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Ixodes ricinus is the dominant questing tick in forest habitats in Romania: the results from a countrywide dragging campaign

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Abstract In 2010 and 2011, questing ticks were collected from 188 forested locations in all the 41 counties of Romania using the dragging method. The total of 13,771 ticks collected belonged to eleven species: *Ixodes ricinus* (86.9 %), *Dermacentor marginatus* (9.5 %), *Haemaphysalis punctata* (2.6 %), *H. concinna* (0.6 %), *H. sulcata* (0.3 %), *H. parva* (0.1 %), *Hyalomma marginatum* (0.02 %), *D. reticulatus* (0.02 %), *I. crenulatus* (0.007 %), *I. hexagonus* (0.007 %) and *I. laguri* (0.007 %). *Ixodes ricinus* was present in 97.7 % (n = 180) of locations, occurring exclusively in 41.7 % of the locations, whereas it was the dominant species in 38.8 % of the other locations, accounting for over 70 % of the total tick community. The following most common questing ticks were *D. marginatus*, *H. punctata* and *H. concinna*. *Ixodes ricinus* co-occurred with one, two or three sympatric species. The occurrence of *D. reticulatus* in forested habitats from Romania was found to be accidental.

Keywords *Ixodes ricinus* · Questing ticks · Tick community · Romania

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Introduction

Understanding the ecology and distribution of ticks is a key step in epidemiological studies of tick-borne zoonoses. The current scientific literature on this subject focuses mostly on molecular epidemiology of certain tick-borne pathogens, in various parts of the world. Among European ticks, *Ixodes ricinus* is considered to be one of the most important vectors of human and zoonotic pathogens (Swanson et al. 2006). *Ixodes ricinus* is a three-host questing tick with very low host specificity, regardless of the developmental stage. It is one of the most widely distributed ticks of the Western Palearctic, ranging from the Atlantic coast to as far East as 50°–60° longitude and from Iceland to as far South as North Africa (Hillyard 1996). More than 300 vertebrate species were reported as hosts for *I. ricinus* ticks (Anderson 1991).

The only exhaustive review on the distribution of ticks (including *I. ricinus*) in Romania was published more than 45 years ago (Feider 1965). Since then, most of the published studies deal with its vectorial capacity (Majláthová et al. 2008; Coipan and Vladimirescu 2010, 2011) or its occurrence on domestic animals (Ioniţă 2003; Chiţimia 2006) or humans (Briciu et al. 2011). The current paper reports the structure of questing tick communities in forested habitats in Romania, based on a countrywide dragging campaign.

Material and methods

Between 01.05.2010 and 18.06.2011 a number of 13,771 ticks were collected using the dragging method (adapted from Estrada-Peña 2001) from 188 locations in all the 41 counties of Romania (Fig. 1). The dragging was performed by five trained people in each location. Each site was examined for questing ticks by dragging a white flannel flag (1.2 m^2) over the vegetation for cca. 30 min. The cloth was examined at 30 s intervals, and ticks were removed with a fine forceps and placed in absolute ethanol. Each site was visited once during the study. No collection was performed on wet vegetation (i.e., significant dew or rain in previous day). GPS coordinates of each location are given in the supplementary electronic material.

Dragging has been performed in randomly chosen forest habitats to result in an overall uniform geographic distribution. The habitats chosen were deciduous or mixed forest margins, with abundant vegetation. The tick species was identified under stereo microscope in the laboratory, based on morphological features using dichotomous keys (Feider 1965; Nosek and Sixl 1972). Ticks were also separated by developmental stages, but the respective data was omitted from the statistical analysis as our paper is focused on community ecology rather than population ecology. All data was introduced in a tabular database specifying the collection date, name of locality, county, GPS coordinates, species and number of individuals. Frequency, prevalence and its 95 % confidence interval were performed using the EpiInfo 2000 software. Digital maps were created using ArcGis/ArcMap 9.2 (ESRI, © 1999–2006).

Results

From the total of 13,771 ticks collected by dragging, eleven species of ticks have been identified: *I. ricinus* (11,965; 86.9 %), *Dermacentor marginatus* (1,310; 9.5 %),

Fig. 1 Spatial distribution of collection sites

Haemaphysalis punctata (354; 2.6 %), *H. concinna* (89; 0.6 %), *H. sulcata* (35; 0.3 %), *H. parva* (11; 0.1 %), *Hyalomma marginatum* (2; 0.02 %), *D. reticulatus* (2; 0.02 %), *I. crenulatus* (1; 0.007 %), *I. hexagonus* (1; 0.007 %) and *I. laguri* (1; 0.007 %). The overall dominant species was *I. ricinus* ($p < 0.001$).

From the 188 locations studied, *I. ricinus* was present in 180 (97.7 %; 95 % CI 91.8–98.1), being the most widespread tick ($p = 0.0001$). In 75 locations (41.7 %; 95 % CI 34.4–49.2), *I. ricinus* was the only species found, while in other 73 sites (38.8 %; 95 % CI 33.31–48.11), it was the dominant species accounting for over 70 % of the local tick community (Table 1, Fig. 2). The second most common species was *D. marginatus* followed by *H. punctata* and *H. concinna*. Other species collected by dragging were found only in few locations (Table 2). However, *D. marginatus*, *H. punctata, H. concinna* and *H. sulcata* were dominant species in some locations (Fig. 3).

In the collection sites were *I. ricinus* was present, there was no statistical significance ($p = 0.08$) between the species of other sympatric ticks (Table 3). There was no statistical difference between tick community structure according to the month or season.

Discussion

The geographical distribution, abundance and population structure of ticks in genus *Ixodes* are related to various and complex factors, including the presence of proper wooded or bushy habitats and the presence and density of suitable hosts for all life stages of ticks (Gern and Humair 2002). For questing ticks, vegetation height is also an important factor. The distribution of questing *I. ricinus* is influenced by sward height and other physical

Table 1 Percentage ranges of *Ixodes ricinus* and respective number of locations

Percent of *I. ricinus*[a]	Number of locations
100	75
90.00–99.99	42
80.00–89.99	19
70.00–79.99	12
60.00–69.99	8
50.00–59.99	8
20.00–49.99	11
0.01–19.99	5
0	8

[a] From the total number of ticks caught in the respective location

Fig. 2 Distribution pattern of *Ixodes ricinus* with the relative prevalence in the local tick communities

properties of the vegetation (Mejlon and Jaenson 1997) but also by host movement and habitat connectivity (Estrada-Peña 2002).

The primary habitat for *I. ricinus* is represented mainly by the leaf litter and the lower vegetation layers of temperate deciduous and mixed forests. In areas with high rainfall, *I. ricinus* also occurs in high densities in coniferous forests and in open areas such as grasslands (Gray et al. 1998). In an extensive study from Spain, Estrada-Peña (2001) showed that *I. ricinus* prefers sites that had substantial secondary plant growth. Under natural conditions, the duration of the life cycle of *I. ricinus* is between 2 and 6 years (average 3 years, if each instar needs one full season to develop to the next stage). However, the duration of the life cycle can vary from one habitat to another and also regionally (Gray 1991). The main factors influencing the duration of any given phase are

Table 2 Frequency and prevalence of ticks species captured by dragging

Species	Frequency (n)	Prevalence (%)	95 % CI
I. ricinus	180	95.7***	91.8–98.1
D. marginatus	83	44.1	36.9–51.6
H. punctata	33	17.6	12.4–23.8
H. concinna	17	9	5.4–14.1
H. sulcata	5	2.7	0.9–6.1
H. parva	7	3.7	1.5–7.5
H. marginatum	2	1.1	0.1–3.8
D. reticulatus	2	1.1	0.1–3.8
I. crenulatus	1	0.5	0–2.9
I. hexagonus	1	0.5	0–2.9
I. laguri	1	0.5	0–2.9

***$p < 0.001$

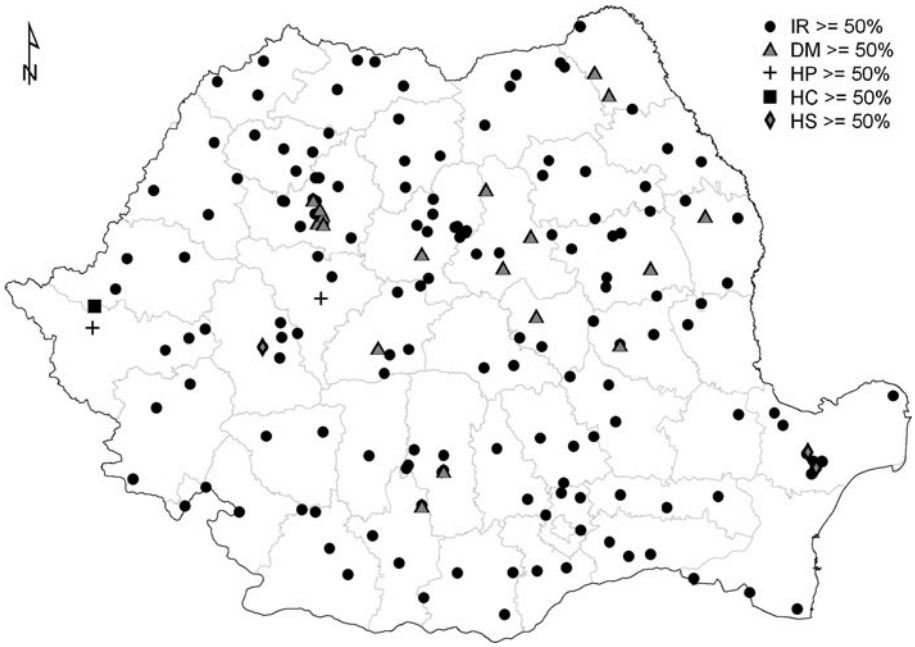

Fig. 3 Geographical distribution of the dominant tick species within its community (*IR Ixodes ricinus*, *DM Dermacentor marginatus, HP Haemaphysalis punctata*, *HC H. concinna, HS H. sulcata*)

microclimate and host density (Gray 1991). *Ixodes ricinus* is active from spring to autumn and seasonal activity of the three active stages shows a bimodal pattern with peaks in spring and autumn. Usually the activity of *I. ricinus* is more pronounced in spring than in autumn (Gern and Humair 2002).

Our study, the first of its kind in Romania confirms the status of *I. ricinus* as the most common questing tick species in Europe. *Ixodes ricinus* dominance over the specific structure of questing ticks communities was already recorded from the former Czechoslovakia (Kmety et al. 1987), Sweden (Tälleklint 1996), France (Pichot et al. 1997), Spain (Barandika et al. 2008) or Russia (Malunov and Egorov 2008). A similar countrywide

Table 3 Frequency (number of locations) and prevalence of *Ixodes ricinus* alone or in association with other tick species

Association	Frequency (n)	Prevalence (%)	95 % CI
I. ricinus alone	75	41.7	34.4–49.2
I. ricinus + 1 tick species	71	39.4	32.3–47
I. ricinus + 2 tick species	31	17.2	12–23.5
I. ricinus + 3 tick species	3	1.7	0.3–4.8
Total	180	95.7	91.8–98.1

$p = 0.08$

survey performed in Bosnia and Herzegovina in 2011, showed the dominance of *I. ricinus* over other questing ticks on various hosts, as well (Omeragic 2011).

Several other studies showed that *I. ricinus* is the most encountered tick in Western, Central and Eastern Europe affecting humans (Manfredi et al. 1999; Briciu et al. 2011) or domestic carnivores (Liebisch et al. 1985; Beichel et al. 1996; Ogden et al. 2000; Földvári and Farkas 2005). Moreover, the majority of the studies performed in wild animals from various European countries showed that *I. ricinus* is the most prevalent tick in small mammals (Siuda et al. 1982; Nilsson 1988; Perez-Eid 1990; Dorn et al. 1999; Estrada-Peña et al. 2005), wild carnivores (Sréter et al. 2005), birds (Comstedt et al. 2006; Poupon et al. 2006) and lizards (Majláthová et al. 2008; Tijsse-Klasen et al. 2010).

Among the Palearctic tick species, *I. ricinus* was shown to be associated with the highest number of pathogens (for a checklist see Briciu et al. 2011). Single *I. ricinus* individuals can harbor simultaneously several pathogens (for details on co-infections in *I. ricinus* see Swanson et al. 2006).

Distribution of *D. marginatus* and *D. reticulatus* ticks in Central Europe is restricted to small regions and has a mosaic-like pattern (Nosek 1972). *D. marginatus* prefers biotopes characterized by xerophilic plant communities, margins of oak forests and bushy ridges (Nosek 1972). In Romania, *D. marginatus* has a wide geographical distribution (Feider 1965). Our data show that this species is the second most common questing tick in forested habitats in Romania. On the other hand, distribution habitats of *D. reticulatus* are in the river basins, in wet (seasonally flooded) woods and wet grasslands rich in bush cover (Nosek 1972). The distribution area of *D. reticulatus* has been reported to expand recently to higher latitudes and altitudes throughout central Europe, namely in Germany, Poland, Hungary and Slovakia (Široký et al. 2011). Nevertheless, our study failed to confirm this range extension for *D. reticulatus* in Romania.

Among Western Palearctic species of genus *Haemaphysalis* the most widespread is *H. punctata*. It is distributed from the British Isles to Japan in east and from southern Scandinavia to the Mediterranean region, Asia Minor and southern part of the former USSR. It occurs mainly on pastures, forest margins and forest-steppes (Nosek 1971a). Thus, its habitat partially overlaps in ecotone habitats with that of *I. ricinus*. The same applies to *H. concinna*, another relatively common tick species in Central Europe (Nosek 1971b). Other species of *Haemaphysalis* (*H. sulcata*, *H. parva*) have patchy distribution, being restricted to more warm and humid areas of Romania (Feider, 1965). Their distribution was reconfirmed by our study almost 50 years later.

The presence of other species captured by dragging in our study is accidental, and their low number is explained by non-questing host-seeking behavior. *Ixodes hexagonus* and *I. laguri* are typical burrow ticks, while *H. marginatum* has an active hunting strategy (Feider 1965).

The abundance of *I. ricinus* together with its wide host specificity, high frequency in humans and significant vectorial capacity together with our results, suggest that this tick species is the most important zoonoses vector in Romania.

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