Prevalence of *Anaplasma phagocytophilum* and *Borrelia burgdorferi* sensu lato, in *Ixodes ricinus* Parasitising on Red Foxes (*Vulpes vulpes*) from Romania

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Abstract

Among wild carnivores, red foxes (*Vulpes vulpes*) are one of the most adapted to anthropic ecosystems and one of the most abundant ones living in close proximity to humans. They are well recognized as important natural reservoirs for a large number of zoonotic agents in Europe, including ticks and tick-borne pathogens. The aim of our study was to establish the prevalence of two zoonotic tick-borne pathogens, *Anaplasma phagocytophilum* and *Borrelia burgdorferi* sensu lato (s.l.), in *Ixodes ricinus* ticks collected from red foxes. A total number of 216 red foxes, from 9 counties, were investigated and 204 *Ixodes ricinus* ticks, of all developmental stages were collected. PCR was performed on all samples to detect *B. burgdorferi* s.l. and *A. phagocytophilum* DNA. Only *A. phagocytophilum* was detected with an overall prevalence of 4.4%. As foxes represent a good model of sentinel species, further studies on the structure of tick communities correlated with the presence of tick-borne pathogens, can be suggestive for the risk posed for public health.

Keywords: *Anaplasma phagocytophilum*, *Borrelia burgdorferi* s.l., *Ixodes ricinus*, *Vulpes vulpes*.

INTRODUCTION

Among wild carnivores, red foxes (*Vulpes vulpes*) are one of the most adapted to anthropic ecosystems and one of the most abundant ones living in close proximity to humans. It is also well recognised that these animals are common hosts for different species of ectoparasites like lice, fleas, mites and ticks. Red foxes are playing an important role in the ecoepidemiology of tick-

transmitted microbes. Foxes act as reservoir host for a large number of zoonotic agents in Europe, including tick-borne pathogens and as host for ticks in certain areas (Martínez-Carrasco et al., 2007), posing a great risk from the public health perspective (Jongejan and Uilenberg, 2004). In this frame, our study aimed to investigate the prevalence of two zoonotic tick-borne pathogens, *Anaplasma phagocytophilum* and *Borrelia*

burgdorferi sensu lato (s.l.), in *Ixodes ricinus* ticks collected from red foxes in Romania.

MATERIALS AND METHODS

The study was conducted in Romania, between November 2011 and September 2014. A total number of 216 red foxes, from 9 counties, collected by the National Sanitary Veterinary and Food Safety Authority during campaigns for rabies screening, were examined. All the animals which were negative for rabies were transferred to the Parasitology Department of the Faculty of Veterinary Medicine in Cluj-Napoca, according to the current laws on dead animals transport and zoonotic risks. A macroscopic inspection was performed on all animals and all parasitizing ticks were collected and preserved in absolute ethanol. The morphological identification was performed individually using morphological keys and descriptions in Feider (1965). Only *Ixodes ricinus* ticks (n=204) were used for further analysis. Genomic DNA extraction was performed individually on all ticks following the protocol with ammonium hydroxide described by Morán-Cadenas et al. (2007). All samples were analysed by PCR technique for the diagnosis of *B. burgdorferi* s.l. and *A. phagocytophilum* DNA. The PCR for detecting *B. burgdorferi* s.l. was carried out by the method described by Priem et al. (1997) and the amplification was performed targeting the *OspA* gene (Priem et al., 1997). The PCR for detecting *A. phagocytophilum* was performed by the method described by Courtney et al. (2004), targeting *msp2* gene. Positive control samples were used (strains of *B. burgdorferi* s.s., *B. afzelii*, *B. garinii*

and DNA extracted from the blood of a dog naturally infected with *A. phagocytophilum*) and laboratory-reared uninfected *I. ricinus* ticks were used as negative controls. Frequency, prevalence, and its 95% confidence interval were performed using the EpiInfo 2000 software. The chi-square test was used on the dataset to evaluate the statistic significance and was represented by a *p* value of <0.05.

RESULTS AND DISCUSSION

A total number of 204 ticks (138 females, 46 males, 15 nymphs and 5 larvae) were morphologically identified as *I. ricinus*. Only *A. phagocytophilum* was detected with a prevalence of 4.4% (n=9). The number of collected ticks and the prevalence of *A. phagocytophilum* for each county are presented in Tabel 1. Frequency and prevalence of *A. phagocytophilum* for each developmental stage are shown in Table 2.

Until several years ago, little information was available regarding infection with *A. phagocytophilum* in foxes. In 1999 Pusterla et al. performed serological studies on red foxes from Switzerland showing that these animals can be carriers of *A. phagocytophilum*. More recently, this tick-borne pathogen was detected by PCR in ticks collected from foxes from Hungary, Slovakia and Austria. In Hungary, six of the 112 pools of *I. ricinus* collected from 100 red foxes were found positive to *A. phagocytophilum* (Sréter et al., 2004). In Slovakia the reported prevalence is 4.4% (Spitalska and Kocianova, 2002) while in Austria, Sixl et al. (2003) found a prevalence of 5.1%. Most registered values on engorged ticks are comparable

Tab. 1. Number of collected ticks and prevalence of *A. phagocytophilum* for each county

County	No of ticks	A. phagocytophilum			
		Frequency	Prevalence %	95% CI	p
Alba	38	2	5.3	0.6-17.7	0.0917
Bihor	17	2	11.8	1.5-36.4	
Braşov	96	2	2.1	0.3-7.3	
Cluj	2	1	50	1.3-98.7	
Covasna	29	1	3.4	0.1-17.8	
Hunedoara	1	0	0	0.0-97.5	
Harghita	2	0	0	0.0-84.2	
Mureş	1	0	0	0.0-97.5	
Satu Mare	18	1	5.6	0.1-27.3	
TOTAL	204	9	4.4	2.0-8.2	

Tab. 2. Frequency and prevalence of *A. phagocytophilum* for each developmental stage

Developmental stage	No of ticks	A. phagocytophilum			p
		Frequency	Prevalence %	95% CI	
Female	138	8	5.8	2.5-11.1	0.5524
Male	46	1	2.2	0.1-11.5	
Nymph	15	0	0	0.0-21.8	
Larvae	5	0	0	0.0-52.2	
TOTAL	204	9	4.4	2.0-8.2	

with the ones on questing *Ixodes ricinus*. Schorn et al. (2011) found a prevalence of 3.9% to 13.3%, in *I. ricinus* collected from different public parks from Germany, depending on the habitat and developmental stage. The overall prevalence was 8.8% in questing *I. ricinus* ticks collected in Norway (Mysterud et al., 2013). Regarding the studies performed on red foxes tissue samples, Ebani et al. (2011) reported a prevalence of 16.6% after investigating 150 foxes from Center Italy. In that area tick vector is present and positive cases in domestic animal were reported (Ebani et al., 2011).

Red foxes are considered competent reservoir host for *B. burgdorferi* s.l., but few epidemiological studies are available. Heidrich et al. (1999) found a prevalence of 7.0% for *B. burgdorferi* s.l. by PCR in skin samples from foxes in Germany. A study conducted in northeast Spain from 1990 and 2006, on serum samples showed that 7 of the 84 foxes (8.3%) were positive for *Borrelia burgdorferi* s.l.. The geographical regions from which animals originated were different in terms of climate and habitat. Positive foxes originated from areas characterised by Atlantic climate, with constant rain and dense forests. The serological negative animals were originated from regions characterized by Mediterranean climate, with lower rainfall in spring and autumn. The authors conclude that the prevalence of *B. burgdorferi* s.l. varies primarily due to differences in habitat, which has a strong influence on tick communities.

In a similar study regarding the prevalence of *B. burgdorferi* s.l. and *A. phagocytophilum* in *I. ricinus* ticks collected from hedgehogs from 12 counties of Romania, Dumitrache et al., (2012) identified both pathogens with prevalence of 0.4% and 12% respectively. In a more recent study, Matei et al., (2015) found a prevalence of 3.42% in questing *I. ricinus* ticks from Romania, after examining an impressing number of 10,438 samples.

None of the tested samples were positive to *B. burgdorferi* s.l.. This can be explained on one hand by the number of ticks analyzed or may be a consequence of a low prevalence of the pathogen in both animals and unfed ticks in the habitats from where foxes originated. Regarding the total prevalence for *A. phagocytophilum*, the value falls within the average values in Europe.

CONCLUSION

Red foxes are hosts for *Ixodes ricinus*, the main vector for zoonotic pathogen *A. phagocytophilum*. The registered prevalence for *A. phagocytophilum*, is similar to other results obtain in different epidemiological studies from Europe. As foxes represent a good model of sentinel species, further studies on the structure of tick communities correlated with the presence of tick-borne pathogens, can be suggestive for the risk posed for public health.

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