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Neglected tick-borne pathogens in the Czech Republic – summary of the EDENext prevalence study

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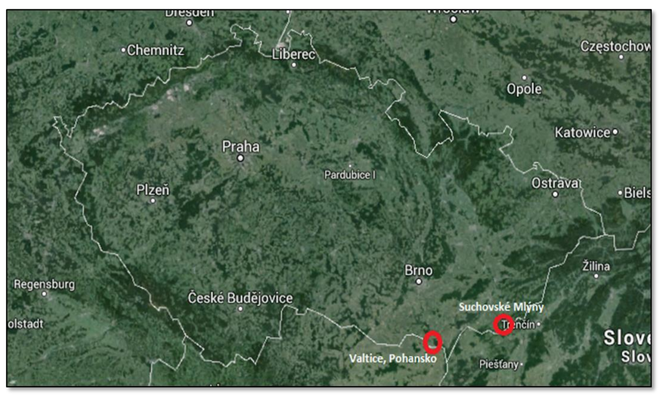

Czech Republic-study sites

Valtice park
URBAN HABITAT

Pohansko
NATURAL HABITAT

Suchovské Mlýny
AGRICULTURAL HABITAT

Introduction

Ixodid ticks (in Central Europe mainly *Ixodes ricinus*) present a significant health risk for humans as vectors of pathogens of which the most important are tick-borne encephalitis virus and *Borrelia burgdorferi*. Nevertheless, this vector species harbours also neglected or emerging pathogens - *Anaplasma phagocytophilum*, *Rickettsia monacensis*, *R. helvetica*, 'Candidatus Neoehrlichia mikurenis' (CNM), *Babesia microti*, *B. divergens* and *B. venatorum* (EU1).

Within the scope of EDENext project we aimed at determination of the prevalence rate of *A. phagocytophilum*, *Rickettsia* spp., CNM and *Babesia* spp. in host-seeking *Ixodes ricinus* ticks in three different South-Moravian ecosystems: natural (game preserve in Pohansko), urban (castle park in Valtice) and agricultural (pastureland in Suchovské mlýny) by using molecular techniques.

Methods

Single-step PCR. Protocols used for PCR were published earlier for *Rickettsia* spp. (Regnery et al. 1991), „*Candidatus* N. mikurensis" (Fertner et al. 2012)and *Babesia* spp. (Casati et al. 2006).
Real-time PCR. *A. phagocytophilum* was detected according to Courtney et al. (2004).
Sequencing. Direct sequencing of the purified PCR product was performed with the BigDye™ Terminator Cycle Sequencing Ready Reaction Kit version 1.1 (Applied Biosystems, U.S.A) and purified with EtOH/EDTA precipitation. The sequencing was performed on an ABI PRISM 310 Genetic Analyzer (Applied Biosystems, USA). PCR amplicons were bidirectionally sequenced once to ensure high quality reads. The DNA sequences were edited and aligned using the Seqman module within Lasergene v. 6.0 (DNASTAR Inc., USA) and also checked manually. The FASTA format and BLAST program (http://www.ncbi.nlm.nih.gov/blast) of the National Center for Biotechnology Information (Bethesda, MD, USA) were used for database searches.

Discussion and Conclusion

In this study we demonstrated the presence of all the previously mentioned pathogens in questing *I. ricinus* ticks. Prevalence data fall within the ranges reported in Europe. These findings and the differences among prevalences and statistical significance of our results are discussed in a manuscript (to be published). Engorged *I. ricinus* females collected from sheep were tested positive for all four pathogens. Interestingly, the prevalence of particular pathogens in questing ticks in Suchovské Mlýny was lower (apart from *Babesia* spp. in 2014) comparing to correponding prevalence in engorged ticks collected from sheep grazing at the same study site.

This work has contributed to the knowledge of ecology and diversity of neglected tick-borne pathogens in various ecosystems. Results might help to complete the map of endemic sites which pose public health risk.

Results

A total of 2473 host-seeking *I. ricinus* ticks (1817 nymphs, 310 females and 346 males) were collected by flagging low vegetation from 2011 through 2014 and tested for the presence of *A. phagocytophilum*, CNM, *Rickettsia* spp. and *Babesia* spp. Here we summarize overall prevalence data in three ecosystems (see Table 1).

In September 2013 and 2014 engorged *I. ricinus* ticks were collected from sheared sheep in Suchovské Mlýny. We tested 199 females ticks in total. Results are shown in Table 2.

Table 1. Prevalence (%) of tick-borne pathogens in questing *I. ricinus* ticks the Czech Republic.

		Valtice (URBAN)				Pohansko (NATURAL)				Suchovské Mlýny (AGRICULTURAL)			
Collection year	Pathogen	Males	Females	Nymphs	Total	Males	Females	Nymphs	Total	Males	Females	Nymphs	Total
2011	*Rickettsia* spp.	4.4	3.2	3.8	**3.8**	5.9	0	4.1	**3.9**	0	11.1	7.7	**7.3**
	"*Candidatus* N. mikurensis"	0	1.6	0.8	**0.8**	0	0	3.1	**2.4**	6.9	11.1	6.5	**6.9**
	A.phagocytophilum	7.4	1.6	1.5	**3.0**	11.8	8.3	0	**2.4**	6.9	5.6	1.6	**2.1**
	Babesia spp.	0	0	0	**0**	0	0	0	**0**	3.5	0	0.3	**0.3**
2012	*Rickettsia* spp.	14.3	11.5	7.6	**9.2**	20	33.3	1.4	**3.8**	13.6	11.8	6.3	**8.4**
	"*Candidatus* N. mikurensis"	4.8	3.9	1.9	**2.7**	20.0	33.0	0	**2.5**	0	5.9	1.3	**1.7**
	A.phagocytophilum	23.8	0	3.9	**6.0**	0	0	0	**0**	9.1	0	1.3	**2.5**
	Babesia spp.	0	0	0	**0**	0	0	0	**0**	0	0	0	**0**
2013	*Rickettsia* spp.	4.2	8.5	4.5	**4.9**	9.1	0	5.9	**5.6**	9.1	0	4.8	**4.7**
	"*Candidatus* N. mikurensis"	8.5	0	1.1	**2.6**	0	5.9	12.7	**11.6**	0	5.6	4.3	**4.2**
	A.phagocytophilum	7.0	4.3	1.6	**3.3**	18.2	5.9	1.0	**2.2**	0	0	0.5	**0.5**
	Babesia spp.	1.4	0	0.5	**0.7**	0	0	1.0	**0.9**	18.2	5.6	3.2	**4.2**
2014	*Rickettsia* spp.	4.9	4.4	8.1	**6.9**	11.8	0	1.6	**2.6**	9.1	0	3.5	**3.9**
	"*Candidatus* N. mikurensis"	4.9	11.1	5	**6.1**	5.9	0	7.8	**7.2**	15.2	19.4	3.5	**9.7**
	A.phagocytophilum	34.1	8.9	7.6	**12.1**	5.9	37.5	1.6	**3.9**	12.1	8.3	2.3	**5.8**
	Babesia spp.	2.4	0	0.6	**0.8**	5.9	12.5	3.9	**4.6**	0	8.3	5.8	**5.2**

Table 2. Prevalence (%) of tick-borne pathogens in engorged *I. ricinus* ticks collected from sheep (Suchovské Mlýny).

Year of collection	*Rickettsia* spp.	*Babesia* spp.	"*Candidatus* N. mikurensis"	*A. phagocytophilum*
2013	7.4	4.4	38.5	18.5
2014	6.3	0	14.1	12.5

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