**BARTONELLA INFECTIONS IN FLEAS (SIPHONAPTERA: PULICIDAE) AND LACK OF BARTONELLA IN TICKS (ACARI: IXODIDAE) FROM HUNGARY**

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**Abstract.** Fleas (95 *Pulex irritans*, 50 *Ctenocephalides felis*, 45 *Ctenocephalides canis*) and ixodid ticks (223 *Ixodes ricinus*, 231 *Dermacentor reticulatus*, 204 *Haemaphysalis concinna*) were collected in Hungary and tested, in assays based on PCR, for *Bartonella* infection. Low percentages of *P. irritans* (4.2%) and *C. felis* (4.0%) were found to be infected. The groEL sequences of the four isolates from *P. irritans* were different from all the homologous sequences for Bartonella previously stored in GenBank but closest to those of *Bartonella* sp. SE-Bart-B (sharing 96% identities). The groEL sequences of the two isolates from *C. felis* were identical with those of the causative agents of cat scratch disease, *Bartonella henselae* and *Bartonella claridgeiae*, respectively. The pap31 sequences of *B. henselae* amplified from Hungarian fleas were identical with that of Marseille strain. No Bartonella-specific amplification products were detected in 95 *P. irritans*, *D. reticulatus* and *H. concinna* pools.

Fleas transmit several bacterial pathogens including genospecies of *Borrelia burgdorferi* sensu lato, *Anaplasma phagocytophilum*, *Rickettsia rickettsii* spp. of the spotted-fever group, *Bartonella henselae* and *Bartonella claridgeiae*, respectively. The *pap31* sequences of *B. henselae* amplified from Hungarian fleas were identical with that of Marseille strain. No Bartonella-specific amplification products were detected in 95 *P. irritans*, *D. reticulatus* and *H. concinna* pools.

Ticks transmit several bacterial pathogens including genospecies of *Borrelia burgdorferi* sensu lato, *Anaplasma phagocytophilum*, *Rickettsia rickettsii* spp. of the spotted-fever group, *Bartonella henselae* and *Bartonella claridgeiae*, respectively. The *pap31* sequences of *B. henselae* amplified from Hungarian fleas were identical with that of Marseille strain. No Bartonella-specific amplification products were detected in 95 *P. irritans*, *D. reticulatus* and *H. concinna* pools.

The tick and flea vectors of some of these Bartonella might be the sheep tick, *Ixodes ricinus* and the cat flea, *Ctenocephalides felis* on the continent (Parola et al. 2005). In the past years, several previously unrecognised bacterial pathogens belonging to the genus Bartonella were also described from ticks and fleas, and several new clinical syndromes caused by these bacteria were reported (Greb and Raoult 2002, Jacomo et al. 2002). From the *Bartonella henselae*-positive pool, fragments of gene coding 60 kDa heat shock protein (*groEL*) were amplified using the primer set of HSPps1, HSPps2 and HSPps4 primers (Zeaiter et al. 2002). From the *Bartonella henselae*-positive pool, fragments of gene coding hemin-binding protein (*pap31*) were also amplified using the primer sets of PAPn1, PAPn2 and PAPn3 (Zeaiter et al. 2002). Amplicons were further characterized by sequencing analysis (Sréter et al. 2000). Sequences were identified by comparison with GenBank entries using the BLAST program (http://www.ncbi.nlm.nih.gov/blast).

No Bartonella-specific amplification products were detected in *I. ricinus*, *D. reticulatus* and *H. concinna* pools. As *B. henselae* and *Bartonella quintana* have been reported from *Ixodes*, *Dermacentor* and *Haemaphysalis* spp. in some countries of Eurasia and North America (Table 1), it cannot be excluded that the lack of bartonellae in Hungarian and Swedish ticks (La Scola et al. 2004) might be attributed to the low prevalence of bartonellae in these ectoparasites in some regions of Europe. Moreover, the surprisingly high infection rate of ticks and the lack of sequencing results in some studies might represent laboratory contamination with DNA originally not present in these ectoparasites or might be attributed to the detection of aspecific products.

The 19 pools of *P. irritans*, Bartonella DNA was detected in four pools coming from four different foxes. As there were four positive pools, there must have been at least four infected fleas among the 95 investigated, giving a minimum prevalence of 4.2% in *P. irritans*. All Bartonella-positive pools appeared to be identical in terms of their groEL sequences, which were deposited in GenBank under the accession no. DQ522300. The sequences were different from all the homologous sequences for bartonellae previously stored in GenBank but closest to those of *Bartonella* sp. SE-Bart-B detected in *Xenopsylla cheopis* fleas from Egypt (GenBank accession no. DQ166942; sharing 96% identities) and those of *Bartonella claridgeiae* detected in *C. felis* fleas from some countries of Eurasia (GenBank accession no. AF014831; sharing 94% identities). Bartonellae have never been reported earlier in *P. irritans* from Europe, and almost no information is available on the infection rate of this flea species on other continents (Table 1). From Gabon and Peru, *B. quintana* and three novel *Bartonella* genotypes were recently detected in *Pulex* fleas (Parola et al. 2002, Rolain et al. 2005). The medical importance of the four new genotypes of Bartonella detected in human fleas from Hungary and Peru has yet to be clarified. Nevertheless, several previously unrecognised bacterial pathogens belonging to the genus *Bartonella* were recently described from ectoparasites including fleas (Greb and...
Raoult 2002, Jacomo et al. 2002, Boulouis et al. 2005, Chomel et al. 2006). As *P. irritans* is a particularly ‘anthrophilic’ flea species, further studies are needed on the transmission potential of this flea species. Although bartonellae have been reported in *C. canis* in Japan (Ishida et al. 2001), no *Bartonella*-specific amplification products were detected in *C. canis* pools from Hungary. Of the 10 pools of *C. felis*, *Bartonella* DNA was detected in two pools coming from two cats. As there were two positive pools, there must have been at least two infected fleas among the 50 investigated, giving a minimum prevalence of 4.0% in *C. felis*. The *groEL* sequences of the two isolates were identical with those of the causative agents of cat scratch disease, *B. henselae* and *B. clarridgeiae*, respectively (GenBank accession nos. AF304019 and AF014831). The *pap31* sequences of *B. henselae* isolate amplified from Hungarian fleas were identical with that of Mar- seille strain (GenBank accession no. AF308169). Of *Bartonella* spp. with known pathogenicity, *B. clarridgeiae*, *B. henselae*, *B. quintana* and *Bartonella koehlerae* were detected in *C. felis* from some other countries of Eurasia, New Zealand and the United States (Chomel et al. 1996, Ishida et al. 2001, Parola et al. 2003, Rolain et al. 2003, Kelly et al. 2004, Shaw et al. 2004, Lappin et al. 2004, La Scola et al. 2004, Shaw et al. 2004, Halos et al. 2005, Kim et al. 2005, Rolain et al. 2005, Kim et al. 2005, Rollin et al. 2005, Holden et al. 2006, Lappin et al. 2006, Lofis et al. 2006, this study). **Prevalence was not determined. ***As-yet-unnamed *Bartonella* genotypes with unknown pathogenicity. ****Bartonellae were not identified at species level.

The role of ixodid ticks and the majority of flea species in the direct transmission of bartonellae is not fully confirmed (Chomel et al. 1996, Eskow et al. 2001, Chomel and Boulouis 2006); nevertheless, the present and other data (Table 1) raise the possibility that several flea and tick species might play a role in the epidemiology of bartonelloses in Eurasia and America. Therefore, studies on the distribution of bartonelloses in fleas and ticks and on the transmission potential of these parasites are encouraged. Recently reported seroprevalences in man (1–62%) indicate that *Bartonella* infections are frequent in human populations in some regions of Eurasia and North America (Breitschwerdt and Kordick 2000, Chomel et al. 2006), and these bacteria are responsible for diverse and in many cases serious clinical manifestations in man (Breitschwerdt and Kordick 2000, Jacomo et al. 2002, Chomel et al. 2006). Considering the relatively high prevalence of various bartonellae in ticks and fleas (Table 1) and the high infection rate of these ectoparasites with several other emerging bacterial pathogens in Hungary and other European countries, clinicians need to be more aware of, and more familiar with, the clinical manifestations of flea- and tick-borne infections including bartonelloses, the best methods for their laboratory confirmation, and the appropriate therapy (Rolain et al. 2004, Boulouis et al. 2005). As the pathogenicity of bartonelloses was recently demonstrated in dogs, and the seroprevalences vary between 1% and 92% in various mammals (Breitschwerdt and Kordick 2000, Chomel et al. 2006), further studies are needed on the veterinary significance of these bacteria.

### Table 1. Current knowledge on the prevalence and geographical distribution of *Bartonella* infections in fleas and ticks.*

<table>
<thead>
<tr>
<th>Flea or tick vector</th>
<th>Bartonella spp. detected in the vector</th>
<th>Recorded prevalence (%) in vector</th>
<th>Known geographical distribution of bartonelloses in vector</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ctenocephalides felis</td>
<td><em>B. henselae, B. clarridgeiae,</em></td>
<td>4–34 19</td>
<td>France, Hungary, Japan, New Zealand, Thailand, United Kingdom, United States (Alabama, Maryland, Texas)</td>
</tr>
<tr>
<td></td>
<td><em>B. quintana, B. koehlerae</em></td>
<td></td>
<td>Japan</td>
</tr>
<tr>
<td>Ctenocephalides canis</td>
<td><em>B. henselae</em></td>
<td>– ND** 7</td>
<td>Gabon**, Hungary, Peru</td>
</tr>
<tr>
<td>Pulex irritans/simulans</td>
<td><em>B. quintana,</em> Bartonella spp.***</td>
<td>4–10 3</td>
<td>Egypt</td>
</tr>
<tr>
<td>Xenopsylla cheopis</td>
<td>Bartonella spp.***</td>
<td>– 4</td>
<td>Thailand</td>
</tr>
<tr>
<td>Leptopsylla segnis</td>
<td>Bartonella spp.***</td>
<td>– 7</td>
<td>United States (Colorado)</td>
</tr>
<tr>
<td>Nosopsylla fasciatus</td>
<td>Bartonella spp.***</td>
<td>– 16</td>
<td>France, Italy, The Netherlands</td>
</tr>
<tr>
<td>Oropsylla hirsuta</td>
<td>Bartonella spp.***</td>
<td>2–13 7</td>
<td>Korea**, Russia (Siberia)</td>
</tr>
<tr>
<td>Ixodes ricinus</td>
<td><em>B. henselae, N</em>****</td>
<td>0–77 11</td>
<td>Korea</td>
</tr>
<tr>
<td>Ixodes persulcatus</td>
<td><em>B. henselae, B. quintana</em></td>
<td>– 38</td>
<td>Korea</td>
</tr>
<tr>
<td>Ixodes turdus</td>
<td>NI*****</td>
<td>– 11</td>
<td>Korea</td>
</tr>
<tr>
<td>Ixodes nipponensis</td>
<td>NI*****</td>
<td>– 5</td>
<td>Korea</td>
</tr>
<tr>
<td>Ixodes scapularis</td>
<td><em>B. henselae</em></td>
<td>– 35</td>
<td>United States (New Jersey)</td>
</tr>
<tr>
<td>Ixodes pacificus</td>
<td><em>B. henselae</em></td>
<td>2–19 9</td>
<td>United States (California)</td>
</tr>
<tr>
<td>Dermacentor reticulatus</td>
<td><em>B. henselae, B. quintana</em></td>
<td>0–21 10</td>
<td>Russia (Siberia)</td>
</tr>
<tr>
<td>Dermacentor variabilis</td>
<td>NI*****</td>
<td>– 14</td>
<td>United States (California)</td>
</tr>
<tr>
<td>Dermacentor occidentalis</td>
<td>NI*****</td>
<td>– 3</td>
<td>United States (California)</td>
</tr>
<tr>
<td>Haemaphysalis longicornis</td>
<td>Bartonella spp.***</td>
<td>– 4</td>
<td>Korea</td>
</tr>
<tr>
<td>Haemaphysalis flavus</td>
<td>NI*****</td>
<td>– 3</td>
<td>Korea</td>
</tr>
</tbody>
</table>

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