Ticks and associated tick-borne pathogens from dogs and red foxes from Bulgaria

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Summary


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-
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together 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 etiology 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 (ATACATGAGCAAATCTCAAC) as described by Inokuma et al. (2002). To detect rickettsiae, a part of the genus-specific gene D was amplified using primers D767f (CGATGGTAGCATTAAAAGCT) and D1390r (CTTGCTTTTCAGCAATATCAC) (Sekeyová et al., 2001). For Ehrlichia canis a region of 16S rRNA gene using the pair of primers EHR 521 (TGTAGGCGGTGGTAGATTAAAG) and EHR 747 (GCCACTCATGTTTACAGCGTG) 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
collected from dogs around Sofia were from the species *Rhipicephalus sanguineus* and 28% – from the species *Ixodes 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 *Rhipicephalus sanguineus* species. These two species have been found in domestic dogs in other regions of Bulgaria and *Rhipicephalus sanguineus* was also more common (Kirkova et al., 2013; Nader et al., 2018). The literature showed that *Rhipicephalus sanguineus* and *Ixodes ricinus* are common ixodid ticks parasitising on domestic dogs in Europe. They have been reported on dogs from Hungary (Földvári & Farkas, 2005), Albania (Xhaxhui et al., 2009; Shukullari et al., 2017), Belgium (Claerebout et al., 2013), Bosnia and Herzegovina (Krečmar 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 – *Ixodes 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; Kocišová 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 *Rhipicephalus sanguineus* ticks were female and 56.4% – male. The opposite was observed for *Ixodes ricinus* ticks – female specimens (86.7%) were significantly more prevalent than males (13.3%). The tendency for *Ixodes 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 *Ixodes ricinus* ticks were mostly female and *Rhipicephalus 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 *Rhipicephalus sanguineus* (93.3%) and only one from the species *Ixodes ricinus* (6.7%). This distribution of the infection in both tick species collected from dogs is not surprising because *Ehrlichia canis* is the aetiologi agent of canine monocytic ehrlichiosis, transmitted by the brown dog tick *Rhipicephalus 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.

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

<table>
<thead>
<tr>
<th>Animals</th>
<th>Tick species</th>
<th>Sex and number of the ticks</th>
<th>Number of PCR positive samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Domestic dogs</td>
<td><em>Rhipicephalus sanguineus</em></td>
<td>Female – 17, Male – 22</td>
<td>Babesia spp. Rickettsia spp. Ehrlichia canis Hepatozoon canis</td>
</tr>
<tr>
<td></td>
<td><em>Ixodes ricinus</em></td>
<td>Female – 13, Male – 2</td>
<td>0 0 8 0</td>
</tr>
<tr>
<td>Red foxes</td>
<td><em>Ixodes ricinus</em></td>
<td>Female – 4, Male – 2</td>
<td>0 1 0 0</td>
</tr>
</tbody>
</table>

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