Demodex canis	1 (0.2)
Ctenocephalides canis	12 (2.0)
C. felis	3 (0.5)
Mixed ectoparasite infections	26 (4.3)
I. ricinus + *R. sanguineus* s. l.	1 (0.2)
I. ricinus + *C. canis*	1 (0.2)
R. sanguineus s. l. + *C. canis*	7 (1.2)
R. sanguineus s. l. + *C. felis*	8 (1.3)
S. scabiei var. canis + *C. canis*	1 (0.2)
C. canis + *C. felis*	3 (0.5)
I. ricinus + *R. sanguineus* s. l. + *C. canis*	1 (0.2)
R. sanguineus s. l. + *C. canis* + *C. felis*	2 (0.3)
R. sanguineus s. l. + *C. canis* + *C. felis* + *Pulex irritans*	1 (0.2)
R. sanguineus s. l. + *C. canis* + *C. felis* + *Trichodectes canis*	1 (0.2)

The most common parasites were ticks which were found attached to 52 dogs (8.6%, 95%CI 6.5 - 11.2) with individual counts ranging from 1 to 61, and fleas which were recovered from 40 dogs (6.6%, 95%CI 4.6 - 8.8) with individual flea counts ranging between 1 and 161. Stratifying the counts of ticks and fleas into three categories using the score established by Marchiondo et al. (2007), approximately 75% of the tick infested dogs carried low and moderate levels of infestation of up to three or four to ten ticks per animal; 75% of the dogs carried a low level infestation of up to five fleas, and the infestation level was moderate (6-20 fleas/animal) or high (>20 fleas/animal) in 12.5% each of the dogs (Figure 3).

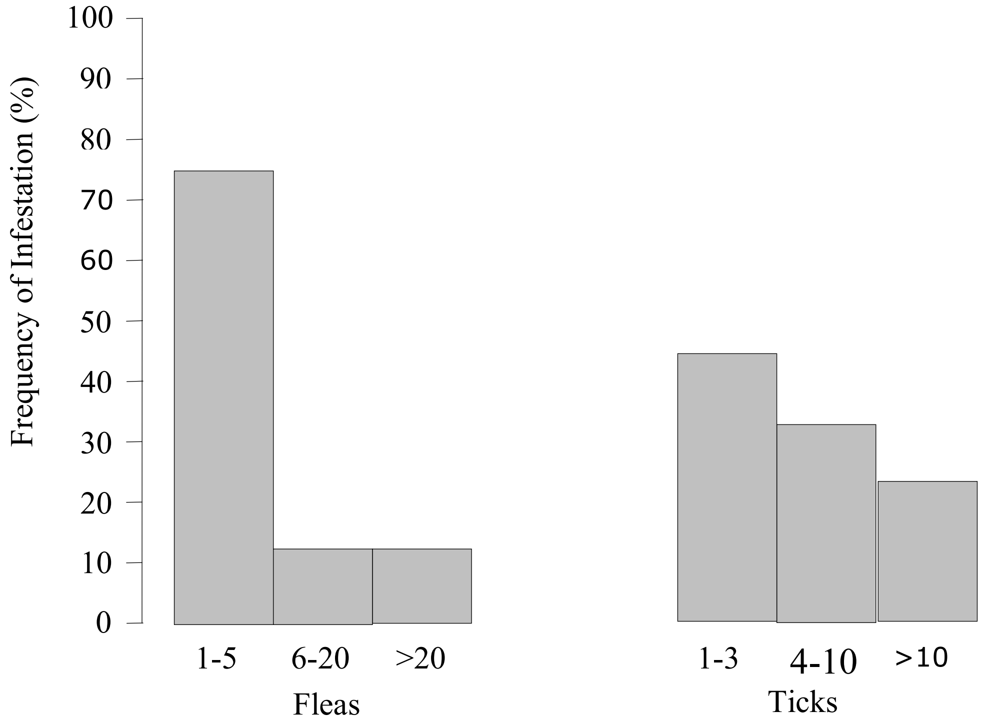

Figure 3: Infestation with fleas and ticks of 40 and 52 client-owned dogs from Albania, respectively, grouped according to the score of Marchiondo et al. (2007) corresponding to low, moderate and high levels of infestation

While fleas (*C. canis* and *C. felis)* were recovered from the dogs in each season, *I. ricinus* were recorded in winter and spring and *R. sanguineaus* s. l. in spring and summer only. Analysis of the prevalence of infestation with any of the three major types of ectoparasites (ticks, fleas, ear mites) revealed a significant association between infestation of ticks and of fleas: dogs infested with fleas were found significantly (p=0.0001) more frequently among dogs infested with ticks (flea-positive/tick-positive: 22/52, 42.3% vs. flea-positive/tick-negative: 18/550, 3.3%).

Testing of prevalence of any type of ectoparasitism vs. grouped reasons for admission of the dogs to the clinics (routine measures, dermatological conditions, other [surgery, gastrointestinal conditions including vomiting, cardiopulmonary and urinary conditions, general abnormal condition]; 12.4%, 45.5%, 12.4%) did reveal a significant association of overall ectoparasitism and dermatological conditions ($p<0.0001$).
The query regarding the use of treatments for the control (treatment and/or prevention) of ectoparasite infestation identified only topical products which release the actives from liquid formulations or polymers embedded in a plastic-like collar (non-systemic compounds which have repellent properties and/or kill the parasites through direct contact to the active ingredient after dispersal over the body surface; deltamethrin, permethrin, tetramethrin, imidacloprid, fipronil).
Dog owners who treated their dogs with ectoparasiticides claimed to deworm the dogs significantly ($p<0.0001$) more often (316/360, 87.8%) than owners who did not use ectoparasiticides (110/242, 45.5%).

Canine parasitism of the tick and flea species has been documented in Albania in prior studies (cf. 2.2.2.1. and 2.2.2.3.). However, the occurrence of all nine species of ectoparasites, including three species of mites and one mallophagan, has been recorded in a recently conducted survey only (Xhaxhiu et al. 2009). All species have been recorded in other countries on the Balkans as discussed exhaustively before (Xhaxhiu et al. 2009) and supported through additional recent work from the geographic region (Omeragic 2011; Pavlović et al. 2011; Kirkova et al. 2013b; Krčmer et al. 2014). Further to the ectoparasite spectrum recorded in this study, the anecdotal collection of a ked (*Hippobosca equina*) from a dog was reported in 1960 (Danielova 1960), and in a tick survey conducted in the early 1970s, *Haemaphysalis inermis* and *H. punctata* ticks were found attached to dogs in Albania (Gina et al. 1975; Gina 1977). Although not specifically studied as parasites of dogs but testified through the endemism of zoonotic canine leishmaniosis and dirofilarioses (cf. 2.2.1.1. and 2.2.1.2.3.), dogs in Albania must be attacked for blood-feeding by the incriminated flying vectors of the agents of the two diseases. Competent vectors of *Leishmania infantum* and *Dirofilaria immitis*, phlebotomine sandflies or mosquitoes of the Culicidae family, respectively, are abundant in the country (cf. 2.2.2.4.).

Of the four species of ticks described attaching to dogs in Albania ticks of the two species *I. ricinus* and *R. sanguineus* s. l. were recorded on the client-owned dogs with the latter outnumbering the former for both prevalence and intensity of infestation. Thus, results of the current study are in agreement with recently conducted studies in dogs from suburban areas of Tirana (Xhaxhiu et al. 2009; Silaghi et al. 2013), and similar results revealed the examination of dogs admitted to veterinary clinics in the city and county of Thessaloniki, Greece (Papazahariadou et al. 2003). Apart from the aspect that *R. sanguineus* is a characteristic representative of the Mediterranean tick fauna which primarily parasitizes dogs while *I. ricinus* ticks are opportunistic feeders infesting virtually all land vertebrates, the predominance of *R. sanguineus* is likely explained through the different habitat preferences of the two species of ticks in relation to the habitats the dogs originated from. More than 80% of the dogs were from urban and suburban habitats which constitute a suitable environment for the endophilous *R. sanguineus* that can infest houses and can reach pest proportions in animal shelters (Dantas-Torres 2008, 2010; Lorusso et al. 2010; Otranto et al. 2012). *Ixodes ricinus* with its very limited tolerance to desiccation, in contrast, has a typical exophilous behaviour and is commonly found in habitats like less maintained meadows, bushy areas and deciduous and mixed forests (Bowman and Nuttall 2008). Although the Mediterranean Basin constitutes the southern edge of the range of distribution of *I. ricinus* in Europe, *I. ricinus* can develop well in the region in natural habitats which provide the specific microclimatic conditions and may complete its life cycle in approximately one year (Dantas-Torres and Otranto 2013). This is apparently applicable to Albania where *I. ricinus* was shown to have a wide geographical distribution (Luli 1963) likewise in the neighbouring Kosovo, former Yugoslav Macedonia and Greece (Papadopoulos et al. 1996; Milutinović et al. 1997; Pavlidou et al. 2008; Pavlović et al. 2014).

While infestation with *I. ricinus* was of a very low level in both the client-owned dogs and dogs with limited or no veterinary care from suburban areas of Tirana (Xhaxhiu et al. 2009), prevalence of *R. sanguineus* s. l. in the client-owned dogs was just one third of the one observed in dogs from suburban areas of Tirana (8.1% vs. 23.8%). Mean level of infestation of the tick-infested client-owned dogs was only slightly lower (~9 ticks per dog) than that of the infested dogs with limited or no veterinary care (~11 ticks per dog) (Xhaxhiu et al. 2009). The relevance of *R. sanguineus* infestation is underlined through the range of pathogens identified in the client-owned dogs enrolled in this survey (cf. 4.1.2.) which are confirmed (*B. vogeli*, *E. canis*, *H. canis*, *M. haemocanis*) or suggested (*A. platys*) to be transmitted by this species of ticks (Senevira et al. 1973; Otranto and Dantas-Torres 2010).

Similarly to the infestation with ticks but much more pronounced, overall infestation with fleas of the client-owned dogs in this study was lower than that of less well-cared-for dogs from suburban areas of Tirana (Xhaxhiu et al. 2009). As with ticks, prevalence of infestation was distinctly different with 6.6% and 75.7%, respectively, while the mean total flea load per infested dog was apparently similar (12.3 vs. 12.0). However, the arithmetic mean flea count of the infested client-owned dogs does not adequately reflect that 75% of the dogs harboured no more than five fleas but it is biased by high counts of single animals. *Ctenocephalides canis* was the most prevalent species of flea in both client-owned, veterinary-cared-for dogs (4.8%) and less well-cared-for dogs from suburban areas of Tirana (75.7%). The comparison of proportions of infested dogs harbouring *C. canis*, *C. felis* and *P. irritans*, respectively, suggest a shift from a *C. canis*-predominated flea population in the dogs with limited or no veterinary care towards a substantial higher percentage of the client-owned dogs from Tirana infested with *C. felis* (100%, 6.5% and 10.9%, respectively, vs. 72.5%, 45.0% and 2.5%, respectively). This is further supported through the flea counts which were higher for *C. felis* than for *C. canis*. The findings in the client-owned dogs from Albania resemble the results of a survey conducted in the early 1990s with dogs presented to the veterinary teaching hospital of Thessaloniki, Greece (Koutinas et al. 1996) where infestation of *C. canis*, *C. felis*, *P. irritans* and *Xenopsylla cheopis* was recorded in 71.3%, 40.3%, 0.8% and 0.8% of the flea-infested dogs, respectively. Globally, *C. felis* is the most prevalent species of flea recorded in domestic dogs (Dobler and Pfeffer 2011). Higher proportions of *C. canis* are found usually in dogs inhabiting rural settings, living in kennels or being kept outdoors, in contrast to dogs from residential habitats which harbour predominately *C. felis* (cf. Xhaxhiu et al. 2009). Based on the findings of this study, further surveys should be conducted to follow-up potential changes in the flea population of client-owned dogs in Albania.

As discussed previously (Xhaxhiu et al. 2009), canine parasitism caused by ectoparasites other than ticks and fleas (e. g., mites and lice) does usually not receive much attention in cross-sectional type of studies and information regarding the prevalence of these parasites is largely lacking, especially in veterinary-cared-for dogs. However, not unexpected, examination of the 602 client-owned dogs revealed lower prevalence rates of infestation with *O. cynotis*, *S. scabiei* var. *canis* and *D. canis* mites and *T. canis* lice than the examination of 181 dogs with limited or no veterinary care from suburban areas of Tirana (3.0%, 0.7%, 0.2% and 0.2%, respectively, vs. 6.7%, 4.4%, 0.6% and 6.6%, respectively; Xhaxhiu et al. 2009).

Risk factors

On univariate analysis, the breed (pure breed dogs vs. mixed-breed dogs), the dog's purpose, the dog's environment, the history of ectoparasiticide treatment and the season of examination were identified as significant ($p<0.05$) factors predisposing dogs to various types of ectoparasitism.

Compared to pure breed dogs, mixed breed dogs were infested more frequently with overall ectoparasites (13.2% vs. 28.7%) and ticks and/or fleas (9.7% vs. 24.1%). Pet dogs were more often infested with ticks and/or fleas than hunting/working dogs (14.6% vs. 6.2%).

Dogs kept mainly indoors/indoors with regular outside walking harboured less frequently overall ectoparasites and ticks and/or fleas (12.4% and 8.5%, respectively) than dogs which were kept in yards/were kennelled (17.7% and 14.2%, respectively). Analysis of general ectoparasiticide use (Yes/No) vs. prevalence of ectoparasitism showed that dogs of owners who used ectoparasiticides in their animals tested significantly less frequently ectoparasite-positive than dogs whose owners did not administer ectoparasiticides for overall ectoparasites (10.8% vs. 31.5%), *C. canis* (1.7% vs. 10.5.5%), *C. felis* (1.1% vs. 5.8%) and *R. sanguineus* s. l. (4.2% vs. 14.0%). However, among the subpopulation of dogs which were administered ectoparasiticides, there was no association of the prevalence of any type of ectoparasitism to the annual frequency of use. Dogs examined during the winter had less evidence of ectoparasite infestation than had the dogs tested in spring, summer and autumn for overall ectoparasites (5.0%, 19.1%, 22.6%, 11.6%), *O. cynotis* (0%, 4.1%, 0.8%, 6.2%), fleas (2.5%, 6.4%, 16.7%, 3.9%), and *R. sanguineus* s. l. (0%, 11.4%, 18.0%, 0%). Figure 4 plots the seasonal percentage prevalence of infestation of the dogs with overall ectoparasites, *R. sanguineus* s. l. ticks and fleas.

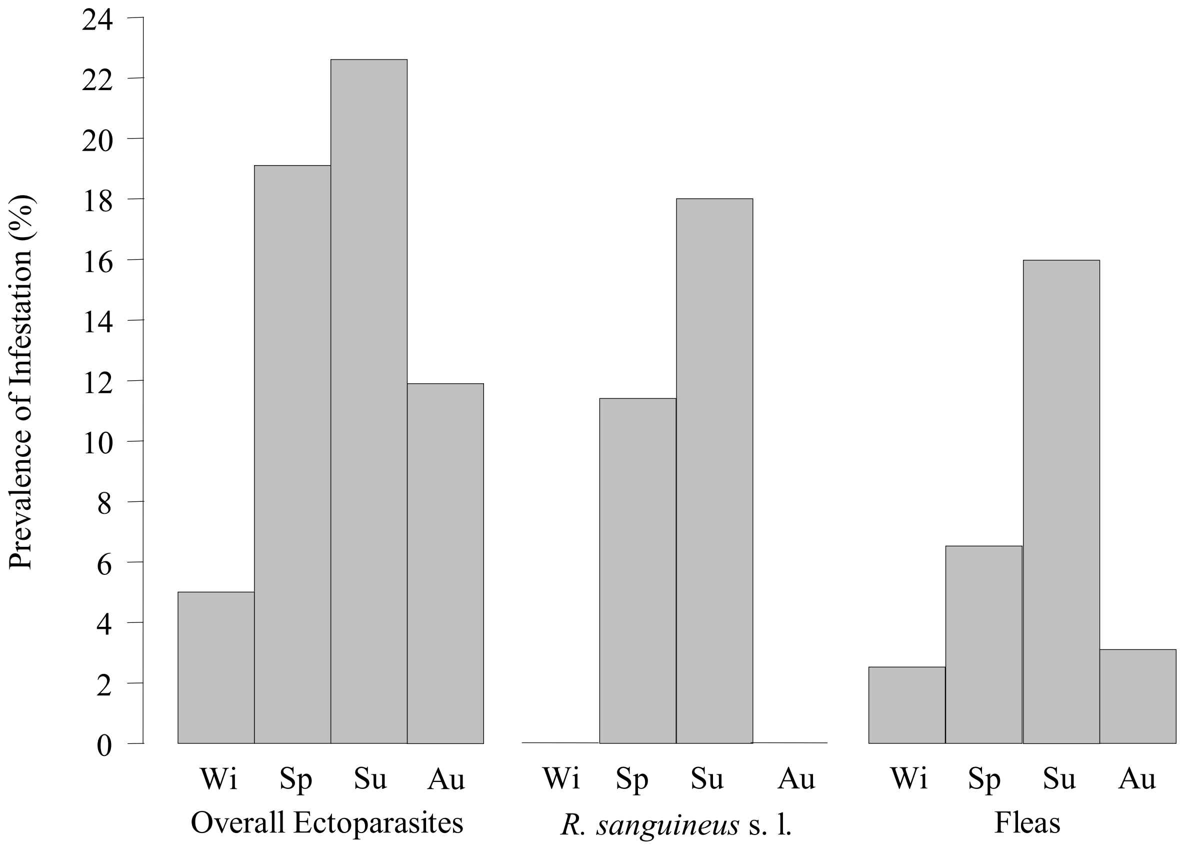

Figure 4: Seasonal prevalence of infestation of client-owned dogs from Albania with overall ectoparasites, *Rhipicephalus sanguineus* s. l. and fleas (Wi, winter; Sp, spring; Su, summer; Au, autumn)

The factors dog's age, gender, the dog's habitat and the presence/absence of other dogs and/or cats were not found to be significant risk factors for ectoparasitism for the examined population of dogs in the univariate analysis.

The final multivariate logistic regression models for risk factors associated with overall ectoparasitism developed on the basis of the results of the examination of the dogs by full body search and total body comb, and examination of ear and scrapings taken from skin lesions suspicious of mite infestation showed that only general ectoparasiticide use and season of examination were the significant predictors (Table 8).

Table 8: Multivariate analysis for risk factors associated with overall ectoparasitism in 602 client-owned dogs from Albania

Factor and group	Odds Ratio	95%CI	P value
Overall Ectoparasitism			
Ectoparasiticide use			
No	Reference	NA[1]	NA
Yes	0.24	0.12 - 0.46	<0.001
Season of Examination			
Winter	Reference	NA	NA
Spring	7.08	2.78 - 18.02	<0.001
Summer	7.43	2.84 - 19.46	<0.001
Autumn	2.48	0.88 - 7.00	<0.001

[1] NA = not applicable

In contrast to endoparasitism (cf. 4.1.1.), there are relatively few studies which evaluated risk factors associated to ectoparasitism and attempted to associate variation in prevalence and/or intensity to certain epidemiological factors. Although univariate analyses identified several risk factors for various types of ectoparasitism in the population of client-owned dogs (e. g., mixed breed dogs, pet dogs, dogs kept in yards and kennel/runs, lack of ectoparasiticide use, warm seasons) only history of ectoparasiticide use and season of examination were identified as strongest predictors for ectoparasitism.

Despite that *R. sanguineus* may infest dogs in the Mediterranean region throughout the year as recently shown in a study conducted in a heavily infested animal shelter in southern Italy (Lorusso et al. 2010), infestation with *R. sanguineus* in the client-owned dogs from Albania was observed in the spring and summer months only. A similar seasonal pattern with *R. sanguineus* found attached to dogs in spring, summer and autumn has been previously recorded in less well-cared-for dogs from suburban areas in Tirana (Xhaxhiu et al. 2009; Silaghi et al. 2013) and was reported from central Italy (Stella et al. 1988; Principato et al. 1989). As found in the client-owned dogs from Albania, field studies conducted in central Europe demonstrated that ticks (mainly *Ixodes* spp. and *Dermacentor reticulatus*) attached less frequently to dogs treated with acaricides than to untreated dogs (Duscher et al. 2013; Beck et al. 2014).

In agreement with other studies from Europe including prior studies conducted in Albania (Koutinas et al. 1995; Beck et al. 2006; Rinaldi et al. 2007; Gracia et al. 2008; Xhaxhiu et al.

2009; Silaghi et al. 2013), infestation of dogs with both *C. canis* and *C. felis* fleas was demonstrated year round with the peak of prevalence seen in summer. In contrast to a study conducted in the South of Italy (Rinaldi et al. 2007), housing with other dogs and/or cats was not identified as risk factor in the client-owned dogs from Albania. Interestingly, while hunting and guard dogs from southern Italy had a higher risk of flea infestation than pet dogs (Rinaldi et al. 2007), examination of the client-owned dogs from Albania revealed the contrary. However, the controversial findings of the two surveys result from univariate analyses only. For the client-owned dogs from Albania, multivariate analyses accounting for several additional factors including use of ectoparasiticides did not provide evidence for the dog's purpose to be considered as a significant predictor for ectoparasitism. The relevance of considering potential confounding factors in this regard was discussed by Rinaldi et al. (2007).

5. DISCUSSION

Related to the specific history of Albania, the status of parasitic infections and vector-borne diseases of dogs was rather undocumented until the recent past when several studies were initiated. As companion animal ownership is a relatively recent phenomenon in Albania only, data on infections with parasitic and non-parasitic vector-borne pathogens for this category of dogs are lacking (cf. 2.1. and 2.2.). Therefore, a prospective study was designed to identify the spectrum and to estimate the prevalence of endoparasite, ectoparasite and non-parasitic vector-borne infections in veterinary-cared-for, client-owned dogs from Albania. For this comprehensive approach, faeces, ectoparasites, and blood and serum samples were collected from 602 dogs presented to four small animal clinics in Tirana monthly from March 2010 to April 2011 inclusive and examined using various techniques. In addition, various variables ranging from dog characteristics to management factors were evaluated for their association to infections.

Most of the (minimum) 14 endoparasites identified in terms of their faecal stages are considered as common canine endoparasites with worldwide and frequent occurrence while some are less often diagnosed (*Hammondia*/*Neospora*, capillarid nematodes, *Angiostrongylus*, *Dicrocoelium*, *Linguatula*). For the first time in Albania, the occurrence in dogs of *Hammondia*/*Neospora*-like, *Angiostrongylus* lungworm, capillariid and *Linguatula* infections was indicated in this survey. The finding of *Linguatula* eggs in the faeces of one dog confirmed the suspected occurrence of this pentastomid in dogs in Albania (cf. 2.2.3.).
With almost 30% and 22% of the dogs testing positive for protozoan infections (mainly *Giardia*) and nematode parasitism, respectively, results indicate that substantial levels of endoparasite infection exist in client-owned dogs in the country. However, prevalence rates of endoparasitism in the dogs examined were substantially lower than those reported from previous studies conducted in Albania which encountered dogs with limited or no veterinary care (Dhamo and Zanaj 2003; Zanaj et al. 2006; Xhaxhiu et al. 2011; Bocari et al. 2014). Although results of different studies cannot necessarily be compared because of the specific procedural methods, overall prevalence and prevalence rates for most individual types of endoparasitism identified in the client-owned dogs in Albania were considerably higher than the rates estimated in several recent faecal surveys conducted in veterinary(well)-cared-for dogs in Central and Western Europe, North America, Japan or Australia which benefit in their environment from a high level of general and veterinary care over several decades (e. g., Claerebout et al. 2007; Batchelor et al. 2008; Palmer et al. 2008; Little et al. 2009; Mohamed

et al. 2009; Barutzki and Schaper 2011; Itoh et al. 2011; Joffe et al. 2011; Globokar Vrhovec 2013). In common with the results of the surveys from central and Western Europe, North America, Japan and Australia, protozoan infections (mainly *Giardia*) were more often identified in the client-owned dogs from Albania than nematode infections. Interestingly, similar prevalence rates of most of the nematode infections identified in the present survey from Albania were reported in the same category of dogs from the closer geographic region, i. e. in Greece and Serbia (Lefkaditis 2003; Lefkaditis et al. 2005, 2009; Papazahariadou et al. 2007; Nikolić et al. 2008), and Southern Italy (Lia et al. 2002; Napoli et al. 2012).
Of the risk factors repeatedly reported to be associated with endoparasitism in dogs, i. e., age, gender, dog's purpose, habitat and environment, use of anthelmintics and geographic location (Visco et al. 1977; Martini et al. 1992; Coggins 1998; Fok et al. 2001; Dubná et al. 2007; Palmer et al. 2008; Gates and Nolan 2009; Little et al. 2009; Mohamed et al. 2009; Barutzki and Schaper 2011; Globokar Vrhovec 2013; Zanzani et al. 2014), the dog's age, the dog's purpose, the dog's habitat and environment, presence/absence of other pets, anthelmintic use and season were identified as significant factors predisposing dogs to various types of endoparasitism while the variables breed, gender and type of food were not significant predictors in the client-owned dogs from Albania. Multivariate logistic regression analysis for factors associated with overall endoparasitism revealed that dogs >1 year of age (odds ratio [OR] = 0.64), dogs dewormed at least once per year (OR = 0.35) and dogs tested during spring, summer and autumn (OR = 0.51, 0.15 and 0.20, respectively) had a lower risk compared to younger dogs, dogs not dewormed or dogs tested during winter. The odds to be diagnosed positive for endoparasites was 1.56 times higher for dogs living together with other pets than that for dogs without other dogs or cats.

Examination of client-owned dogs from Albania revealed the presence of nine arthropod parasites including three species each of mites and fleas, two species of ticks and one biting louse which all are common canine ectoparasites with worldwide occurrence but variability as to prevalence and importance related to geographical region and category of dogs. The occurrence of all nine species of ectoparasites concurrently has been recorded before only recently in a survey of less well-cared-for dogs in Albania (Xhaxhiu et al. 2009). Overall prevalence of ectoparasitism was approximately 15% and thus significantly lower than that of almost 80% established in a comparable study which enrolled 181 less well-cared-for dogs from suburban Tirana (Xhaxhiu et al. 2009).

As expected from prior studies conducted in Albania (Xhaxhiu et al. 2009; Silaghi et al. 2013) and neighbouring Greece (Papazahariadou et al. 2003), *R. sanguineus* was the predominant species of ticks. *Rhipicephalus sanguineus* is a characteristic representative of the Mediterranean tick fauna, and urban and suburban habitats constitute a suitable environment for this endophilous tick that can infest houses (Dantas-Torres 2008, 2010). The relevance of infestation with *R. sanguineus* is associated with the range of pathogens identified in the examined client-owned dogs (*B. vogeli*, *H. canis*, *A. platys*, *E. canis*, *M. haemocanis*; cf. 4.1.2.) which are confirmed or supposed to be transmitted by this tick (Senevira et al. 1973; Otranto and Dantas-Torres 2010). Similarly to the infestation with *R. sanguineus*, mites and biting lice, but much more pronounced, infestation with fleas of the client-owned dogs in this study was lower than that of less well-cared-for dogs from suburban Tirana (Xhaxhiu et al. 2009). Although *C. canis* was the most prevalent species of flea in both client-owned, veterinary-cared-for dogs and less well-cared-for dogs in Albania (Xhaxhiu et al. 2009), proportions of infested dogs harbouring *C. canis*, *C. felis* and *P. irritans*, suggest a shift from a *C. canis*-predominated flea population in the dogs with limited or no veterinary care towards a population with a substantial higher percentage of *C. felis* in the client-owned dogs (100%, 6.5% and 10.9%, respectively, vs. 72.5%, 45.0% and 2.5%, respectively). This pattern, which might be related to the keeping of a high percentage of the client-owned dogs in residential habitats (cf. Xhaxhiu et al. 2009), should be followed-up in future surveys in client-owned dogs in the country. In contrast to endoparasitism (cf. 4.1.1.), there are relatively few studies which evaluated risk factors associated to ectoparasitism and attempted to associate variation in prevalence and/or intensity of infestation to certain epidemiological factors. Although univariate analyses identified several risk factors for various types of ectoparasitism in the sample of client-owned dogs (mixed breed dogs, pet dogs, dogs kept in yards and kennel/runs, lack of ectoparasiticide use, warm seasons) only history of ectoparasiticide use and season of examination were identified as strong predictors for ectoparasitism through multivariate logistic regression analyses. As discussed by Rinaldi et al. (2007) with respect to the infestation of dogs with fleas, consideration of potential confounding variables is crucial for a meaningful analysis and interpretation of importance of potential risk factors for ectoparasitism.

Examination of whole blood, blood smears and serum confirmed the presence in dogs of exposure to *Neospora caninum* and *Toxoplasma gondii* and of infection with eight vector-borne pathogens (*B. vogeli*, *H. canis*, *L. infantum*, *D. immitis*, *A. phagocytophilum*, *A. platys*, *E. canis*, *M. haemocanis*). Seroprevalences for *N. caninum* and *T. gondii* (18.3% and 51.7%, respectively) and spectrum and prevalence of the vector-borne pathogens (range, 1% to 9%) in the client-owned dogs from Albania were comparable to that reported in other countries in the Mediterranean Basin. Seroprevalence for *Babesia* spp., *L. infantum*, *Anaplasma* spp. and *E. canis* infections were at the lower range of that reported in studies from the Balkan Peninsula (cf. 4.1.2.). As discussed before, several of the pathogens are associated to the presence of *R. sanguineus*, which was found attached to approximately 8% of the client-owned dogs enrolled in the study (cf. 4.2.).

This study presents the first record of detection of *A. phagocytophilum, A. platys* and *M. haemocanis* by PCR and of antibodies against *T. gondii* and *N. caninum* in dogs from Albania. The occurrence of *A. platys* and *M. haemocanis* had been indicated before through the detection of their DNA in *R. sanguineus* ticks or *C. canis* fleas, respectively, which were collected from dogs in Tirana (Silaghi et al. 2013). Similarly, seropositivity in cats indicated previously the presence of *N. caninum* in the dog population of Albania (Silaghi et al. 2014).

Univariate analysis identified the dog's age, the dog's habitat, season of examination and gender were identified as significant factors predisposing dogs to the presence of/exposure to various vector-borne pathogens while the dog's purpose, environment, breed and presence/absence of other pets were not significant predictors. In contrast to endoparasitism, multivariate logistic regression analyses for risk factors for the presence of/exposure to pathogens identified higher age as the overall strongest risk factor (OR 1.7 to 2.8). Potential reasons for the positive association of sero-/antigen positivity and age may be repeated re-infection, general increasing probability of exposure to pathogens with age and/or persisting antibodies. However, there are several studies reporting controversial findings regarding the relationship of age and prevalence of the pathogens/infections considered (e. g., Zaffaroni et al. 1999; Jensen et al. 2003; Solano-Gallego et al. 2006; Dubey et al. 2007; Yildrim et al. 2007; Cortes et al. 2012; Miró et al. 2013).

Conclusion

This survey is the first which evaluated the parasite status of client-owned, veterinary-cared-for dogs from Albania and covered endo- and ectoparasite infections as well as the presence of/exposure to vector-borne diseases. More canine samples were examined in this study than in any other prevalence study previously conducted in Albania. The broad spectrum of parasites and vector-borne pathogens as well as the seroprevalence rates established through the examination of samples from 602 client-owned dogs were comparable to those reported from other countries in the Mediterranean region in this category of dogs. The prevalence rates for most type of infections in the dogs under veterinary care were lower than those in less well-cared dogs from the Balkan region, probably reflecting increasing responsible ownership and veterinary care. However, the results indicate that veterinarians and dog owners need to continue and increase their efforts in the periodic examination for and treatment of endo- and ectoparasitism including general improvement of dog husbandry practices and routine prophylactic control measures. This will reduce the parasitism in the dogs, limit the exposure of dogs to pathogens/vectors and thus lower the potential for transmission of zoonotic agents to humans.

This study provided current information on the prevalence of parasites and other pathogens in client-owned dogs in Albania but sampling was restricted mainly to the Tirana area as was the sampling of previously conducted work in less well-cared-for dogs. Therefore, future studies should enroll dogs from other geographic locations too in order to establish regional data for the country.

6. SUMMARY

From March 2010 to April 2011 inclusive, faeces, ear swabs, ectoparasites collected by body search and full body combing, and blood samples were obtained from 602 client-owned dogs and examined for stages of endoparasites, ectoparasite infestation or presence of/exposure to vector-borne and other pathogens. Dog owners were invited to participate with their dogs in the study when attending four small animal clinics in Tirana, Albania. A questionnaire was completed for each dog in order to collect demographic and management information.

Faecal samples of 245 dogs (40.7%) tested positive for at least one type of faecal **endoparasite** (protozoan, helminth and/or pentastomid) stage, of which 180 (29.9%) and 129 (21.9%) tested positive for protozoan or nematode endoparasites, respectively. Faecal forms of at least 14 parasites were identified. The most frequently identified stages were those of *Giardia* (26.4%), *Trichuris* (9.5%), *Toxocara* (8.0%), hookworms (7.1%), *Cystoisospora ohioensis* (4.3%) and *Cy. canis* (3.0%). For the first time for dogs in Albania, this survey indicated the occurrence of *Hammondia*/*Neospora*-like (0.2%), *Angiostrongylus* lungworm (0.3%), capillariid (2.8%) and *Linguatula* (0.2%) infections. Single and multiple infections with up to seven parasites concurrently were found in 152 (25.2%) and 93 dogs (15.4%), respectively. On univariate analysis, the dog's age, the dog's purpose (pet, hunting dog, working dog), the dog's habitat (city, suburban, rural) and environment (mainly indoors, indoors with regular outside walking, yard, kennel/run), presence/absence of other pets, anthelmintic use and season were identified as significant ($p<0.05$) factors predisposing dogs to various types of endoparasitism while the variables breed (pure breed vs. mixed-breed dogs), gender and type of food were not significant predictors. Multivariate logistic regression analysis for factors associated with overall endoparasitism revealed that dogs >1 year of age (odds ratio [OR] = 0.64), dogs dewormed at least once per year (OR = 0.35) and dogs tested during spring, summer and autumn (OR = 0.51, 0.15 and 0.20, respectively) had a significantly lower risk compared with ≤1 year old dogs, dogs not dewormed or dogs tested during winter. The odds to be diagnosed positive for endoparasites was 1.56 times higher for dogs living together with other pets than that for dogs without other dogs or cats.

Ninety-three dogs (15.4%) were demonstrated to be infested with **ectoparasites**, and nine species were identified: *Ixodes ricinus*, 0.8%; *Rhipicephalus sanguineus* s. l., 8.1%; *Demodex canis*, 0.2%; *Sarcoptes scabiei* var. *canis* 0.7%; *Otodectes cynotis*, 2.8%; *Ctenocephalides canis*, 4.8%; *C. felis*, 3.0%; *Pulex irritans*, 0.2%; *Trichodectes canis*, 0.2%. Single and multiple infestations with up to four species of ectoparasites concurrently were recorded in 67

(11.1%) and 26 dogs (4.3%), respectively. On univariate analysis, breed, the dog's purpose, the environment, use of ectoparasiticides and the season were identified as significant ($p<0.05$) factors predisposing dogs to various types of ectoparasitism while the variables dog's age, gender, the dog's habitat and the presence/absence of other pets were no significant predictors. Multivariate logistic regression analysis for factors associated with overall ectoparasitism revealed that dogs treated with ectoparasiticides at least once per year (OR = 0.24) had a significantly lower risk of infestation compared with dogs not treated against ectoparasite infestation and that dogs examined during spring, summer and autumn (OR = 7.08, 7.43 and 2.48, respectively) had a significantly higher risk of infestation than dogs examined during winter.

Whole blood, blood smears and serum were examined for the presence of **arthropod-borne infections** as well as ***Neospora caninum*** and ***Toxoplasma gondii***. Eight different pathogens (*Babesia vogeli*, *Hepatozoon canis*, *Leishmania infantum*, *Dirofilaria immitis*, *Anaplasma phagocytophilum*, *A. platys*, *Ehrlichia canis*, *Mycoplasma haemocanis*) were detected by direct methods with prevalences 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 tested significantly more often positive for vector-borne infections 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*. On univariate analysis, the dog's age, the dog's habitat, season of examination and gender were identified as significant ($p<0.05$) factors predisposing dogs to the presence of/exposure to various vector-borne pathogens while the dog's purpose, environment, breed and presence/absence of other pets were not significant predictors. Multivariate logistic regression analyses for risk factors for the presence of/exposure to pathogens showed that the higher age was overall the strongest risk factor (OR 1.7 to 2.8).

This survey is the first which evaluated the status of client-owned, veterinary-cared-for dogs from Albania for endo- and ectoparasite infections and the presence of/exposure to vector-borne diseases. Results show that dogs are at risk of a broad spectrum of parasites and bacterial vector-borne agents. These findings highlight the need for both veterinarians and dog owners to continue and to increase their efforts in the examination for and treatment of endo- and ectoparasitism including improvement of dog husbandry practices and routine prophylactic control measures.

9. ZUSAMMENFASSUNG

Von März 2010 bis einschließlich April 2011 wurden Kot, Ohrtupferproben, vom Körper abgesammelte und abgekämmte Ektoparasiten sowie Blutproben von 602 Hunden auf Fäkalstadien von Endoparasiten, Ektoparasitenvorkommen bzw. das Vorliegen von Infektionen mit Vektor-übertragenen Erregern untersucht. Den Besitzern der Hunde wurde beim Besuch von vier Tierarztpraxen in Tirana, Albanien, angeboten, mit ihren Hunden an dieser Studie teilzunehmen. Für jeden Hund wurde ein Fragebogen ausgefüllt mit Angaben zum Tier und zu seinen Haltungsbedingungen.

Im Kot von 245 Hunden (40,7%) wurde mindestens ein Typ fäkaler Stadien von **Endoparasiten** (Protozoen, Helminthen und/oder Pentastomiden) gefunden. Dabei waren 180 Proben (29,9%) positiv für Protozoen und 129 (21,9%) positiv für Nematoden. Insgesamt ließen sich Stadien von mindestens 14 Parasiten identifizieren. Die am häufigsten nachgewiesenen Stadien waren die von *Giardia* (26,4%), *Trichuris* (9,5%), *Toxocara* (8,0%), Hakenwürmern (7,1%), *Cystoisospora ohioensis* (4,3%) and *Cy. canis* (3,0%). In dieser Studie konnte erstmalig für Hunde in Albanien das Vorkommen von Infektionen mit *Hammondia/Neospora* (0,2%), *Angiostrongylus*-Lungenwürmern (0,3%), Haarwürmern (2,8%) und *Linguatula* (0,2%) nachgewiesen werden. Infektionen mit einem Parasiten-Typ wurden bei 152 (25,2%) Hunden und multiple Infektionen mit bis zu sieben Parasiten-Typen bei 93 (15,4%) Hunden diagnostiziert. Eine univariante Datenanalyse identifizierte folgende Parameter als signifikante ($p<0,05$) Faktoren für die Prädisposition für einzelne Endoparasiten: Alter des Hundes, Verwendung des Hundes (Haustier, Jagdhund, Arbeitshund), Lebensraum (Stadt, Vorort, ländlicher Raum) und Haltung (vorwiegend im Haus, im Haus mit regulärem Freilauf, Hof/Zwinger) des Hundes, Vorhandensein anderer Hunde/Katzen, anthelminthische Behandlung sowie Zeitpunkt der Untersuchung (Saison); Rasse (reinrassige Hunde – Mischlingshunde), Geschlecht und Ernährung wurden nicht als signifikante Faktoren identifiziert. Die multivariante Regressionsanalyse für mit 'Endoparasitenbefall(gesamt)' assoziierte Faktoren ergab, dass Hunde mit einem Alter von mehr als einem Jahr (odds ratio [OR] = 0,64), Hunde, die mindestens einmal im Jahr entwurmt wurden (OR = 0,35) und Hunde, die in den Frühjahrs-, Sommer- und Herbstmonaten untersucht worden waren (OR = 0,51, 0,15 bzw. 0,20) ein signifikant geringeres Befallsrisiko hatten als jüngere Hunde, nicht entwurmte Hunde bzw. Hunde, die in den Wintermonaten untersucht wurden. Hunde, die gemeinsam mit anderen Hunden/Katzen gehalten wurden, hatten ein höheres Befallsrisiko (OR = 1,56) als einzeln gehaltene Hunde.

Ein Befall mit **Ektoparasiten** wurde bei 93 Hunden (15,4%) nachgewiesen, wobei Ektoparasiten von neun Arten nachgewiesen wurden: *Ixodes ricinus*, 0,8%; *Rhipicephalus sanguineus* s. l., 8,1%; *Demodex canis*, 0,2%; *Sarcoptes scabiei* var. *canis* 0,7%; *Otodectes cynotis*, 2,8%; *Ctenocephalides canis*, 4,8%; *C. felis*, 3,0%; *Pulex irritans*, 0,2%; *Trichodectes canis*, 0,2%. Mono- und Mischinfektionen mit bis zu vier Arten von Ektoparasiten wurden bei 67 (11,1%) und 26 Hunden (4,3%) festgestellt. Eine univariante Datenanalyse identifizierte folgende Parameter als signifikante (p<0,05) Faktoren für die Prädisposition für einzelne Ektoparasiten: Rasse, Verwendung des Hundes, Haltung, antektoparasitäre Behandlung sowie Zeitpunkt der Untersuchung; Alter, Geschlecht, Lebensraum sowie Vorhandensein anderer Hunde/Katzen wurden nicht als signifikante Faktoren identifiziert. Die multivariante Regressionsanalyse für mit 'Ektoparasitenbefall(gesamt)' assoziierte Faktoren ergab, dass Hunde die mindestens einmal im Jahr mit einem Antektoparasitikum behandelt wurden ein signifikant geringeres Befallsrisiko hatten (OR = 0,24) und dass Hunde, die in den Frühjahrs-, Sommer- und Herbstmonaten untersucht worden waren, ein signifikant größeres Befallsrisiko hatten als in den Wintermonaten untersuchte Hunde (OR = 7,08, 7,43 bzw. 2,48).

Vollblut, Blutausstriche und Serum wurden zum Nachweis von Infektionen mit **Vektor-übertragenen Erregern**, ***Neospora caninum*** und ***Toxoplasma gondii*** untersucht. Acht verschiedene Erreger wurden dabei mit Hilfe direkter Methoden nachgewiesen (*Babesia vogeli*, *Hepatozoon canis*, *Leishmania infantum*, *Dirofilaria immitis*, *Anaplasma phagocytophilum*, *A. platys*, *Ehrlichia canis*, *Mycoplasma haemocanis*) mit Befallsraten von 1% bis 9%. Die Seroprävalenzen für *Babesia* spp., *L. infantum*, *Anaplasma* spp. und *E. canis* betrugen 6,6%, 5,1%, 24,1% bzw. 20,8%. Hunde mit einem Alter von mehr als einem Jahr wurden häufiger positiv für Vektor-übertragene Infektionen getestet als jüngere Tiere (p=0,003). Mehr als die Hälfte der Hunde hatte Antikörper gegen *T. gondii* und 18,3% der Hunde war seropositiv für *N. caninum*. Eine univariante Datenanalyse identifizierte folgende Parameter als signifikante (p<0,05) Faktoren für die Prädisposition für einzelne Infektionen mit Vektor-übertragenen Erregern: Alter, Lebensraum, Geschlecht und Zeitpunkt der Untersuchung; Rasse, Verwendung des Hundes, Haltung sowie Vorhandensein anderer Hunde/Katzen wurden nicht als signifikante Faktoren identifiziert. Die multivariante Regressionsanalyse für mit 'Vektor-übertragene Infektionen' assoziierte Faktoren ergab, dass ältere Hunde insgesamt ein höheres Infektionsrisiko hatten (OR = 1,7 - 2,8).

In dieser Arbeit wurde zum ersten Mal das Vorkommen von Infektionen mit Endo- und Ektoparasiten sowie Vektor-übertragenen Erregern bei veterinärmedizinisch betreuten Hunden von privaten Besitzern in Albanien untersucht. Die Ergebnisse der Untersuchungen zeigen, dass die Hunde einem breiten Spektrum von parasitären und bakteriellen, Vektor-übertragenen Erregern ausgesetzt sind. Diese Ergebnisse indizieren, dass sowohl Tierärzte als auch Hundebesitzer ihre Bemühungen hinsichtlich der Überwachung des Befalls mit und der Behandlung von Endo- und Ektoparasiteninfektionen verstärken müssen, einschließlich der Verbesserung der Haltungsbedingungen und regelmäßiger prophylaktischer Maßnahmen.

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9. PRESENTATION of PRELIMINARY RESULTS

Conference Abstracts (Oral Presentations)

Shukullari E, Hamel D, Visser M, Winter R, Rapti D, Pfister K, Rehbein S (2013) Parasitenbefall und Vektor-übertragene Infektionen bei tierärztlich betreuten Hunden in Albanien: Parasiten des Gastrointestinaltraktes und der Atmungsorgane. Tagung der DVG-Fachgruppe Parasitologie und parasitäre Krankheiten, 08-10 July 2013, Gießen, Germany, Abstracts, 26-27

Hamel D, Silaghi C, Shukullari E, Rapti D, Pfister K, Rehbein S (2013) Parasitenbefall und Vektor-übertragene Infektionen bei tierärztlich betreuten Hunden in Albanien: Vektor-übertragene und parasitäre Infektionen. Tagung der DVG-Fachgruppe Parasitologie und parasitäre Krankheiten, 08-10 July 2013, Gießen, Germany, Abstracts, 24-25

Conference Abstracts (Poster Presentation)

Shukullari E, Rapti D, Xhaxhiu D, Pfister K, Visser M, Rehbein S (2013) Survey of ectoparasite infestation of dogs under veterinary care in Albania. 12th International Symposium on Ectoparasites of Pets (ISEP) joint with EVPC Annual Meeting, 07-10 April 2013, Munich, Germany, Final Program and Proceedings, 72

10. APPENDIX

Questionnaire – Parasitological Status I ID ....

Date ....

Owner Information

Name .... Phone ....

Address (city, street) ....

Dog Information

Name .... Age (or date of birth) .... Sex ....

Breed .... Weight (kg) ....

Dog's Purpose: Pet ☐ Working Dog (watch dog, military dog, herding, hunting) ☐

Dog's Habitat: City (central parts) ☐ City (suburban) ☐ Rural (village) ☐

Dog lives at/in: Home ☐ Kennel/Run ☐ Yard ☐ Free ☐

Does the dog walk? Yes ☐ No ☐

Other pets in household / home? Yes ☐ No ☐

If Yes: No. of dogs .... No. of cats ....

Dog's Feed: Fresh (uncooked) meat/organs Regularly ☐ Occasional ☐

Cooked/Canned/Dry Feed Regularly ☐ Occasional ☐

Antiparasitic Treatments

Anthelmintic Treatment (Deworming)

No (0) ☐ 1-2x/year ☐ >2x/year ☐ Date last treatment: ....

Product(s) used: ....

Ectoparasiticide Treatment (flea and/or tick control)

No (0) ☐ 1-2x/year ☐ >2x/year ☐ Date last treatment: ....

Product(s) used: ....

Heartworm Prophylaxis

No (0) ☐ 1-2x/year ☐ >2x/year ☐ Date last treatment: ....

Product(s) used: ....

Questionnaire – Parasitological Status II ID ……….

Reason for Visiting the Clinic - Anamnesis

Major Clinical Signs (e. g., cough, heart problems, body temp if increased …)

…..………

..……

………

………

………

………

Hair coat/Skin Status (i. e., lesions suspicious for ectoparasites – scraping!) Body Search

Normal/Inconspicuous ☐

Abnormal (describe, incl. location) ………..……………………………………………………………

………

Hair length: Short ☐ Long ☐

Pruritus: Yes ☐ No ☐

Skin Scraping Taken: Yes ☐ No ☐

Diarrhea Yes ☐ No ☐

Samples to be collected from each dog

Ear Swab (both ears) ☐

Body Comb Ectoparasites (ticks, fleas, lice) collected: Yes ☐ (ambient) No ☐

Blood EDTA-Blood (freezer) ☐ Serum (freezer) ☐ Blood Smears (dry, ambient) ☐

Faeces Sample (minimum 10-15 g) (refrigerator) ☐

11. ACKNOWLEDGEMENTS

Firstly, my deep gratitude goes to my principal supervisor, Dr. Steffen Rehbein, for the guidance, suggestions, expertise, input, encouragement and continuous support with any detail of this thesis. I am most grateful for the opportunity to work at the Kathrinenhof Research Center laboratories and for the support of Merial GmbH.

I am grateful to my thesis advisor, Prof. Kurt Pfister, who enabled me to work on this project in the format of a thesis, for his interest and support including the possibility to perform work at the Institute of Comparative Tropical Medicine and Parasitology.

I am indebted to Martin Visser for his dedication and patience and his efforts in regards to sample processing and identification of parasites. Very special thanks go to the whole staff of the Kathrinenhof Research Center for their generous assistance and hospitality.

For teaching me in serology and examination of samples in the Diagnostic Unit of the Institute of Comparative Tropical Medicine and Parasitology, I am extremely grateful to Dr. Dietmar Hamel.

I wish to thank Prof. Dhimitër Rapti for the opportunity to enroll dogs in his small animal clinic, for his support throughout the sample collection period and the advice during the completion of this thesis.

Special thanks go to the veterinarians of the 'Xhimi' clinic for their assistance in the collection of the samples and all data necessary for this project. Many thanks also go to Dr. Gerta Dhamo, Dres. Olsi and Blerina Doku, and Lavdrim Balla, MSc for their interest and their contribution and support in the collection of dog samples for my project.

Sanisys SA, Switzerland, is thanked for their assistance in the data analysis.

Last but not least, my deepest and warmest thanks go to my family for their support throughout this work, and for always being there and encouraging me over the years.