Molecular Detection of Bartonella alsatica in Rabbit Fleas, France

To the Editor: Bartonella alsatica was first isolated from the blood of wild rabbits from the Alsace region in France (1). This bacterium is now considered an emerging infectious disease zoonotic agent in persons in close contact with rabbits; at least 2 human cases of endocarditis and 1 human case of lymphadenitis have been reported (2–4). In this study, we report the molecular detection of B. alsatica in fleas (Spilopsyllus cuniculi) collected from rabbits in southern France.

During January and February 2008, a total of 60 fleas were collected from wild rabbits (Oryctolagus cuniculus) from 3 regions in southern France: Canohes (42°38′N, 2°51′E), Pollestres (42°38′N, 2°52′E), and Toreilles (42°45′N, 2°58′E). The fleas were collected and identified phenotypically, kept in ethanol, and sent to Unité de Recherche sur les Maladies Infectieuses et Tropicales Emergentes in September 2009.

DNA from these fleas, as well as negative controls from uninfected lice maintained as colonies in our laboratory, were extracted by using a QIAmp Tissue Kit (QIAGEN, Hilden, Germany), as described (5). Identification of flea species at the molecular level was achieved by PCR amplification and sequencing of partial siphonapteran 18S rDNA gene (1.95 kbp) as described (5). Sequences were assembled in Sequencher 4.2 (GeneCodes Corporation, Ann Arbor, MI, USA). DNA was used as templates in a real-time quantitative PCR specific for a portion of the Bartonella genus 16S–23S intergenic spacer (ITS) performed in a Smart cycler instrument (Cepheid, Sunnyvale, CA, USA), as described (2). Positive samples at the genus level were confirmed by PCR amplification and sequencing of the Bartonella ITS region, as described (2). Finally, B. alsatica amplification and specific identification was confirmed by using 2 new specific PCRs with primers and TaqMan probes (Applied Biosystems, Courtaboeuf, France) specific for a portion of the heat shock protein 60 (hsp60) and the DNA gyrase subunit B (gyrB) genes of B. alsatica (Table). Specificity of these 2 PCRs was verified in silico (computer simulation) and by using a panel of 14 Bartonella species available in our laboratory (data not shown).

All fleas were morphologically identified as S. cuniculi by using current taxonomic criteria (6). Moreover, the 18S rRNA gene amplified and sequenced as described (6) from fleas gave a sequence with 100% similarity with the sequence of S. cuniculi fleas deposited in GenBank (accession no. EU336097). B. alsatica was detected by ITS reverse transcription–PCR in 8 (13.3%) of 60 fleas: 6 from Toreilles (17.6%, 6/34) region, 2 from Canohes (10.5%; 2/19), and none from Pollestres (0/7). Sequences obtained after PCR amplification and sequencing of partial ITS showed 96.6% identity with B. alsatica (GenBank accession no. HM060955). Using our 2 new PCRs specific for partial hsp60 and gyrB genes from B. alsatica, we identified all Bartonella spp.–positive fleas, which had cycle threshold values ranging from 12.15 to <32.35 and 13.21 to <36.99 for hsp60 and gyrB genes, respectively.

We report the specific detection of B. alsatica in S. cuniculi rabbit fleas from southern France using 4 different PCRs and sequencing, including 2 new reverse transcription PCRs described in this study. There is 1 report of molecular detection of B. alsatica from S. cuniculi fleas from a European wildcat (Felis silvestris silvestris) in Andalusia, Spain (7). Although S. cuniculi fleas are rare on cats, this study demonstrates that cats in contact with rabbits may be infected by these fleas and consequently become a potential source for B. alsatica transmission to humans. Márquez has also recently reported the molecular detection of B. alsatica in blood from 48/279 (17.2%) of wild rabbits (O. cuniculus) in Andalusia, Spain (8).

In conclusion, further research is needed to better understand the mode of transmission of B. alsatica in humans and mammals and the role of rabbit fleas for potential transmission for these bacteria. The recent description of B. alsatica as a human pathogen and the discovery of rabbit fleas as a potential vector reemphasize the emergence potential of this bacterium in humans who have close contact with rabbits.

Table. Oligonucleotide primers and TaqMan* fluorescent probe sequences of hsp60 and gyrB genes used for reverse transcription PCRs of Bartonella alsatica†

<table>
<thead>
<tr>
<th>Gene</th>
<th>Oligonucleotide</th>
<th>Sequence (5′→3′)</th>
<th>Length, bp</th>
<th>Amplicon size, bp</th>
</tr>
</thead>
<tbody>
<tr>
<td>hsp60</td>
<td>B_alsa_hsp60_F</td>
<td>TGCTAAGCTCTAGGAAAAGTGT</td>
<td>23</td>
<td>108</td>
</tr>
<tr>
<td></td>
<td>B_alsa_hsp60_R</td>
<td>CCAGATCAGACGTCCGTTCC</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td></td>
<td>B_alsa_hsp60_P</td>
<td>6FAM-TGTCGAAGAGCGAAACGGCTGACAAAC-TAMRA</td>
<td>28</td>
<td></td>
</tr>
<tr>
<td>gyrB</td>
<td>B_alsa_gyrB_F</td>
<td>CGAAGCAAACTTCTTATTAGTAAGGT</td>
<td>27</td>
<td>126</td>
</tr>
<tr>
<td></td>
<td>B_alsa_gyrB_R</td>
<td>GCAAGGTTTCTGCGGAG</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td></td>
<td>B_alsa_gyrB_P</td>
<td>6FAM-ATAGAGGCTGCTGGCGCGCG-TAMRA</td>
<td>20</td>
<td></td>
</tr>
</tbody>
</table>

*Applied Biosystems, Courtaboeuf, France.
†hsp60, heat shock protein 60; gyrB, DNA gyrase subunit B.
Acknowledgments

We are grateful to Jean-Claude Beau-cournu for help in flea identification. We also thank Juline Collin and Oleg Medianikov for their technical assistance.

Tahar Kernif, Philippe Parola, Jean-Claude Ricci, Didier Raoult, and Jean-Marc Rolain

Author affiliations: Université de la Méditer-
ranée, Marseille, France; email: jean-marc.
rolain@univmed.fr

References


French patient in close contact with rab-
DOI: 10.1111/j.1469-0691.2008.02187.x

4. Angelakis E, Lepidi H, Canel A, Rispal
Pouedras P, Fournier PE, Raoult D, et
al. First isolation of Bartonella alsatica in
In press. DOI: 10.1089/vbz.2009.0135

5. Márquez FJ. Molecular detection of Bar-
tonella alsatica in European wild rabbits
In press. DOI: 10.1089/vbz.2009.0135

Address for correspondence: Jean-Marc Rolain,
Université de la Méditerranée, URMI TE UMR
6236 CNRS-IRD, Faculté de Médecine et de
Pharmacie, Marseille, France; email: jean-marc.
rolain@univmed.fr

-----

Cutaneous Myiasis Caused by
Chrysomya bezziana
Larvae, Mexico

To the Editor: We report a case of cutaneous myiasis caused by Chry-
somya bezziana larvae in a 62-year-old
woman who had a complex vascular
cutaneous anomaly in her lower right
extremity for 8 years. On physical ex-
amination, in September 2009, she had
a nonlimping walk with pink and pain-
ful feet and an ulcerative lesion on the
internal surface of the right leg above
the internal malleolus. This ulcer was
large, clean, without evidence of in-
fec tion, and had tissue in the process of
granulation. Adjacent to the upper
edge of this lesion, we observed a sec-
don, crater-like ulcer ≈2.5 cm in diam-
eter from which drained an abundant,
highly purulent, serohematic material
(Figure, panel A).

Approximately 10 days earlier,
the patient had detected dis charge of
worms from the second lesion, moti-
vating her to seek medical consulta-
tion. We performed surgical cleaning
and manual removal of worms (Fig-
ure, panel B) and referred the patient
external consultation to control
vascular, metabolic, and parasitologic
and for instruction in proper
hygiene. The worms were identified as
C. bezziana larvae by the Parasitology
Laboratory of the Microbiology and
Parasitology Department, Faculty of
Medicine, National Autonomous Uni-
versity of Mexico.

Myiasis, a zoonotic disease, is
defined as invasion of human living
tissue by eggs or larvae from flies of
the order Diptera. Among the diverse
types of human myiasis that can occur
in tropical regions, those in skin tis-
sue are the most frequent, especially
those generated by flies of the family
Calliphoridae, of which the predomi-
nant species are Cordylobia anthropo-
phaga (tumbu fly); C. bezziana, and
Oestrus ovis in Africa (1) and Derma-
tobia hominis (American warble fly)
in Central and South America.

Myiases have become increasingly
relevant, particularly when human
activity is carried out in environments
with poor hygiene or in close prox-
imity to domestic and peridomestic
animals, such as dogs and rats (2). Hu-
man myiases generally are present in
cavities or wounds but also can affect
tissue, such as the skin, eyes, oral cav-
ity, intestines, or urogenital area. C.
bezziana larvae can usually be found
infecting wounds or cutaneous ulcers
but are occasionally found in normal
skin (3–5).

Tegumentary and exposed-cavity
myiases are relatively easy to diag-
nose because the source larvae can be
observed directly. As a result of the
taxonomic study of the larvae based on
their morphologic characteristics (6),
we searched the Medline, PubMed,
Scielo, and Lilacs databases for artic-
les describing myiasis caused by the
identified species. The published lit-
erature showed that no prior cases had
been documented in Mexico, and only
a few cases had been documented in
other regions of North America.

Old World flies, such as C. bezz-
iana and O. ovis, are the most im-
portant producers of myiasis from an
economic perspective (7). The larvae
feed on living tissue causing highly
traumatic lesions in a great variety of
warm-blooded animals. These