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**SHORT COMMUNICATION**

# Detection of *Borrelia burgdorferi* Sensu Lato and *Anaplasma phagocytophilum* in Small Mammals and Ectoparasites in Hungary

Krisztina Rigó,[1] Miklós Gyuranecz,[2] Ádám György Tóth,[3] and Gábor Földvári[1]

## Abstract

The aim of our study was to investigate the presence of *Borrelia burgdorferi* sensu lato (s.l.) and *Anaplasma phagocytophilum* in small mammals and ticks using polymerase chain reaction and to gain information about the prevalence and possible coexistence of these pathogens at a selected site in Hungary. Two hundred seventy-seven small mammals were trapped in South-Eastern Hungary during 2009. Tissue samples and a total of 831 ectoparasites (*Ixodes ricinus, Ixodes acuminatus, Haemaphysalis concinna, Ctenophtalmus assimilis*, and *Nosopsyllus fasciatus*) were collected from small mammals. One thousand one hundred and six *I. ricinus* and 476 *H. concinna* were collected from the vegetation during the investigation. Neither *A. phagocytophilum* nor *B. burgdorferi* s.l. was detected in any of the mammal tissue samples. *A. phagocytophilum* was not found in ticks collected from small mammals. Very low minimum prevalence was found for all pathogens (0.62% for *Borrelia afzelii* in ticks collected from small mammals, and 0.57%, 0.06%, and 0.19% for *A. phagoctyophilum, B. afzelii*, and *Borrelia garinii*, respectively, in questing ticks). The present study is the first report of borreliae from *I. acuminatus* and *H. concinna* from Hungary.

**Key Words:** *Anaplasma phagocytophilum*—*Borrelia afzelii*—*Borrelia garinii*—*Borrelia burgdorferi* sensu lato—*Haemaphysalis concinna*—Hungary—*Ixodes acuminatus*—*Ixodes ricinus*—Reservoirs—Rodents—Small mammals.

## Introduction

Small mammals are important hosts for the larval and nymphal stages of numerous hard tick species and, as a consequence, they may serve as reservoirs for many tick-borne pathogens, including *Borrelia burgdorferi* sensu lato (s.l.) and *Anaplasma phagocytophilum*.

*Borrelia burgdorferi* s.l. is the causative agent of Lyme borreliosis, which is the most prevalent vector-borne human disease in the temperate zone of the Northern Hemisphere. *Borrelia afzelii, Borrelia garinii, B. burgdorferi* sensu stricto, and *Borrelia spielmanii* are the most important pathogenic *Borrelia* species in Europe and each is associated with different vertebrate reservoirs (Bowman and Nuttall 2008).

Granulocytic anaplasmosis is caused by *A. phagocytophilum*. This pathogen has also been found in several vertebrate species (rodents, carnivores, equids, and ruminants), but the main reservoir in Europe is as yet unknown (Woldehiwet 2010).

The most important vector for both *B. burgdorferi* s.l. and *A. phagocytophilum* in Europe is *Ixodes ricinus*, which is also one of the most prevalent tick species in Hungary (Sréter et al. 2005, Földvári et al. 2007).

The aim of this preliminary study was to investigate the presence of Lyme disease spirochetes and *A. phagocytophilum* in wild-living small mammals, in their ectoparasites, and in field-collected ticks in Hungary and to gain information about the occurrence and possible coexistence of these pathogens at a selected region.

## Materials and Methods

The study area is a mosaic of grasslands and oak woods situated in southeastern Hungary. Small mammals were live-trapped in April, July, October, and November 2009 and common hamsters were obtained from trappers in May and October 2009. Ectoparasites were combed off and lung, liver, spleen, and kidney samples were collected. After identification,

[1]Department of Parasitology and Zoology, Faculty of Veterinary Science, Szent István University, Budapest, Hungary.
[2]Veterinary Medical Research Institute, Hungarian Academy of Sciences, Budapest, Hungary.
[3]Central Agricultural Office Veterinary Diagnostic Directorate, Budapest, Hungary.

ticks were pooled according to species (30 larvae, 15 nymphs, 10 or fewer males or females per pool). DNA was extracted by an X-tractor Gene nucleic acid extraction robot (Corbett Robotics Pty. Ltd., Queensland, Australia) with the Total RNA Isolation Kit, Nucleospin 96 RNA (Macherey-Nagel GmbH & Co. KG, Düren, Germany), except for the DNase incubation step as described in Gyuranecz et al. (2010a, 2010b) *A. phagocytophilum* and *B. burgdorferi* s.l. were detected by polymerase chain reaction. Primers SL forward and reverse were used to amplify a portion of the *Borrelia* osp A gene as described in Demaerschalck et al. (1995). *Borrelia* species were determined by sequencing and analyzing the amplicons. *A. phagocytophilum* DNA was detected and identified by species-specific primers MSP4AP3 and MSP4AP5 (de la Fuente et al. 2005).

## Results

Overall, 277 small mammals (100 common hamsters [*Cricetus cricetus*], 38 common voles [*Microtus arvalis*], 110 yellow-necked mice [*Apodemus flavicollis*], and 15 striped field mice [*Apodemus agrarius*] were trapped, and 8 Eurasian pygmy shrews [*Sorex minutus*] and 6 common shrews [*Sorex araneus*]) were by-catched. Neither *A. phagocytophilum* nor *B. burgdorferi* s.l. was detected in any of the tissue samples.

A total of 806 ticks (404 *I. ricinus*, 374 *Ixodes acuminatus*, and 28 *Haemaphysalis concinna*) and 25 fleas (15 *Ctenophtalmus assimilis* and 10 *Nosopsyllus fasciatus*) were removed from small mammals. *A. phagocytophilum* was not found in any of the feeding ticks. Three pools containing *Ixodes* larvae (collected from one *A. flavicollis* and two *A. agrarius*), one pool of *I. ricinus* nymphs (collected from *M. arvalis*), and one pool of *I. acuminatus* females (collected from *C. cricetus*) contained *B. afzelii* (Table 1). None of the flea samples were positive for any of the examined pathogens.

A total of 1106 *I. ricinus* and 476 *H. concinna* were collected from the vegetation. Six *I. ricinus* pools and three *H. concinna* pools contained *B. afzelii. B. garinii* was found in one *I. ricinus* pool. We detected *A. phagocytophilum* in three *I. ricinus* pools (two female and one nymph pools).

## Discussion

A large number of *I. acuminatus* ticks were found, but only on common hamsters (*C. cricetus*), not on other rodents. Only a few *I. acuminatus* were collected previously in Hungary: three females from hedgehogs (Babos 1965) and four females from dogs (Földvári et al. 2007). According to our results, *I. acuminatus* might be more prevalent in Hungary than we previously thought. An explanation for the low number of previously collected specimens of this species is that these nidiculous ticks are usually found on rodents and small insectivores and in their burrows (Bowman and Nuttall 2008), so it is unlikely that they are collected by flagging or dragging.

Four pools of *Ixodes* larvae were found positive for *B. afzelii*. Larvae in three out of the four pools were removed from small mammals. The infection of these larvae with borreliae could be the result of either cofeeding or that the small mammals were infected with *Borrelia*, but the infection was not detected. The ticks in the fourth positive larva pool were collected from the vegetation. These infected larvae may be evidence for transovarial transmission, although it was reported that *B. afzelii* is rarely, if ever, inherited by ticks (Bowman and Nuttall 2008).

The present study is the first report of borreliae from *I. acuminatus* and *H. concinna* from Hungary.

*A. phagocytophilum* was previously detected in *I. ricinus* collected from foxes (Sréter et al. 2004) and in field-collected ticks (this study) in Hungary, but we have not been able to find it in small mammals yet, despite the fact that recent studies suggest that rodents can be important reservoirs of this pathogen (Woldehiwet 2010). The small number of positive samples in field-collected ticks suggests a low prevalence of this pathogen at the study site. This provides a possible explanation for our lack of success in detecting these bacteria in small mammal tissue samples.

The prevalence of pathogens in field-collected ticks and in ticks collected from small mammals was very low and, despite the fairly high number of captured rodents, we were not able to detect them in any tissue samples. This may reflect the fact that prevalence varies greatly between different regions. There is also a possibility that the incompetent or less competent host species present at the study site outnumber the reservoir animals, thus diluting the prevalence of these pathogens. However, to prove the presence and determine the characteristics of this dilution effect, further information about the host community of the area and the analysis of tick blood-meals is needed.

Table 1. Results of *Borrelia*-Specific Polymerase Chain Reaction and Sequencing

| *Species* | *Tick stage* | *Source* | *No. of ticks* | *No. of pools/ positive pools* | *Minimum prevalence (%)* | Borrelia *species found* | *GenBank accession numbers* |
|---|---|---|---|---|---|---|---|
| *Ixodes* spp. | Larva | Vegetation | 173 | 7/1 | 0.58 | *Borrelia garinii* | HQ739057 |
| *Ixodes ricinus* | Nymph | Vegetation | 744 | 51/4 | 0.54 | *Borrelia afzelii* | HQ739053, HQ739054, HQ739055, HQ739061 |
| *I. ricinus* | Female | Vegetation | 96 | 10/2 | 2.08 | *B. afzelii* | HQ739056, HQ739060 |
| *Haemaphysalis concinna* | Nymph | Vegetation | 388 | 28/3 | 0.77 | *B. afzelii* | HQ739059, HQ739062, HQ739063 |
| *Ixodes* spp. | Larva | Small mammals | 362 | 34/3 | 0.83 | *B. afzelii* | HQ739058, HQ739065, HQ739066 |
| *I. ricinus* | Nymph | Small mammals | 40 | 10/1 | 2.5 | *B. afzelii* | HQ739067 |
| *Ixodes acuminatus* | Female | Small mammals | 386 | 36/1 | 0.26 | *B. afzelii* | HQ739064 |

Only categories with >0% *Borrelia* prevalence are presented.

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## Disclosure Statement

No competing financial interests exist.

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Address correspondence to:
*Krisztina Rigó*
*Department of Parasitology and Zoology*
*Faculty of Veterinary Science*
*Szent István University*
*István u. 2.*
*Budapest H-1078*
*Hungary*

*E-mail:* rigo.krisztina@aotk.szie.hu