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Bartonella infection in small mammals and their ectoparasites in Lithuania

Indre Lipatova [a], Algimantas Paulauskas [a,*], Irma Puraite [a], Jana Radzijevskaja [a], Linas Balciauskas [b], Vaclovas Gedminas [c]

[a] *Department of Biology, Vytautas Magnus University, Vileikos Str. 8, LT-44404 Kaunas, Lithuania*
[b] *Nature Research Centre, Akademijos Str. 2, LT-08412 Vilnius, Lithuania*
[c] *Kaunas Tadas Ivanauskas Zoological Museum, Laisves Av. 106, LT-44253 Kaunas, Lithuania*

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Abstract

The *Bartonella* pathogen is an emerging zoonotic agent. Epidemiological studies worldwide have demonstrated that small mammals are reservoir hosts of *Bartonella* spp. and their ectoparasites are potential vectors. The aim of this study was to investigate the prevalence of *Bartonella* infections in small mammals (Rodentia, Insectivora) and their ectoparasites (fleas and ticks) in Lithuania. A total of 430 small mammals representing nine species were captured with live-traps in Lithuania during 2013–2014. A total of 151 fleas representing eight species were collected from 109 (25.8%) small mammals. Five hundred and seventy ticks (*Ixodes ricinus*) were collected from 68 (16.1%) small mammals. *Bartonella* DNA was detected in 102 (23.7%) small mammals, 44 (29.1%) fleas and five (3.7%) pooled tick samples. Sequence analysis of 16S–23S rRNA ITS region showed that sequences were identical or similar to *Bartonella grahamii, Bartonella taylorii* and *Bartonella rochalimae*. This study is the first investigating the distribution and diversity of *Bartonella* species in small mammals and their ectoparasites in Lithuania. *B. grahamii, B. taylorii,* and *B. rochalimae* were detected in small mammals and their fleas, and *B. grahamii* in ticks obtained from small mammals.

Keywords: *Bartonella*; Fleas; Ticks; ITS; Lithuania

1. Introduction

Bartonella is a gram-negative bacteria, a facultative intracellular parasite that is transmitted by arthropod vectors such as fleas, ticks, sand flies, lice and mites. *Bartonella* species have been identified in a wide range of wild and domestic mammals [1]. This pathogen is an emerging zoonotic agent. The genus *Bartonella* has about 30 species or subspecies, of which at least 14 have been associated with a variety of human diseases [2–4]. Epidemiological studies worldwide have demonstrated that small mammals (Rodentia, Insectivora) are reservoir hosts of *Bartonella* spp. and their ectoparasites are potential vectors.

Populations of small mammals such as rodents and insectivores that have close contact with human populations can influence the transmission of *Bartonella* pathogen from animals to humans [2]. More than 15 *Bartonella* species have been isolated from rodents and insectivores [5]. Some of them are causative agents of human diseases.

Arthropods feeding on small mammals are involved in the transmission routes of *Bartonella* species. Fleas are important vectors for the maintenance and transmission of many *Bartonella* species among populations of small mammals [6,7]. Ticks have also been identified as a risk factor associated with the *Bartonella* pathogens [8,9].

In Lithuania, the first case of cat scratch disease caused by *B. henselae* was reported in a 16 year old boy in 2007 [10]. The most recent clinical case of cat scratch neuroretinitis was diagnosed in a patient in a Lithuanian hospital in 2014 [11]. These recent

* Corresponding author. Tel.: +370 37 327901; fax: +370 37 327916.
E-mail address: a.paulauskas@gmf.vdu.lt (A. Paulauskas).

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events involving this emerging zoonose underline the necessity to determine the vectors of *Bartonella* infection. The identification of the arthropod vectors for the *Bartonella* pathogen is important in understanding host, vector and pathogen relationships. The aim of this study was to investigate the prevalence of *Bartonella* infections in small mammals (Rodentia, Insectivora) and their ectoparasites (fleas and ticks) in Lithuania.

2. Materials and methods

2.1. Sample collecting

Small mammals were captured with live-traps in three locations (coordinates: 55°32′N 21°7′E, 55°4′N 25°15′E, 55°54′N 24°19′E) in Lithuania during 2013–2014. A total of 430 small mammals representing nine species (*Apodemus flavicollis*, Micromys minutus, *Arvicola amphibius*, Myodes glareolus, *Microtus arvalis*, *Microtus agrestis*, *Microtus oeconomus*, *Sorex minutus*, *Sorex araneus*) were trapped. Captured rodents and insectivores were dispatched by cervical dislocation and put into individual plastic bags. Small mammals were marked and identified by species and sex. All ectoparasites were collected from infested small mammals. All fleas and ticks were put into test tubes with 70% ethanol and it kept at 4 °C until investigation. Flea and tick species were identified by morphological criteria [12,13].

2.2. DNA extraction

DNA from small mammals was extracted by using a Genomic DNA Purification Kit (Thermo Fisher Scientific Baltics, Lithuania), according to the manufacturer's instructions. DNA from fleas and ticks was extracted by using 2.5% ammonium hydroxide [14].

2.3. PCR amplification

Bartonella DNA in samples was detected using a PCR of the *gltA* gene (primers BhCS.781p-BhCS.1137n) [15]. The obtained specific products of 379 base pairs were considered as a positive result.

All positive samples for *Bartonella* species were chosen for 16S–23S rRNA ITS region amplification. A nested-PCR of the ITS region was performed using an external primer (WITS-F and WITS-R) and internal primers (Bh311-332F and Bh473-452R) [4,16].

2.4. Electrophoretic analysis of the PCR

PCR products were subjected to electrophoresis on 1,5-3% agarose gel. The gels were visualized on a UV light transilluminator and photographed.

2.5. DNA sequencing

PCR products selected for DNA sequencing were purified with a GeneJET™ Gel Extraction Kit (Thermo Fisher Scientific Baltics, Lithuania). A BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) was used for DNA sequencing reaction, according to the manufacturer's recommendations. Sequencing was performed using a 3130xl DNA automated sequencer (Applied Biosystems). The obtained sequences were edited using Mega6 [17] programs and aligned using the BLAST algorithm with *Bartonella* spp. gene sequences registered in the GenBank database.

Sequences obtained in this study were deposited in the GenBank with accession numbers KR922899 to KR922905 and KT032220 to KT032221. Q1

3. Results

From 430 small mammals, 109 (25.3%) were infested by fleas. A total 151 fleas representing eight species were collected: *Ctenophthalmus agyrtes* – 71 fleas (47%), *Ct. assimilis* – one flea (0.7%), *Ct. uncinatus* – seven fleas (4.6%), *Hystrichopsylla talpae* – 10 fleas (6.6%), *Megabothris turbidus* – 44 fleas (29.1%), *M. walkeri* – 13 fleas (8.6%), *Palaeopsylla soricis* – three fleas (2%) and *Peromyscopsylla bidentata* – two fleas (1.3%).

A total of 570 ticks (*Ixodes ricinus*) were collected from 137 (31.9%) small mammals: seven (1.2%) were nymphs and 563 (98.8%) were larvae.

Bartonella species were detected in 102 (23.7%) out of 430 small mammal DNA extractions using *gltA* gene primers. *Bartonella* spp. was detected in 33.2% of *A. flavicollis,* 23.7% of *M. minutus,* 15.5% of *Myodes glareolus* and 12.5% of *M. oeconomus*. One sample of two sampled from *M. arvalis* was positive for *Bartonella* gltA gene. Four small mammal species (*A. amphibius, M. agrestis, S. minutus, S. araneus)* out of nine were not positive for the *gltA* gene fragment. Ninety-six (94.1%) out of 102 positive samples were found positive for the *Bartonella* 16S–23S ITS region. The ITS region sequences showed that *Bartonella grahamii, Bartonella taylorii* and *Bartonella rochalimae* were detected in small mammals. Fifty-four positive samples contained *B. grahamii*, 23 positive samples contained *B. taylorii*, 13 positive samples contained both *B. grahamii* and *B. taylorii* and one positive sample contained *B. rochalimae*.

A total of 44 out of 151 (29.1%) fleas collected from small mammals contained *Bartonella* spp. *gltA* fragments. All positive fleas were tested for the *Bartonella* 16S–23S ITS region and 33 out of 44 (75%) fleas were found positive. Five positive samples contained *B. grahamii*, 18 positive samples contained *B. taylorii,* 7 positive samples contained both *B. grahamii* and *B. taylorii* and two positive samples contained *B. rochalimae*. *B. grahamii* was detected in 7.6% of *M. walkeri*, 6.8% of *M. turbidus* and 1.4% of *Ct. agyrtes*. *B. taylorii* was detected in 19.7% of *Ct. agyrtes*, 14.3% of *Ct. uncinatus*, 10% of *H. talpae*, 9.1% of *M. turbidus* and 7.6% of *M. walkeri*. Both *B. grahamii* and *B. taylorii* species were detected in 7.1% of *Ct. agyrtes* and 4.5% of *M. turbidus*. *B. rochalimae* was detected in 4.5% of *M. turbidus*. The *Bartonella* pathogen was not detected in *Ct. assimilis, P. soricis* or *P. bidentata*.

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In five out of 135 (3.7%) pools of *I. ricinus* larvae, *Bartonella gltA* and 16S–23S ITS fragment were detected, but they were not detected in nymph tick DNA samples. The ITS region sequences showed that *B. grahamii* was detected in all positive tick samples.

4. Discussion

Several studies in Europe, Asia and the USA have suggested that fleas and ticks are potential vectors of *Bartonella* infection in small mammals [2,5,18,19]. Rodents are one of

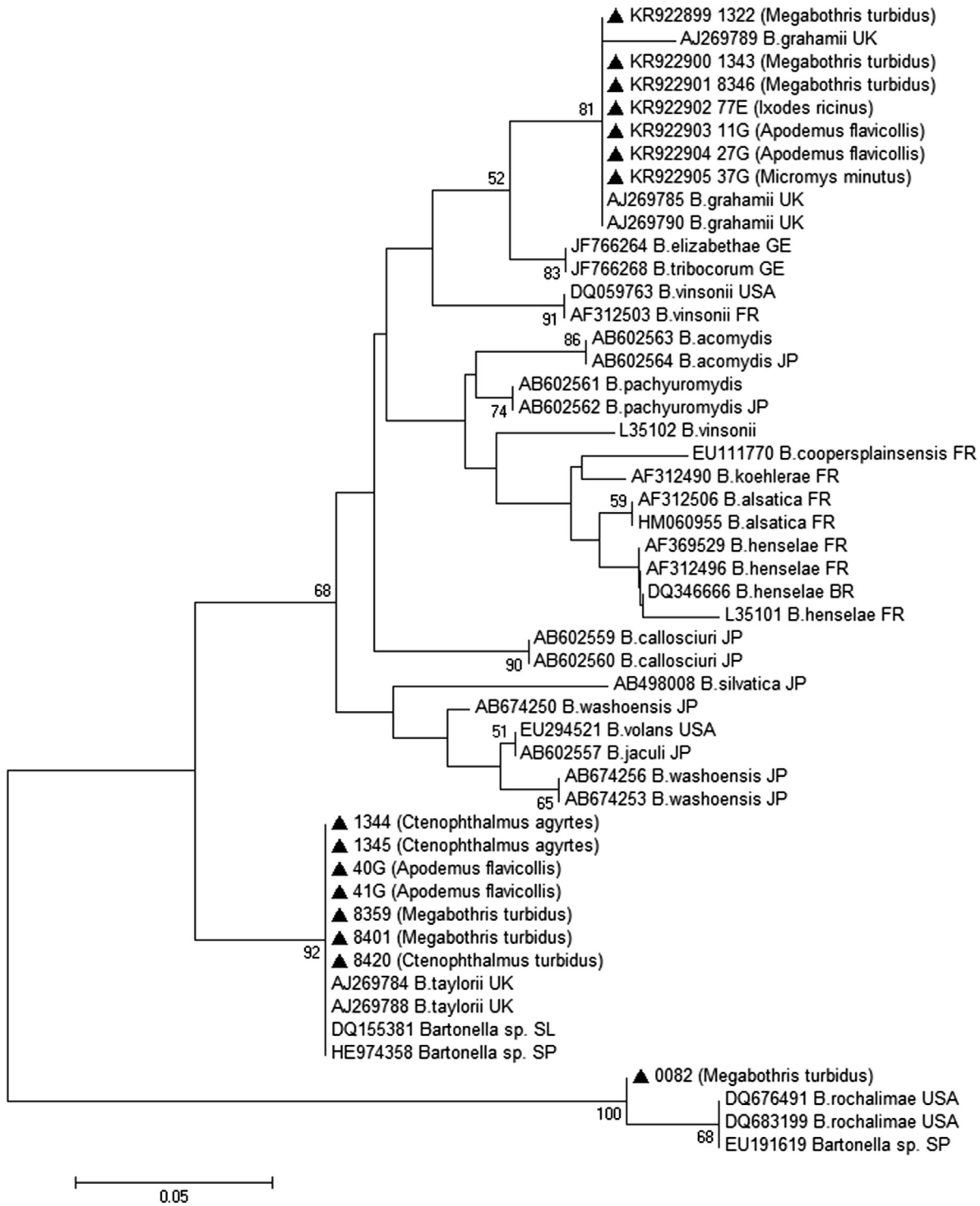

Fig. 1. Phylogenetic tree of the 16S–23S ribosomal RNA intergenic spacer partial sequences of *Bartonella* spp. created using the Neighbour-joining method and Bootstrap analysis of 1000 replicates. The scale bar indicates nucleotide substitutions per site. Strains with sequence accession numbers have been taken from the GenBank for comparison. Samples sequenced in the present study are indicated by the ▲ symbol. Abbreviations: BR – Brazil; FR – France; GE – Georgia; JP – Japan; SL – Slovenia; SP – Spain, UK – the United Kingdom; USA – the United States of America.

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the most important reservoirs for *Bartonella* pathogens [7,18]. This study is the first report on the distribution and diversity of *Bartonella* species in small mammals and their ectoparasites in Lithuania. Sequence analysis showed that sequences were identical or similar to *B. grahamii, B. taylorii, B. rochalimae* (Fig. 1). Moreover, *B. grahamii, B. taylorii* and *B. rochalimae* were detected for the first time in rodents and fleas in Lithuania, and *B. grahamii* in ticks obtained from rodents.

B. grahamii is a causative agent of human illnesses [20] and infects different rodent species over a worldwide geographic distribution. In this study, *B. grahamii* was detected in three rodent species (*A. flavicollis, M. glareolus, M. oeconomus*), three flea species (*Ct. agyrtes, M. turbidus, M. walkeri*) and *I. ricinus* larvae. The phylogenetic tree of 16S–23S rRNA ITS sequences showed that rodents, fleas and ticks harboured the same *B. grahamii* genotype in Lithuania (Fig. 1). The ITS sequences of these samples were 100% identical to *B. grahamii* previously detected in the United Kingdom (AJ269785, AJ269790).

B. rochalimae is a zoonotic infection agent. This pathogen has been isolated from a human in the USA after travel to a region where the *Bartonella* pathogen is endemic [21]. It was previously reported that rodents could be a reservoir for *B. rochalimae* [7]. We amplified *B. rochalimae* from the *M. glareolus* rodent species and *M. turbidus* flea species. The 16S–23S rRNA ITS sequence of *M. turbidus* flea species (0082 sample) was 99% similar to *B. rochalimae* from human blood in the USA (DQ683199) and to *Bartonella* sp. from a flea (*Pulex irritans*) in Spain (EU191619).

B. taylorii can cause infection in animals, but the real pathogenic potential of this *Bartonella* species is as yet unknown. In this study, *B. taylorii* was detected in four rodent species (*A. flavicollis, M. minutus, M. arvalis, M. glareolus*) and five flea species (*Ct. agyrtes, Ct. uncinatus, H. talpae, M. turbidus, M. walkeri*). This pathogen has also been isolated from *M. glareolus* in the United Kingdom [22] and Siberia [5], and from the *Xenopsylla cheopis* flea species in Afghanistan [23]. The phylogenetic tree of 16S–23S rRNA ITS sequences showed that rodents and fleas harboured a different *B. taylorii* genotype in Lithuania (Fig. 1). They are branched together and the similarity value of the ITS fragment was ≥98% with the validated species.

The results of this study suggest that fleas may be substantial vectors for the transmission of *B. grahamii, B. taylorii* and *B. rochalimae* in rodents. Furthermore, we detected that *I. ricinus* does not play an important role in the transmission of *Bartonella* species in small mammal populations in Lithuania, therefore only *B. grahamii* was present in all positive tick samples.

Conflict of interest

None of the authors have any conflict of interest.

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