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SHORT COMMUNICATION

Hard ticks (Ixodidae) in Romania: surveillance, host associations, and possible risks for tick-borne diseases

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Abstract Ticks and tick-borne diseases represent a great concern worldwide. Despite this, in Romania the studies regarding this subject has just started, and the interest of medical personnel, researchers, and citizens is increasing. Because the geographical range of many tick-borne diseases started to extend as consequences of different biological and environmental factors, it is important to study the diversity of ticks species, especially correlated with host associations. A total number of 840 ticks were collected between 1 April and 1 November 2010, from 66 animals, from 17 species in 11 counties, spread all over Romania. Four Ixodidae species were identified: *Dermacentor marginatus* (49.2%), *Ixodes ricinus* (48.3%), *Hyalomma marginatum* (2.4%), and *Rhipicephalus sanguineus* (0.1%). The obtained results indicate that *D. marginatus* is the most abundant tick species and *I. ricinus* is the most prevalent. As both of them are important vectors for human and animal diseases, the present paper discusses the associated risks for tick-borne diseases.

Introduction

Ticks are notorious as vectors of human and other animal disease agents. They transmit a greater variety of infectious organisms than any other group of blood-sucking arthropods. Worldwide, they are the most important vectors in veterinary field. Ticks transmit numerous protozoan (*Babesia*), viral (TBEV, Colorado tick fever virus, and Crimean–Congo hemorrhagic fever), bacterial (*Borrelia burgdorferi* sensu lato, *Rickettsia rickettsii*, and *Anaplasma phagocytophilum*), and fungal pathogens (*Paecilomyces farinosus* and *Verticillium lecanii*) (Nicholson et al. 2009). Beside the medical importance of ticks, the economical damages that they can cause are also significant. Although difficult to measure precisely, the global economic impact of ticks and tick-borne diseases is believed to be in many billions of dollars (Jongejan and Uilenberg 2004).

The complex cycles of vector-borne diseases often include multiple mammalian and non-mammalian vertebrate and invertebrate species. Ticks can transmit microbes from evolutionarily commensalistic reservoir species (i.e., rodents) to incidental susceptible species (i.e., humans). The risk of disease transmission therefore is determined by the prevalence of infectious ticks, a function of the number and infection prevalence of the pathogen's reservoir host, and by the likelihood of an encounter between an infected tick and a susceptible host—a function of both the numbers of ticks and susceptible hosts within a fixed area and their respective behaviors. Therefore, the distribution of disease risk is restricted by the necessity for sympatric coexistence of the microbial pathogen, a competent vector tick, a reservoir host, and a susceptible host (Fritz 2009).

The aim of this study was to establish the tick species that occur in the studied areas in accordance with their animal host, as possible risk for tick-borne diseases. From a public health perspective, our study aims to improve our ability to predict human outbreaks of vector-borne zoonoses and to prepare intervention strategies. All this requires

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consideration of invertebrate and vertebrate ecology and biology.

A total number of 840 ticks were collected between 1 April and 1 November 2010, from 66 hosts, belonging to 17 species (reptiles, birds, and mammals) (Table 1) in 11 counties, spread all over Romania. All the ticks were removed by medical personnel, collected to plastic bags and registered. Each tick was individually identified based on morphological features, recording the genus and species characteristics, developmental stage, and sex (only for adult ticks) (Estrada-Peña et al. 2004).

Frequency, prevalence, and its 95% confidence interval were performed using the EpiInfo 2000 software. A p value of <0.05 was considered statistically significant. The statistic significance of dataset was evaluated by using chi-square test with GraphPad InStat software.

Four Ixodidae species were identified: *Dermacentor marginatus* (49.2%), *Ixodes ricinus* (48.3%), *Hyalomma marginatum* (2.4%), and *Rhipicephalus sanguineus* (0.1%). The most abundant developmental stages were adults. Preadult stages (larvae and nymph) were found only in the case of *I. ricinus* (Table 2).

Most hosts were parasitized by *I. ricinus* (86.4%; $p<0.001$). *D. marginatus* was involved in 11.4% of cases. *H. marginatum* was identified in two hosts from two different species (*Ovis aries* and *Capra hircus*), while *R. sanguineus* was found only in *Canis aureus* (Table 3). Most hosts (97%) of the animals were parasitized only by one tick species. In 3% of the cases, we identified tick associations. One goat (*C. hircus*) was coinfected by *I. ricinus* and *H. marginatum* and one golden jackal (*C. aureus*) by *I. ricinus* and *R. sanguineus* (Table 3).

From the total number of 17 species of animal hosts, 16 of them were parasitized by *I. ricinus*. *D. marginatus* was identified in five species of animals (*Canis familiaris*, *Capreolus capreolus*, *Equus caballus*, *O. aries*, and *Sus scrofa ferus*) (Table 1). Golden jackal (*C. aureus*) and corn crake (*Crex crex*) are new host record for hard ticks in Romania.

Four tick species (*I. ricinus*, *D. marginatus*, *H. marginatum*, and *R. sanguineus*) from order Metastigmata, family Ixodidae were identified, from 17 species of animal hosts. In a study from Romania performed between 1984 and 1995 on domestic animal host (bovines, equines, and sheep), Teodorescu and Popa (2002) found six Ixodidae species: *Rhipicephalus (Boophilus) annulatus*, *Dermacentor reticulatus, I. ricinus*, *Haemaphysalis punctata*, *H. marginatum*, and *Rhipicephalus bursa*. In a similar study, Popa and Teodorescu (2006) found five tick species: *R. (Boophilus) annulatus*, *Dermacentor pictus*, *I. ricinus*, *H. marginatum*, and *R. bursa*, on the same domestic animal host. Chițimia (2006) found in domestic animal host from southwest part of Romania, during 2003–2006, eight tick species: *I. ricinus*, *D. marginatus*, *H. punctata*, *Haemaphysalis concinna*, *Haemaphysalis parva*, *Haemaphysalis sulcata*, *Hyalomma detritum scupense*, and *R. sanguineus*. Compared with the first two studies, our research reveals the presence of other two species: *D. marginatus* and *R. sanguineus*. The prevalence of *I. ricinus* found by Teodorescu and Popa (2002) (80.4%) and by Popa and Teodorescu (2006) (84%) are

Table 1 Ticks species in correlation with animal species

Animal species	*Ixodes ricinus n* (%)	*Dermacentor marginatus n* (%)	*Hyalomma marginatum n* (%)	*Rhipicephalus sanguineus n* (%)
Canis familiaris (*n*=33)	32 (97)	1 (3)	0 (0)	0 (0)
Felis catus (*n*=5)	5 (100)	0 (0)	0 (0)	0 (0)
Ovis aries (*n*=5)	0 (0)	4 (80)	1 (20)	0 (0)
Equus caballus (*n*=2)	1 (50)	1 (50)	0 (0)	0 (0)
Capra hircus (*n*=1)	1 (100)	0 (0)	1 (100)	0 (0)
Capreolus capreolus (*n*=4)	3 (75)	1 (25)	0 (0)	0 (0)
Sus scrofa ferus (*n*=2)	1 (50)	1 (50)	0 (0)	0 (0)
Vulpes vulpes (*n*=2)	2 (100)	0 (0)	0 (0)	0 (0)
Erinaceus europaeus (*n*=2)	2 (100)	0 (0)	0 (0)	0 (0)
Arvicola terrestris (*n*=2)	2 (100)	0 (0)	0 (0)	0 (0)
Canis aureus (*n*=1)	1 (100)	0 (0)	0 (0)	1 (100)
Canis lupus (*n*=1)	1 (100)	0 (0)	0 (0)	0 (0)
Crex crex (*n*=2)	2 (100)	0 (0)	0 (0)	0 (0)
Parrus major (*n*=1)	1 (100)	0 (0)	0 (0)	0 (0)
Turdus merula (*n*=1)	1 (100)	0 (0)	0 (0)	0 (0)
Turdus philomelos (*n*=1)	1 (100)	0 (0)	0 (0)	0 (0)
Lacerta agilis (*n*=1)	1 (100)	0 (0)	0 (0)	0 (0)

Table 2 Frequency and prevalence of ticks related to their development stage/sex

Ticks species	Total *n* (%)	Larvae *n* (%)	Nymphs *n* (%)	Females *n* (%)	Males *n* (%)	*p*
Dermacentor marginatus	413* (49.2%)	0 (0%)	0 (0%)	190 (46%)	223* (54%)	0.0001
Ixodes ricinus	406* (48.3%)	20* (4.9%)	43* (10.6%)	248* (61.1%)	95 (23.4%)	0.0001
Hyalomma marginatum	20 (2.4%)	0 (0%)	0 (0%)	9 (45%)	11 (55%)	0.0001
Rhipicephalus sanguineus	1 (0.1)	0 (0%)	0 (0%)	0 (0%)	1 (100%)	0.26
Total	840	20	43	437	330	0.0001

*$p<0.001$

similar with the value of prevalence that was obtained in our study (86.4%).

Only few recent records of species diversity of ticks from Europe are published. However, more information is available on epidemiological studies on tick-borne pathogens, frequently correlated with animal host species. In Hungary Sréter et al. (2005) found the prevalence of *I. ricinus* in red foxes 34% and 24% for *D. reticulatus*, on the same animal host. In Sweden, Jaenson et al. (1994) consider that because of its wide geographic distribution, great abundance, and wide host range, *I. ricinus* is medically the most important arthropod in northern Europe. *I. ricinus* is common in southern and south central Sweden and along the coast of northern Sweden and has been recorded from 29 mammal species, 56 bird species, and 2 species of lizards. In the same study, a list of recorded tick species from northern Europe is presented: *Ixodes trianguliceps*, *Ixodes uriae*, *Ixodes caledonicus*, *Ixodes unicavatus*, *I. ricinus*, *Ixodes persulcatus*, *Ixodes arboricola*, *Ixodes canisuga*, *Ixodes hexagonus*, *Ixodes lividus*, *Ixodes apronophorus*, *Ixodes festai*, *Ixodes rugicollis*, *Ixodes simplex*, *H. punctata*, *H. concinna*, *Haemaphysalis leachi*, *H. marginatum*, *R. sanguineus*, *D. reticulatus*, *D. marginatus*, *Argas polonicus* and *Argas reflexus*. The data regarding prevalence of tick species are not provided.

The epidemiology of tick-borne diseases is determined by encounter rates between vectors and hosts. Our study showed that a large number of species have the capacity of hosting different species of ticks. Nicholson et al. (2009) considers that *I. ricinus* is an opportunistic species, with over 300 species of vertebrates recorded as hosts. *D. marginatus* adults parasitize on sheep, cattle, deer, dogs, men, hares, and hedgehogs. Nymph and larvae feed on small mammals, insectivores, and birds (Soulsby 1982). The main hosts for the larvae and nymphs of *H. marginatum* include many species of wild and domestic birds, European brown hares, and four-toed and eared hedgehogs. Rodents are of no significant importance for ticks as a feeding source. Adult ticks parasitize on agricultural animals, domestic dogs, and attack humans (Kotti et al. 2001). *R. sanguineus*, commonly referred to as the “kennel tick” or “brown dog tick”, is a cosmopolitan tick species that will feed on a wide variety of mammals, but dogs are the preferred host (Pegram et al. 1987).

All four tick species that were identified in our study are involved in tick-borne diseases transmission. *I. ricinus* is vector for one of the most important tick-borne diseases: Lyme disease, tularemia, tick-borne encephalitis, and babesiosis (Goddard 2003), but also of other bacteria, rickettsia, and viruses. In Slovenia, in a study on 496 *I. ricinus* ticks, 20 out of 85 adult ticks (23.5%) and 18 out of 411 nymphs (4.4%) were infected with *B. burgdorferi* (Ruzic-Sabluic et al. 1993). In another study, from France, percentages of infection with *B. burgdorferi*, according to the various stages of *I. ricinus* were as follows: 4.95% in 3,247 nymphs, 11.2% in 699 males, and 12.5% in 727 females (Gilot et al. 1996). In Poland, Michalik et al. (2003) found an infection rate of 16.2% with *B. burgdorferi*, in 1,123 questing *I. ricinus* ticks. In The Netherlands, the DNA of the human pathogen *Rickettsia conorii* as well as *Rickettsia helvetica*, *Rickettsia* sp. IRS, and *Rickettsia bellii-like* and unexpectedly, the DNA of the highly pathogenic *Rickettsia typhi* and *Rickettsia prowazekii* and four other uncharacterized *Rickettsia* spp. related to the typhus group were detected in *I. ricinus* (Sprong et al. 2009). Of 1931 *I. ricinus* ticks, collected within the territory of Belluno, Italy, 8.23% were positive for *B. burgdorferi*, 4.4% were positive for *Ehrlichia*, 1.6% were positive for *Rickettsia*, and 1.6% were positive for *Babesia*. The copresence of *Borrelia* and *Ehrlichia* (1.2%)

Table 3 Frequency and prevalence of ticks species correlated with number of animal host

Species	Frequency (*n*)	Prevalence (%)	95% CI
Ixodes ricinus	57*	86.4%	73.6–91.9%
Dermacentor marginatus	8	12.1%	5.1–21.3%
Hyalomma marginatum	2	3%	0.3–9.9%
Rhipicephalus sanguineus	1	1.5%	0.0–7.7%

*$p<0.001$

and *Babesia* (0.5%), *Borrelia*, *Ehrlichia*, and *Rickettsia* (0.1%) was also found (Piccolin et al. 2006). The screening for vector-borne infections of 216 canine EDTA blood samples from Hungary and Romania resulted in the detection of 11 different pathogens by molecular methods-*Babesia canis canis*, *Babesia canis vogeli*, *Babesia gibsoni*, *Babesia felis-like*, *Leishmania* spp., *Hepatozoon canis*, *Dirofilaria immitis*, *Dirofilaria repens*, *Acanthocheilonema reconditum*, *Mycoplasma haemocanis*, and *A. phagocytophilum* (Hamel et al. 2011). *H. marginatum* is one of the vectors involved in transmission of Crimean–Congo hemorrhagic fever (Goddard 2003). *D. marginatus* is one of the most frequent tick vectors for tick-borne encephalitis and babesiosis (Heyman et al. 2010). *R. sanguineus* is an important vector of tick-borne diseases such as boutonneuse fever to man and babesiosis and ehrlichiosis to dogs (Walker et al. 2000). It is the major vector of *Babesia vogeli*, *Ehrlichia canis*, *H. canis*, and *R. conorii* and it is a putative vector of many other pathogens including *Babesia Canis*, *Babesia microti*-like piroplasm, *Anaplasma platys* and *A. phagocytophilum* (Otranto and Dantas-Torres 2010). In a study developed in central and northern Italy regarding tick reservoirs for piroplasms, a total of 11 ixodid tick species were identified, five of them proving to be piroplasm positive. Molecular diagnostics identified *Theileria equi* and eight *Babesia* species. The highest infection rate was recorded in *H. marginatum* (9.1%), followed by *I. ricinus* (5.1%), *D. marginatus* (5%), *Rhipicephalus turanicus* (3.1%) and *R. sanguineus* (1.2%) (Iori et al. 2010).

In times of intensive globalization and global warming, studies on the biology, vectorship, invasion, and spreading of wanted vectors belonging to the field of arachno-entomology are urgently needed (Mehlhorn et al. 2011). The risk of infection by tick-borne pathogens is represented by the outcome of ticks' interactions with their natural environment and hosts, therefore the prevention measures of tick-borne diseases must be focused on each of the risk factors.

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