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Prevalence and diversity of human pathogenic rickettsiae in urban versus rural habitats, Hungary

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Abstract Tick-borne rickettsioses belong to the important emerging infectious diseases worldwide. We investigated the potential human exposure to rickettsiae by determining their presence in questing ticks collected in an urban park of Budapest and a popular hunting and recreational forest area in southern Hungary. Differences were found in the infectious risk between the two habitats. *Rickettsia monacensis* and *Rickettsia helvetica* were identified with sequencing in questing *Ixodes ricinus*, the only ticks species collected in the city park. Female *I. ricinus* had a particularly high prevalence of *R. helvetica* (45 %). Tick community was more diverse in the rural habitat with *Dermacentor reticulatus* ticks having especially high percentage (58 %) of *Rickettsia raoultii* infection. We conclude that despite the distinct eco-epidemiological traits, the risk (hazard and exposure) of acquiring human pathogenic rickettsial infections in both the urban and the rural study sites exists.

Keywords *Rickettsia helvetica* · *Rickettsia monacensis* · *Rickettsia raoultii* · Urban · Recreational area · Hungary

Tick-borne rickettsioses are produced by spotted fever group rickettsiae and cause an expanding spectrum of clinical signs. Until recently, Mediterranean spotted fever caused by *Rickettsia conorii* was considered the only tick-borne rickettsiosis in Europe (Oteo and Portillo 2012). In the last decade many other species and subspecies of *Rickettsia* have been discovered and implicated as human pathogens, and new rickettsial syndromes have been described. Lack of (local) awareness among medical health professionals and availability of diagnostic tools (case definitions, serology, PCR etc.) hamper the identification of clinical cases.

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The aim of this study was to investigate potential human exposure to rickettsiae by determining their presence in questing ticks collected in an urban park of Budapest (Margaret Island), and a popular hunting and recreational forest area in Hungary (Gemenc). Ticks collected with flagging in 2011–2012 were identified and stored in 70 % ethanol until DNA extraction by alkaline hydrolysis then analyzed with a multiplex qPCR assay as previously described (de Bruin et al. 2015). This included a primer pair specific for *R. helvetica* gltA gene and another amplifying a different region of gltA of rickettsiae in general (Stenos et al. 2005). Positive samples were analyzed with conventional PCR and sequencing (de Bruin et al. 2015).

We collected 534 *Ixodes ricinus* from Margaret Island and 162 ticks (34 *I. ricinus*, 64 *Dermacentor reticulatus*, 2 *D. marginatus* and 62 *Haemaphysalis concinna*) from Gemenc. *Rickettsia helvetica* was detected in 139 (26 %) out of 534 and 9 (26.5 %) out of 34 questing *I. ricinus* from the urban park and the rural site, respectively (Table 1). The qPCR specific for *Rickettsia* spp. was positive in 88 (16.5 %) out of 534 *I. ricinus* ticks from the urban and 41 (25.3 %) out of 162 ticks from the rural habitat. Prevalence of rickettsiae in *I. ricinus* did not differ significantly in the two study sites (Table 1). However, both components of infection risk, the hazard (density of infected *I. ricinus*) and exposure (human presence) is higher in the urban park (Földvári et al. 2014) compared to the rural site. Female *I. ricinus* ticks in the urban parks were found to have a particularly high prevalence of *R. helvetica* (44.6 %) suggesting a higher infection risk when humans are bitten by this tick stage. The significantly higher prevalence of *R. helvetica* and *Rickettsia* spp. in the adult stages of *I. ricinus* compared to nymphs from the urban habitat (Fisher's exact test: $p < 0.05$) suggests the important role of transstadial infection in the eco-epidemiology of these pathogens (Table 1).

From the urban habitat 22 *R. monacensis* and 9 *R. helvetica* out of 534 questing *I. ricinus* were identified with the less sensitive conventional PCR and sequencing. Compared to the average ratio of these two rickettsiae in other European studies (Rizzoli et al. 2014; Špitalská et al. 2014), the relatively high prevalence of *R. monacensis* (originally described from a city park in Germany (Rizzoli et al. 2014)) appears unique probably as a consequence of the eco-epidemiology of the closed island park habitat (Földvári et al. 2014).

Rickettsiae were detected in 57.8 % of *D. reticulatus* which is much higher compared to the prevalence of 15.5 % reported previously in questing ticks of the same species collected throughout the country (Hornok et al. 2010). We identified *R. raoultii* infection with sequencing in 31 qPCR-positive *D. reticulatus* samples from the rural habitat. Tick-borne lymphadenopathy (TIBOLA) patients (Lakos 1997) and *R. slovaca* and *R. raoultii* in ticks removed from them have been reported in Hungary (Földvári et al. 2013). Both female (53.7 %) and male (65.2 %) *D. reticulatus* ticks in the present study had high prevalence of *Rickettsia* spp. This corroborates with previous findings about the equal role of both tick sexes and both *Dermacentor* spp. in TIBOLA epidemiology (Földvári et al. 2013).

Our results showed considerable difference between the dominant rickettsial agents in the city park (*R. helvetica* and *R. monacensis*) and natural forest habitat (*R. raoultii*). This is due to the differences of these habitats in their vector diversity. In urban settings, usually *I. ricinus* dominates (Rizzoli et al. 2014), whereas in natural habitats there is a more diverse tick community even visible in the small rural sample size of the present study (Szekeres et al. 2015). This more diverse tick community extends the range of possible human pathogenic rickettsiae, including newly emerging ones. Both our study sites have frequent human visitors: Margaret Island is a popular recreational and jogging park in the center of Budapest and Gemenc is a popular hunting and hiking area with over 50,000

Table 1 Prevalence and diversity of rickettsiae in questing ticks from an urban (Margaret Island) and a rural (Gemenc) habitat in Hungary

Tick species	Urban site			Rural site		
	R. helvetica +/Tested/prevalence	Rickettsia spp.	Confirmed with sequencing[d]	R. helvetica +/Tested/prevalence	Rickettsia spp.	Confirmed with sequencing[d]
I. ricinus						
Female	78/166/44.6 %	40/166/24.1 %	9 *R. helvetica*, 4 *R. monacensis*	1/5/20 %	1/5/20 %	1 *R. monacensis*
Male	45/214/21 %	34/214/15.9 %	14 *R. monacensis*	1/8/12.5 %	3/8/37.5 %	1 *R. helvetica* 1 *R. monacensis*
Nymph	20/150/13.3 %	14/150/9.3 %	4 *R. monacensis*	7/21/33.3 %	0/21/–	6 *R. helvetica*
Larva	0/4/–	0/4/–		–	–	
***I. ricinus* Sum**	**139/534/26 %**	**88/534/16.5 %**	**9 *R. helvetica*, 22 *R. monacensis***	**9/34/26.5 %**	**4/34/11.8 %**	**7 *R. helvetica* 2 *R. monacensis***
D. reticulatus[a]	–	–		0/64/–	37/64/57.8 %	Female: 18 *R. raoultii* Male: 13 *R. raoultii*
D. marginatus[b]	–	–		0/2/–	0/2/–	
H. concinna[c]	–	–		0/62/–	0/62/–	
Sum	**139/534/26 %**	**88/534/16.5 %**	**9 *R. helvetica*, 22 *R. monacensis***	**9/162/5.5 %**	**41/162/25.3 %**	**7 *R. helvetica* 2 *R. monacensis* 31 *R. raoultii***

[a] Only females and males

[b] Only females

[c] All stages presented

[d] GenBank accession numbers: LC060664 and LC060713 to LC060722

tourists per year. Since all rickettsiae (*R. helvetica, R. monacensis* and *R. raoultii*) detected in this study are proven human pathogens (Fournier et al. 2000; Jado et al. 2007; Jia et al. 2014), we can conclude that despite the distinct eco-epidemiological traits, the risk (hazard and exposure) of acquiring rickettsial infections in both the urban and the rural study sites exists.

Compliance with ethical standards

Conflict of interest None.

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