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Short communication

TICKS AND ASSOCIATED TICK-BORNE PATHOGENS FROM DOGS AND RED FOXES FROM BULGARIA

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Summary

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Climate changes in recent years led to a sharp rise in the tick population and an increase in the number of animals and people with tick-borne infections. The domestic and wild carnivores, especially the dogs, have a huge role for the distribution of ticks in certain areas. In this study 60 ixodid ticks collected from domestic dogs and red foxes from Bulgaria have been investigated for infection with *Ehrlichia canis*, *Hepatozoon canis*, *Babesia* spp., and *Rickettsia* spp. The results showed that the dogs were infected with two tick species – *Rhipicephalus sanguineus* (72%) and *Ixodes ricinus* (28%). The red foxes were infected with only one species – *I. ricinus*. Out of all *R. sanguineus* ticks, 43.6% were female and 56.4% male. The opposite was observed for *I. ricinus* – female specimens (86.7%) were significantly more prevalent than males (13.3%). Similar trend was found out for *I. ricinus* collected from red foxes – 66.7% of the ticks were female and 33.3% male. Infectious agents were found in 31.7% of the investigated ticks. *Ehrlichia* spp. was established in 79% and *Rickettsia* spp. in 21% of the infected ticks. *Ehrlichia* spp. was found only in ticks collected from dogs. The majority of the ticks infected with *Ehrlichia* spp. were *Rh. sanguineus* (93.3%) and only one tick was *I. ricinus* (6.7%). Four ticks were positive for *Rickettsia* spp., two were *Rh. sanguineus* and two – *I. ricinus*, one of the latter was found on a fox. This is the first report about detection of *Ehrlichia* spp. in *Rh. sanguineus* ticks from Bulgaria as well as *Rickettsia* spp. in *I. ricinus* ticks collected from red foxes from this country.

Key words: Bulgaria, dog, *Ehrlichia canis, Ixodes ricinus*, red fox, *Rhipicephalus sanguineus*, *Rickettsia* spp.

Currently, multiple anthropogenic stressors including climate change, habitat loss and fragmentation, urbanisation, agricultural expansion and intensification, to-

gether with other changes in the use of water and land resources, are directly or indirectly impacting all species on earth. Such processes have also significant effects on host-parasite interactions and infectious disease risks. Due to global changes (climate, economic and social) in the last decades, emergence of new serious infectious diseases of different aetiology as well as spreading of already known diseases can be seen.

Ixodid ticks are involved in the emergence and circulation of dangerous diseases of different nature, including such with a zoonotic character. They have been described as vectors of human diseases with bacterial origin as spotted fever rickettsioses, recurrent fever borrelioses, tularemia, and Q fever (Parola & Raoult, 2001). A number of Gram-negative bacteria have been identified in ticks, including *Chromobacterium violaceum, Pasteurella haemolytica, Pseudomonas aeruginosa*, and *Serratia marcescens* potentially pathogenic for man or animals (Stojek & Dutkiewicz, 2004). Tick-borne encephalitis is considered the most important tick-borne viral disease of humans in Eurasia (Pfeffer & Dobler, 2011). Ixodid ticks are also vectors for parasitic pathogens like *Babesia* and *Theileria*, in many cases they appear to be the leading factors in the development and distribution of these infections (Perez de Leon *et al.,* 2013).

This work presents the results from investigation of species composition of ixodid ticks and associated pathogens circulating among domestic dogs and red foxes from two regions of Western Bulgaria.

Sixty ixodid ticks were examined, 54 of them were from domestic dogs and 6 from red foxes. The ticks were collected in 2018 during checkup of house dogs in veterinary practices in Sofia and necropsies of two red foxes found dead on roadways in Blagoevgrad district. The identification of the found ticks was performed according to guides by Estrada-Pena *et al.* (2004) and Georgieva & Gecheva (2013). The ticks were investigated for the following pathogens: protozoans from genus *Babesia* and the species *Hepatozoon canis* and bacteria from genus *Rickettsia* and the species *Ehrlichia canis*.

The genomic DNA was extracted with NucleoSpin® Tissue kit (MACHEREY-NAGEL, Germany) according to the producer`s manual. To detect babesiae, the genus-specific *18S rDNA* gene was amplified following the methodology and using the primers described by Casati *et al.* (2006). For *H. canis* a portion of *18S rRNA* gene was amplified, using primers HepF (CTTATTATTCCATGCTGCAG) and HepR (ATACATGAGCAAAATCT CAAC) as described by Inokuma *et al.* (2002). To detect rickettsiae, a part of the genus-specific gene D was amplified using primers D767f (CGATGGTAG CATTAAAAGCT) and D1390r (CTT GCTTTTCAGCAATATCAC) (Sekeyová *et al.*, 2001). For *Ehrlichia canis* a region of *16S rRNA* gene using the pair of primers EHR 521 (TGTAGGCGGTT CGGTAAGTTAAAG) and EHR 747 (GCACTCATCGTTTACAGCGTG) was amplified as described by Pancholi *et al.* (1995). For electrophoresis of the PCR products obtained, 1.5% agarose and TAE buffer were used.

Thirty-nine of the ticks collected from dogs were of the species *Rhipicephalus sanguineus* (17 female and 22 male) and 15 were identified as *Ixodes ricinus* (13 female and 2 male). Ticks from foxes were only *I. ricinus* (4 females and 2 males).

The PCR results are summarised in Table 1. They show that 72% of the ticks

Table 1. Investigations of ixodid ticks collected from carnivores from Bulgaria for pathogens

Animals	Tick species	Sex and number of the ticks	Number of PCR positive samples			
			Babesia spp.	*Rickettsia* spp.	*Ehrlichia canis*	*Hepatozoon canis*
Domestic dogs	*Rhipicephalus sanguineus*	Female – 17	0	0	8	0
		Male – 22	0	2	6	0
	Ixodes ricinus	Female – 13	0	1	1	0
		Male – 2	0	0	0	0
Red foxes	*Ixodes ricinus*	Female – 4	0	1	0	0
		Male – 2	0	0	0	0

collected from dogs around Sofia were from the species *Rh. sanguineus* and 28% – from the species *I. ricinus.* Our unpublished data from other studies have shown that ixodid ticks infecting house dogs in the region of Sofia were also predominantly of the *Rh. sanguineus* species. These two species have been found in domestic dogs in other regions of Bulgaria and *Rh. sanguineus* was also more common (Kirkova *et al.*, 2013; Nader *et al.*, 2018). The literature showed that *Rh. sanguineus* and *I. ricinus* are common ixodid ticks parasitising on domestic dogs in Europe. They have been reported on dogs from Hungary (Földvári & Farkas, 2005), Albania (Xhaxhiu *et al.*, 2009; Shukullari *et al.*, 2017), Belgium (Claerebout *et al.*, 2013), Bosnia and Herzegovina (Krčmar *et al.*, 2014), Germany (Rehbein *et al.*, 2016), and Greece (Lefkaditis *et al.*, 2016).

Only one tick species was found on red foxes in the present study – *I. ricinus*. It is among the common parasites of this wild carnivore in Europe. Besides Bulgaria, *I. ricinus* has been collected from red foxes in Hungary, Slovakia, Spain and Italy (Sréter *et al.*, 2003; Kočišová *et al.*, 2006; Széll *et al.*, 2006; Martínez-Carrasco *et al.*, 2007; Lorusso *et al.*, 2011).

Our current study shows that 43.6% of the *Rh. sanguineus* ticks were female and 56.4% – male. The opposite was observed for *I. ricinus* ticks – female specimens (86.7%) were significantly more prevalent than males (13.3%). The tendency for *I. ricinus* collected from red foxes was similar – 66.7% of the specimens were female and 33.3% – male. The results of Nader *et al.* (2018) in relation to the sex of ticks collected from different hosts are similar to ours – the authors have established that *I. ricinus* ticks were mostly female and *Rh. sanguineus* ticks were predominantly male.

The results showed that 31.7% of all investigated ticks were vectors of different pathogens. *Ehrlichia* sp. was identified in 79% of the infected ticks, while *Rickettsia* spp. –in 21%. *Ehrlichia* spp. was present only in ticks collected from dogs. Fourteen of the ticks infected with *Ehrlichia* spp. were from the species *Rh. sanguineus* (93.3%) and only one from the species *I. ricinus* (6.7%). This distribution of the infection in both tick species collected from dogs is not surprising because *Ehrlichia canis* is the aetiological agent of canine monocytic ehrlichiosis, transmitted by the brown dog tick *Rh. sanguineus*, whose geographical distribution in the Neotropics overlaps with that of the pathogen (Otranto *et al.*, 2015). *Ehrlichia* spp. has been previously established in *I.*

ricinus collected from areas or dogs from Bulgaria (Christova *et al.*, 2001; 2003; Tsachev *et al.,* 2008; 2017; Pantchev *et al.*, 2015). So far, no studies have reported *Rh. sanguineus* as a vector of *Ehrlichia* sp. in our country.

In the present study *Rickettsia* spp. were found in four ticks – two *Rh. sanguineus* and two *I. ricinus*, one of the latter found on a fox. *Rickettsia* spp. have been also found in *Rhipicephalus* spp. and *I. ricinus* collected from dogs from South-Eastern Bulgaria (Nader *et al.*, 2018). The worldwide distribution of *Rickettsia* spp. in dogs is discussed in a review by Otranto *et al.* (2015). The authors point out that no *Rickettsia* has been detected so far in wild carnivores in Europe. However, since some *Rickettsia* spp. are transmitted by ixodid species (which also feed on carnivores), the prevalence of these pathogens in wildlife is likely underestimated (Otranto *et al.*, 2015). The detection of *Rickettsia* spp. in *I. ricinus* from a red fox in the current study supports this suggestion and confirms the potential role of foxes in the epidemiology of rickettsioses in animals and humans.

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