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 *gyr*B 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 *hsp*60 and *gyr*B genes used for reverse transcription PCRs of *Bartonella alsatica*†

Gene	Oligonucleotide	Sequence (5′ → 3′)	Length, bp	Amplicon size, bp
hsp60	B_alsa_hsp60_F	TGCTAACGCTATGGAAAAAGTTG	23	108
	B_alsa_hsp60_R	CCACGATCAAACTGCATTCC	20	
	B_alsa_hsp60_P	6FAM-TGTCGAAGAAGCAAAAACGGCTGAAACC-TAMRA	28	
gyrB	B_alsa_gyrB_F	CGAAGCAAAACTTCTTATTAGTAAGGT	27	126
	B_alsa_gyrB_R	GCAAGCTTTCCTGGCAGAG	19	
	B_alsa_gyrB_P	6FAM-ATAGAGGCTGCTGCGGCGCG-TAMRA	20	

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

Acknowledgments

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

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

Author affiliations: Université de la Méditerranée, Marseille, France (T. Kernif, P. Parola, D. Raoult, J.-M. Rolain); and Institut Méditerranéen du Patrimoine Cynégétique et Faunistique, Vergèze, France (J.-C. Ricci)

DOI: 10.3201/eid1612.100696

References

1. Heller R, Kubina M, Mariet P, Riegel P, Delacour G, Dehio C, et al. *Bartonella alsatica* sp. nov., a new *Bartonella* species isolated from the blood of wild rabbit. Int J Syst Bacteriol. 1999;49:283–8. DOI: 10.1099/00207713-49-1-283
2. Raoult D, Roblot F, Rolain J-M, Besnier J-M, Loulergue J, Bastides F, et al. First isolation of *Bartonella alsatica* from a valve of a patient with endocarditis. J Clin Microbiol. 2006;44:278–9. DOI: 10.1128/JCM.44.1.278-279.2006
3. Jeanclaude D, Godmer P, Leveiller D, Pouedras P, Fournier PE, Raoult D, et al. *Bartonella alsatica* endocarditis in a French patient in close contact with rabbits. Clin Microbiol Infect. 2009;15:110–1. DOI: 10.1111/j.1469-0691.2008.02187.x
4. Angelakis E, Lepidi H, Canel A, Rispal P, Perradeau F, Barre I, et al. Human case of *Bartonella alsatica* lymphadenitis. Emerg Infect Dis. 2008;14:1951–3. DOI: 10.3201/eid1412.080757
5. Whiting MF. Mecoptera is paraphyletic: multiple genes and phylogeny of Mecoptera and Siphonaptera. Zool Scr. 2002;31:93–104. DOI: 10.1046/j.0300-3256.2001.00095.x
6. Bitam I, Parola P, Dittmar DL, Matsumoto K, Belkacem B, Rolain J-M, et al. First molecular detection of *Rickettsia felis* in fleas from Algeria. Am J Trop Med Hyg. 2006;74:532–5.
7. Márquez FJ, Millán J, Rodríguez-Liébana JJ, García-Egea I, Muniain MA. Detection and identification of *Bartonella* sp. in fleas from carnivorous mammals in Andalusia, Spain. Med Vet Entomol. 2009;23:393–8. DOI: 10.1111/j.1365-2915.2009.00830.x
8. Márquez FJ. Molecular detection of *Bartonella alsatica* in European wild rabbits (*Oryctolagus cuniculus*) in Andalusia (Spain). Vector Borne Zoonotic Dis. 2010. In press. DOI: 10.1089/vbz.2009.0135

Address for correspondence: Jean-Marc Rolain, Université de la Méditerranée, URMITE 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 *Chrysomya 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 examination, in September 2009, she had a nonlimping walk with pink and painful 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 infection, and had tissue in the process of granulation. Adjacent to the upper edge of this lesion, we observed a second, crater-like ulcer ≈2.5 cm in diameter from which drained an abundant, highly purulent, serohematic material (Figure, panel A).

Approximately 10 days earlier, the patient had detected discharge of worms from the second lesion, motivating her to seek medical consultation. We performed surgical cleaning and manual removal of worms (Figure, panel B) and referred the patient for external consultation to control vascular, metabolic, and parasitologic evolution 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 University 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 tissue are the most frequent, especially those generated by flies of the family *Calliphoridae*, of which the predominant species are *Cordylobia anthropophaga* (tumbu fly); *C. bezziana*, and *Oestrus ovis* in Africa (*1*) and *Dermatobia 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 proximity to domestic and peridomestic animals, such as dogs and rats (*2*). Human myiases generally are present in cavities or wounds but also can affect tissue, such as the skin, eyes, oral cavity, 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 diagnose 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 articles describing myiasis caused by the identified species. The published literature 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. bezziana* and *O. ovis*, are the most important 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