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Research Note

Preliminary study on the prevalence of endoparasite infections and vector-borne diseases in outdoor dogs in Bulgaria

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Summary

The present work was designed to evaluate the prevalence of gastrointestinal parasites and some vector-borne pathogens in dogs in Bulgaria. A total of 172 owned dogs, keeping outside, were included in the study. Fecal samples were examined using standard flotation and sedimentation methods. Blood samples were processed by Knott's technique, SNAP™ 4Dx Plus Test (IDEXX) and Angio Detect™ Test (IDEXX). The overall prevalence of gastrointestinal parasites was 64.5%. Eggs of hookworms (*Ancylostoma* sp. and *Uncinaria* sp.) were the most frequently detected (54.1%), followed by *Trichuris vulpis* (15.1%), *Capillaria* sp. (11.0%), *Toxocara canis* (6.4%), *Cystoisospora* sp. (4.1%), *Sarcocystis* sp. (2.3%), *Toxascaris leonina* (1.7%), *Taenia* sp. (1.2%) and *Linguatula serrata* (0.6%). In addition, hookworms were the most commonly involved in the cases of single infection (20.3%). Combinations between *Capillaria* sp./hookworms and *T. vulpis*/hookworms were the most common co-infections (4.1% and 2.9%, respectively). Blood samples revealed the presence of antibodies against *Ehrlichia* sp. (13.4%), *Anaplasma* sp. (13.4%) and *Borrelia burgdorferi* (1.7%). Antigens of *Dirofilaria immitis* and *Angiostrongylus vasorum* were detected in 10.5% and 0.6% of the samples tested, respectively. Microfilariae of *Dirofilaria repens* were found in 5.8% of the blood samples. Additionally, the prevalence of *D. immitis* and *Ehrlichia* sp. was significantly higher in adult than in young dogs ($p<0.05$). In contrast, the gender was not considered as a risk factor contributing to the occurrence of infections.

Keywords: prevalence; dogs; gastrointestinal parasites; *Angiostrongylus vasorum*; Bulgaria

Introduction

Dogs are still the most common companion animals establishing more frequent and closer contact with humans than any other pets. Furthermore, various canine parasites are involved in the epidemiology of many parasitic diseases affecting a wide range of domestic and wild herbivorous and omnivorous. Also, the canids act as reservoirs and sources of several zoonotic parasites posing a serious threat to the human health (Xhaxhiu *et al.,* 2011). The children are generally at higher risk of acquiring infections with some parasites (e.g. *Toxocara canis*) than adults due to the habit of placing their fingers in the mouth after playing with dogs or after contact with contaminated soil. In contrast, human hydatid disease is mainly associated with persons, practicing hunting and sheep farming. This dangerous and life-threatening infection is caused by the larval stage of *Echinococcus granulosus*, a cestode, inhabiting the small intestines of various canids as final hosts (Gillespie & Bradbury, 2017).

The prevalence of parasitic infections in dogs depends on several factors such as the lifestyle, deworming frequency, climate

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conditions, and contacts with stray dogs or wildlife (Roussel *et al.,* 2019). Additionally, some owners neglect the prophylaxis of their dogs resulting in contamination of environment with infective parasite stages of high tenacity. Also, the shepherd dogs often have no prophylactic therapy, and the occurrence and intensity of parasite infections, especially with tapeworms, usually results from scavenging sheep carcasses (Vasileiou *et al*., 2015; Rehbein *et al*., 2016).

Canine parasitic fauna has been a purpose of many studies conducted all around the world over the last few decades. Numerous Bulgarian investigations on the same focus have also been carried out, including different categories of dogs, originating from many regions of the country. In addition, gastrointestinal (GI) parasitism of *Trichuris vulpis* and *Capillaria* sp., hookworms (*Ancylostoma caninum*, *Uncinaria stenocephala*), ascarids (*T. canis*, *Toxascaris leonina*), cestodes (*Dipylidium caninum*, *Mesocestoides lineatus*, *Taenia hydatigena*, *Taenia ovis*, *Taenia multiceps*, *Taenia pisiformis*, *E. granulosus*), protozoans (*Cystoisospora* sp., *Sarcocystis* sp., *Giardia* sp.) has been recorded in studies based on necropsy (Kamenov *et al*., 2009); combined necropsy/coproscopy (Georgieva *et al*., 1999; Lalkovski & Sabev, 2009) or coproscopy (Kirkova *et al*., 2006; Kirkova *et al.,* 2013; Kanchev *et al*., 2014; Radev *et al*., 2016; Iliev *et al*., 2017).

Canine vector-borne diseases (CVBDs) constitute an important group of illness caused by a diverse range of pathogens, which are transmitted to both animals and humans by different blood-sucking arthropods (Dantas-Torres, 2008). Those diseases are usually endemic in tropics and subtropics, but an increasing prevalence has also been recognized in temperate regions (Dantas-Torres, 2008; Beugnet & Chalvet-Monfray, 2013). The prevalence of CVBDs in Bulgaria has been frequently reported over the past few years, including infections with *Dirofilaria immitis*, *Dirofilaria repens*, *Ehrlichia canis*, *Anaplasma* sp., and *Borrelia burgdorferi* (Georgieva *et al*., 2001; Tsachev *et al*., 2006; Tsachev *et al*., 2006a; Tsachev *et al*., 2008; Panayotova-Pencheva *et al*., 2016; Radev *et al*., 2016; Iliev *et al*., 2017). Considering the data mentioned above as well as the trend towards increase in canine population in the country focused our attention on performing this epidemiological work. We aimed both at corroborating previously published findings and providing updated information on the prevalence of GI parasites as well as some arthropod-transmitted pathogens in outdoor dogs in Bulgaria.

Materials and Methods

Animals and study areas

This study was performed from July 2016 to September 2017 on 172 owned dogs (115 males and 57 females) aged from 1 month to 12 year, reared outdoor. The animals were recruited from outskirts of Rousse (43º50'N, 25º57'E), Razgrad (43º32'N, 26º32'E), Sofia (42º41'N, 23º19'E) (Northern Bulgaria), Stara Zagora (42º25'N, 25º38'E), and Plovdiv (42º8'N, 24º44'E) (Southern Bulgaria).

Table 1. Overall prevalence (%) of the pathogens in dogs (n=172)

Species	Southern Bulgaria (n=124)	Northern Bulgaria (n=48)	Total (n=172)		Test applied
	Positive (%)		Positive (%)	95% CIs	
D. repens	4 (3.2)	6 (12.5)	10 (5.8)	2.3 - 9.3	Knott's test
D. immitis	14 (11.3)	4 (8.3)	18 (10.5)	5.9 – 15.0	Serology
A. vasorum	1 (0.8)	-	1 (0.6)	0 – 1.7	
T. vulpis	14 (11.3)	12 (25.0)	26 (15.1)	9.8 – 20.5	Flotation
Capillaria sp.	10 (8.1)	9 (18.8)	19 (11.0)	6.4 – 15.7	
T. canis	9 (7.3)	2 (4.2)	11 (6.4)	2.7 – 10.1	
Ancylostoma/Uncinaria spp.	57 (46.0)	36 (75.0)	93 (54.1)	46.6 – 61.5	
T. leonina	1 (0.8)	2 (4.2)	3 (1.7)	0 – 3.7	
Taenia sp.	1 (0.8)	1 (2.1)	2 (1.2)	0 – 2.8	
L. serrata	-	1 (2.1)	1 (0.6)	0 – 1.7	Sedimentation
Cystoisospora sp.	6 (4.8)	1 (2.1)	7 (4.1)	1.1 – 7.0	Flotation
Sarcocystis sp.	4 (3.2)	-	4 (2.3)	0.1 – 4.6	
Anaplasma sp.	16 (12.9)	7 (14.6)	23 (13.4)	8.3 – 18.5	Serology
Ehrlichia sp.	22 (17.7)	1 (2.1)	23 (13.4)	8.3 – 18.5	
B. burgdorferi	2 (1.6)	1 (2.1)	3 (1.7)	0 – 3.7	

Table 2. Cases of single infection in dogs (n=172).

Species	Positive (%)	95% CIs
D. immitis	2 (1.2)	0 – 2.8
Ehrlichia sp.	6 (3.5)	0.7 – 6.2
T. vulpis	6 (3.5)	0.7 – 6.2
T. canis	5 (2.9)	0.4 – 5.4
Ancylostoma/Uncinaria spp.	35 (20.3)	14.3 – 26.4
Anaplasma sp.	6 (3.5)	0.7 – 6.2
D. repens	2 (1.2)	0 – 2.8
Cystoisospora sp.	1 (0.6)	0 – 1.7
A. vasorum	1 (0.6)	0 – 1.7
T. leonina	1 (0.6)	0 – 1.7
B. burgdorferi	1 (0.6)	0 – 1.7

Sampling and assaying

Fecal samples were obtained manually from *ampulla recti*, placed into plastic bags, stored at 4ºC, and processed (within 24 hours) by following methods: direct smear for detection of motile trophozoites or cysts of protozoa; flotation technique, using 3 grams feces and saturated sodium chloride (sp. gr. 1.20), for extraction of lighter helminth eggs and coccidian oocysts or sporocysts; routine sedimentation, using 3 grams feces, for recovering heavier helminth and pentastomid eggs.

Blood samples were collected by venipuncture of *v. cephalica antebrachii*, from each animal, into vacutainers. After clotting the samples, the sera were stored at 4ºC and assayed within 24 hours for detection of *D. immitis* antigens; antibodies against *Anaplasma* sp., *Ehrlichia* sp., and *B. burgdorferi* (SNAP® 4Dx Plus Test, IDEXX) as well as *A. vasorum* antigens (Angio Detect™ Test, IDEXX). Both assays were performed according to manufacturer's instructions. Additional blood samples were collected into sterile tubes with anticoagulant (K_2EDTA) and processed by Knott's technique for detection of filariid first stage larvae. Isolated microfilariae were identified on basis of their morphometrical characteristics (Zajac & Conboy, 2012).

Statistical analysis

All data were analysed by means of MedCalc v.10.2.0.0, MedCalc Software (Belgium). The prevalence and its 95 % confidence interval (CI) were calculated for each parasitic species, including co-infections. The difference of prevalence among groups (regarding to the gender and age) was evaluated by Chi-square (χ^2) test and was considered significant at $P \leq 0.05$.

Ethical Approval and/or Informed Consent

Approvals for using animals in the current study were obtained from the Bulgarian Food Safety Agency (registration of the permits: №85/09.01.2014 and №138/28.06.2016). The research has been complied with all the relevant national regulations and institutional policies for the care and use of animals.

Results

The overall prevalence of GI parasites was 64.5 %. Eggs of hookworms were the most commonly observed in feces (54.1 %), followed by *T. vulpis* (15.1 %), *Capillaria* sp. (11.0 %), *T. canis* (6.4 %), *Cystoisospora* sp. (4.1 %), *Sarcocystis* sp. (2.3 %), *T. leonina* (1.7 %) and *Taenia* sp. (1.2 %) (Table 1). Eggs of the nasal pentastomid *Linguatula serrata* were also detected (0.6 %). Cases of single infection were found in 38.4 % of dogs sampled (Table 2). Mixed infections with two or more species were observed in 20.3 % and 18.0 % of dogs, respectively (Tables 3). The most frequently detected co-infections were with *Capillaria* sp./hookworms (4.1 %) and *T. vulpis/*hookworms (2.9 %).

The serum analysis identified antigens of *D. immitis* and *A. vasorum* (10.5 % and 0.6 % of the samples, respectively) and antibodies against *Anaplasma* sp. (13.4 %), *Ehrlichia* sp. (13.4 %) and *B. burgdorferi* (1.7 %). *Dirofilaria repens* microfilariae were found in 5.8 % of the dogs (Table 1).

The age was identified as a risk factor for *D. immitis* infection (χ^2=4.358). The highest prevalence was observed in dogs above 12 months of age and no case was recognized in younger dogs. Similarly, antibodies against *Ehrlichia* sp. were more commonly detected in the adult animals (χ^2=3.740). Statistical analysis showed no significant association between the gender and prevalence of infections.

Discussion

The findings of this study showed that more than half of the dogs (64.5 %) were infected by at least one species of GI parasite.

Table 3. Cases of mixed infections in dogs (n=172)

Combinations	Positive (%)	95% CIs
Ancylostoma/Uncinaria spp. + *Anaplasma* sp.	2 (1.2)	0 – 2.8
Ancylostoma/Uncinaria spp. + *D. immitis*	5 (2.9)	0.4 – 5.4
Ancylostoma/Uncinaria spp. + *Ehrlichia* sp.	2 (1.2)	0 – 2.8
D. immitis + *Ehrlichia* sp.	1 (0.6)	0 – 1.7
Capillaria sp. + *Ancylostoma/Uncinaria* spp.	7 (4.1)	1.1 – 7.0
Ancylostoma/Uncinaria spp. + *Cystoisospora* sp.	2 (2.1)	0 – 2.8
Ancylostoma/Uncinaria spp. + *Sarcocystis* sp.	1 (0.6)	0 – 1.7
Cystoisospora sp. + *Ehrlichia* sp.	1 (0.6)	0 – 1.7
T. vulpis + *Ancylostoma/Uncinaria* spp.	5 (2.9)	0.4 – 5.4
D. immitis + *T. vulpis*	1 (0.6)	0 – 1.7
T. vulpis + *Anaplasma* sp.	1 (0.6)	0 – 1.7
D. repens + *Ancylostoma/Uncinaria* spp.	4 (2.3)	0.1 – 4.6
T. canis + *Ancylostoma/Uncinaria* spp.	1 (0.6)	0 – 1.7
Ancylostoma/Uncinaria spp. + *T. leonina*	1 (0.6)	0 – 1.7
L. serrata + *Ancylostoma/Uncinaria* spp.	1 (0.6)	0 – 1.7
T. vulpis + *Capillaria* sp. + *Ehrlichia* sp.	2 (1.2)	0 – 2.8
D. immitis + *Anaplasma* sp. + *Ehrlichia* sp.	1 (0.6)	0 – 1.7
D. immitis + *Ancylostoma/Uncinaria* spp. + *Ehrlichia* sp.	1 (0.6)	0 – 1.7
D. immitis + *T. vulpis* + *Ancylostoma/Uncinaria* spp.	2 (1.2)	0 – 2.8
Ancylostoma/Uncinaria spp. + *Anaplasma* sp. + *Ehrlichia* sp.	2 (1.2)	0 – 2.8
D. immitis + *Capillaria* sp. + *Ancylostoma/Uncinaria* spp.	1 (0.6)	0 – 1.7
Cystoisospora sp. + *Ancylostoma/Uncinaria* spp. + *Sarcocystis* sp.	1 (0.6)	0 – 1.7
D. immitis + *Ancylostoma/Uncinaria* spp. + *Sarcocystis* sp.	1 (0.6)	0 – 1.7
T. canis + *Ancylostoma/Uncinaria* spp. + *Sarcocystis* sp.	1 (0.6)	0 – 1.7
T. vulpis + *Capillaria* sp. + *Ancylostoma/Uncinaria* spp.	3 (1.7)	0 – 3.7
Capillaria sp. + *Ancylostoma/Uncinaria* spp. + *Anaplasma* sp.	1 (0.6)	0 – 1.7
D. repens + *Capillaria* sp. + *Ancylostoma/Uncinaria* spp.	1 (0.6)	0 – 1.7
T. vulpis + *Ancylostoma/Uncinaria* spp. + *Anaplasma* sp.	3 (1.7)	0 – 3.7
D. immitis + *T .canis* + *Ancylostoma/Uncinaria* spp. + *Anaplasma* sp.	1 (0.6)	0 – 1.7
B. burgdorferi + *Anaplasma* sp. + *Ehrlichia* sp.	1 (0.6)	0 – 1.7
D. immitis + *D. repens* + *Ancylostoma/Uncinaria* spp. + *Ehrlichia* sp.	1 (0.6)	0 – 1.7
Ancylostoma/Uncinaria spp. + *Taenia* sp. + *Anaplasma* sp. + *Ehrlichia* sp.	1 (0.6)	0 – 1.7
D. repens + *B. burgdorferi* + *Capillaria* sp. + *Ancylostoma/Uncinaria* spp.	1 (0.6)	0 – 1.7
D. repens + *T. vulpis* + *Capillaria* sp. + *Ancylostoma/Uncinaria* spp.	1 (0.6)	0 – 1.7
T. vulpis + *Capillaria* sp. + *Ancylostoma/Uncinaria* spp. + *Taenia* sp. + *Ehrlichia* sp.	1 (0.6)	0 – 1.7
T. vulpis + *T. canis* + *Ancylostoma/Uncinaria* spp. + *Anaplasma* sp. + *Ehrlichia* sp.	1 (0.6)	0 – 1.7
T. canis + *Ancylostoma/Uncinaria* spp. + *Cystoisospora* sp. + *Anaplasma* sp. + *Ehrlichia* sp.	1 (0.6)	0 – 1.7
T. canis + *Ancylostoma/Uncinaria* spp. + *T. leonina* + *Anaplasma* sp. + *Ehrlichia* sp.	1 (0.6)	0 – 1.7
D. immitis + *Capillaria* sp. + *Ancylostoma/Uncinaria* spp. + *Cystoisospora* sp. + *Anaplasma* sp.	1 (0.6)	0 – 1.7

Numerous recent surveys performed on the Balkans have disclosed comparable results indicating the presence of GI parasitism in 67.1 % of dogs in Romania (Ursache *et al*., 2016), 75.4 % in Serbia (Sommer *et al*., 2017), between 48.1 % and 64.9 % in Croatia (Brezak *et al*., 2017), 26 % in Greece (Papazahariadou *et al*., 2007), 30.4 % in Turkey (Senlik *et al*., 2006) and 40.7 % in Albania (Shukullari *et al*., 2015). All the mentioned results were obtained by coprological exaninations. According to the regional reports, between 24.8 % and 65 % of dogs in Bulgaria harbor several species of endoparasites including ascarids, hookworms, whipworms, tapeworms and coccidians (Kirkova *et al*., 2006; Lalkovski & Sabev, 2009; Kirkova *et al*., 2013; Radev *et al*., 2016), which seems to be in general agreement with our finding. The differences between aforementioned results are not unexpected and could be due to various factors. Those investigations include either well- or not well-cared dogs of different ages and categories (e.g. hunting, shepherd, military, pet, shelter and stray dogs) with different deworming frequency (e.g. regular or not), clinically healthy or under veterinary care (with GI disorders). Taking into consideration the above, it can be argued that the combined influence of the age and purpose of dogs, the general hygiene, and the access to regular deworming may exert a marked effect on the prevalence and species variety of GI parasites among the canine population (Shukullari *et al*., 2015). In addition, the strong influence of the geographic location and climate conditions is confirmed by the results of a recent study, demonstrating lower prevalence of GI parasitic infections (9.4 %) even in stray and not well-cared dogs in Germany (Becker *et al*., 2012).

In this study, hookworms were recognized as the most common enteric pathogens, which were found in the fecal samples of more than half of dogs (54.1 %) In similar investigation conducted in Stara Zagora (Bulgaria), the prevalence of *A. caninum* and *U. stenocephala* in stray dogs was even higher, reaching 90 % and 60 %, respectively (Georgieva *et al*., 1999). Other local investigations have indicated that between 6.18 % and 37.8 % of the dogs harbor hookworms (Kirkova *et al*., 2013; Iliev *et al*., 2017). Those findings were expected, taking into consideration the modes of infection transmission. Also, it should not be underestimated the zoonotic potential of hookworms. Once entered the body, the infective larvae of *A. caninum* may induce two serious conditions known as human gut disease (eosinophilic enteritis) and cutaneous larva migrans (CLM) or creeping eruption (Katagiri & Oliveira-Sequeira, 2008). The relationship between *U. stenocephala* and CLM remains unclear and still debated (Villeneuve *et al*., 2015).

The canine whipworm, *T. vulpis*, was the second most frequent GI parasite detected in this study (15.1 %). Our results are in general agreement with those of comparable surveys in dogs in Bulgaria (Kirkova *et al*., 2006; Lalkovski & Sabev, 2009; Kirkova *et al*., 2013; Radev *et al*., 2016; Iliev *et al*., 2017).

The overall number of dogs infected with *T. canis* and *T. leonina* in this study was substantially low (6.4 % and 1.7 %, respectively). As observed in similar Bulgarian investigations, the prevalence of those ascarids ranged from 6.8 % to 17.8 % for *T. canis* and from 0.78 % to 3.1 % for *T. leonina* (Kirkova *et al*., 2006; Kirkova *et al.,* 2013; Kanchev *et al*., 2014; Iliev *et al*., 2017). More importantly, *T. canis* is better recognized as the most common causative agent of visceral and ocular larva migrans in people; both syndromes might lead to severe damages of different tissues, especially in children (Villeneuve *et al*., 2015).

The results of our study showed that 1.2 % of dogs are infected with *Taenia* sp., which is in general agreement with findings of Kirkova *et al*. (2006) and Iliev *et al.* (2017). Those authors also reported a substantially low prevalence of taeniid infections (0.8-1.16 %) in dogs in Bulgaria.

Numerous studies regarding to the prevalence of aforementioned helminth species have been conducted in closer geographical regions, including Croatia (Brezak *et al*., 2017); Romania (Ursache *et al*., 2016), Serbia (Sommer *et al*., 2017); Greece (Papazahariadou *et al*., 2007); Albania (Shukullari *et al*., 2015) and Turkey (Senlik *et al*., 2006). Summarized results indicate the presence of GI parasitism ranging from 1.2 % to 41 % for hookworms, from 2.9 % to 9.6 % for *T. vulpis*, from 3 % to 34.8 % for *T. canis*, from 0.7 % to 21.8 % for *T. leonina* and from 0.3 % to 1.5 % for *Taenia* sp.

Our results showed *Capillaria* sp. infection, which is probably caused by the capillariid lungworm *Eucoleus aerophilus*. We detected eggs of such helminth in 11 % of the fecal samples. That value is very likely to be lower than real percentage because of the eggs may originate not only from adult lungworms, but also after passing through the alimentary tract following ingestion of contaminated food or after coprophagy (Shukullari et al., 2015). The prevalence of *E. aerophilus* ranges from 0.2 % to 2.8 % in European and Balkan countries (Traversa *et al*., 2010; Shukullari *et al*., 2015; Ursache *et al*., 2016; Brezak *et al*., 2017) and from 2 % to 6.8 % in Bulgaria (Kirkova *et al*., 2006; Kirkova *et al.,* 2013).

One case of nasal linguatulosis due to the pentastomid *L. serrata* was recorded here. The adult parasites reside in nasal cavities in dogs but the eggs pass from the respiratory system to intestines and release into the environment through feces. The total prevalence of *L. serrata* in dogs in Bulgaria reaches to 0.7 % (Kirkova *et al*., 2013). However, nymphs of this parasite (known as *Pentastomum denticulatum*) have been recovered from the lungs, liver and mesenteric lymph nodes in Bulgarian goats as intermediate hosts (Ivanov *et al*., 2012). This pentastomid is considered responsible for important zoonotic disease. Furthermore, visceral linguatulosis (pentastomosis) in Bulgaria was reported in a 9-year-old boy in Pleven (Mateva *et al*., 2013).

Our investigation presents the first serologically proven case of angiostrongylosis due to the cardiopulmonary nematode *A. vasorum*. In contrast, several studies have shown such infection in canids in the European countries (Traversa *et al*., 2010; Elsheikha *et al*., 2014) and on the Balkans (Papazahariadou *et al*., 2007; Shukullari *et al*. 2015; Ilie *et al*., 2016).

Protozoa infections were less often identified in this study than helminth infections (4.1 % for *Cystoisospora* sp. and 2.3 % for *Sarcocystis* sp.). The prevalence of GI protozoa in dogs in Bulgaria varies from 1.97 % to 7.72 % for *Cystoisospora* sp. and from 0.39 % to 0.5 % for *Sarcocystis* sp. (Kirkova *et al*., 2006; Lalkovski & Sabev, 2009; Radev *et al*., 2016; Iliev *et al*., 2017). Our findings are in general agreement with those reported in closer geographical region (Croatia, Romania, Albania, Serbia, Greece), where the overall prevalence ranges from 3 % to 16.1 % for *Cystoisospora* sp. and from 0.3 % to 4.5 % for *Sarcocystis* sp. (Papazahariadou *et al*., 2007; Shukullari *et al*. 2015; Ursache *et al*., 2016; Brezak *et al*., 2017; Sommer *et al*., 2017).

Almost half of the dogs (44.8 %) were positive for vector-borne parasites and bacteria such as *D. immitis*, *D. repens*, *Ehrlichia* sp., *Anaplasma* sp. and *B. burgdorferi*. The prevalence of those pathogens usually depends on several factors, but the age of dogs is considered as an important parameter as we found for *D. immitis* and *Ehrlichia* sp. in current study. Our findings showed that 10.5 % and 5.8 % of dogs were infected with *D. immitis* and *D. repens*, respectively. According to other Bulgarian researchers, between 7.4 % and 16.2 % of clinically healthy dogs and 34.33 % of dogs with cardiopulmonary disorders and under veterinary care are infected with *D. immitis* (Georgieva *et al*., 1999; Georgieva *et al*., 2001; Pantchev *et al*., 2015; Radev *et al*., 2016; Iliev *et al*., 2017). The prevalence of canine heartworm disease due to *D. immitis* is also reported on the Balkans and ranges from 0.7 % to 17.9 % in Greece, from 7.2 % to 22.01 % in Serbia, from 1 % to 27 % in Turkey and from 8 % to 16 % in Croatia (Morchon *et al*., 2012).

Our results also showed infections with *Anaplasma* sp. (13.4 %), *Ehrlichia* sp. (13.4 %) and *B. burgdorferi* (1.7 %). Data of several researches regarding the overall seroprevalence of those tick-borne pathogens among canine population in Bulgaria have been previously reported, indicating occurrence of infections ranging from 21 % to 37.5 % for *E. canis*, from 3.5 % to 46.1 % for *A. phagocytophilum* (Tsachev, 2006; Tsachev *et al*., 2006; Tsachev *et al*., 2006a; Pantchev *et al*., 2015) and from 22.74 % to 74.5 % for *B. burgdorferi* (Angelov *et al*., 1993; Zarkov & Marinov, 2003). Those findings are much higher and are not in agreement with our results. One reason could be that the authors present data obtained from dogs originated from enzootic regions or under veterinary care. However, our results are similar with the findings published by Pantchev *et al*. (2015) who also found a low seroprevalence (2.4 %) of *B. burgdorferi* among dogs in Bulgaria.

The results obtained here indicated that elder dogs (irrespective of sex) were commonly affected by *D. immitis* and *Ehrlichia* sp. Similar findings have also been published earlier (Villeneuve *et al*., 2011; Volgina *et al*., 2013; Hamel *et al*., 2016; Pantchev *et al*., 2015). Other authors have reported that elder dogs, kept outdoor, were more commonly infected by *D. immitis* (Yildirim *et al*. 2007; Cardoso *et al*. 2012; Mircean *et al*. 2012). According to Hamel *et al*. (2016), the occurrence of CVBDs is significantly higher in dogs over one year of age. The gender of animals included in our work was not considered as a risk factor for *Ehrlichia* sp. and *Anaplasma* sp., which coincides with findings reported from other authors (Solano-Gallego *et al*., 2006; Tsachev *et al*., 2006; Tsachev *et al*., 2006a; Villeneuve *et al*., 2011; Miro *et al*., 2013).

Conclusion

This study presents an overview of the prevalence of GI parasites and some vector-borne helminths and bacteria in outdoor dogs in Bulgaria as well as the first report of *A. vasorum*. Our findings demonstrate a wide variety of endoparasites and high prevalence rates of parasitism, suggesting environmental contamination with infective stages of parasites and presence of arthropods carrying different pathogens. Therefore, both the arthropods and infected dogs could be responsible for occurrence of several zoonotic diseases; particularly *T. canis*, *Taenia* sp. (refers to *E. granulosus*), *B. burgdorferi* and hookworms. This statement thus should increase the efforts of veterinarians and owners on performing a regular and proper prophylaxis of dogs against ecto and endoparasites resulting in lower levels of parasitism in both animals and humans.

Conflict of Interest

Authors state no conflict of interest.

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