Candidatus
Bartonella mayotimonensis
and Endocarditis

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We describe a new Bartonella species for which we propose the name Candidatus Bartonella mayotimonensis. It was isolated from native aortic valve tissue of a person with infective endocarditis. The new species was identified by using PCR amplification and sequencing of 5 genes (16S rRNA gene,ftsZ, rpoB, gltA, and internal transcribed spacer region).

Bartonella species are small, fastidious, gram-negative, intracellular bacteria that cause culture-negative infective endocarditis. Six species have been documented to cause endocarditis in humans: B. quintana (1), B. henselae (2), B. elizabethae (3), B. vinsonii subsp. berkhoffii (4), B. koehlerae (5), and B. altsatica (6). We report a case of culture-negative endocarditis caused by a new Bartonella species, for which we propose the name Candidatus Bartonella mayotimonensis.

The Patient

A 59-year-old man was initially hospitalized at Sartori Memorial Hospital (Cedar Falls, IA, USA) from April 14 through 19, 2008, for progressive shortness of breath, weight loss, fatigue, and altered mental status. He was then transferred to the Mayo Clinic (Rochester, MN, USA). Physical examination identified a new diastolic heart murmur. He was afebrile and did not have peripheral stigmata of endocarditis. Two sets of blood cultures obtained before antimicrobial drug therapy showed negative results for all bacteria tested after 5 days of incubation. A transesophageal echocardiogram showed a bicuspid aortic valve, mobile components on the left cusp of the aortic valve suggesting vegetations, and a 5.3-cm ascending aortic aneurysm. Empiric antimicrobial drug therapy, including vancomycin and ceftriaxone, was initiated. Subsequently, acute renal dysfunction, possibly secondary to vancomycin exposure, developed in the patient.

The patient lived alone on a farm in Iowa, USA, and had not had recent exposure to animals. However, he had observed murine fecal droppings in his house and mice on the farm. He had had a house cat for 18 years until its death a few years before his hospitalization and had intermittent contact with cats when he visited his daughter. Serum immunoglobulin G titers were positive for B. henselae and B. quintana (≥1.024). Oral doxycycline and rifampin were prescribed for treatment of presumed Bartonella endocarditis. Gentamicin was not administered because of development of the acute renal dysfunction. Two weeks later, he underwent aortic valve and aortic root replacement. Results of gram staining, acid-fast staining, fungal staining, anaerobic bacterial culture, aerobic bacterial culture, mycobacterial culture, and fungal culture on resected aortic valve tissue were negative for Bartonella species.

PCR performed at the Mayo Clinic on resected aortic valve tissue detected part of the citrate synthase gene (gltA) of Bartonella species. However, the melting temperature was not characteristic of B. quintana or B. henselae (7). Oral doxycycline and rifampin were continued for 12 weeks after aortic valve resection. The patient was well and had no signs of relapsing infection at a follow-up visit 11 months after valve surgery.

Aortic valve tissue and serum were tested at the Unité des Rickettsies, Marseille, France. B. quintana Oklahoma, B. henselae Houston (ATCC 49882), B. vinsonii subsp. berkhoffii (URBVAIE25), B. vinsonii subsp. arupensis (ATCC 700727), and B. altsatica (CIP 105477 T) strains were used for immunofluorescent assays and Western blotting (6). Valve tissue was injected into human endothelial cells in a shell vial assay and onto Columbia 5% sheep blood agar plates and incubated at 37°C in an atmosphere of 5% CO2, as described (6).

A Bartonella species was detected in a shell vial by immunofluorescence after 15 days of culture; identification was confirmed by PCR. DNA was extracted from valve specimen and injected cells by using the QIAamp Tissue Kit (QIAGEN, Hilden, Germany). DNA was used as a template in a genus Bartonella Lightcycler assay with primers and a Taqman probe specific for the internal transcribed spacer (ITS) gene (6) and in standard PCR assays specific for the 16S rRNA, ITS, rpoB, gltA, and ftsZ genes (8). Sequences from both DNA strands were determined twice for all PCR products. These products were resolved in an ABI 3100 automated sequencer (PerkinElmer, Waltham, MA, USA). Sterile water was used as a negative control in each assay. Percentages of similarity among sequences were determined by using MEGA 2.1 software (9). Phylogenetic relationships among Bartonella strains were inferred from concatenated sequences by using MEGA 2.1 software (9).

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Surgically resected aortic valve tissues were fixed in formalin, embedded in paraffin, and sectioned to a thickness of 5 μm. Sections were stained with periodic acid–Schiff, Giemsa, Gram, Grocott-Gomori methenamine silver, and Warthin-Starry stains. Immunohistochemical analysis was performed by using a procedure described elsewhere (10) and polyclonal antibody against *B. vinsonii* at a dilution of 1:1,000.

Serum samples showed immunoglobulin G endpoint titers of 50 against all *Bartonella* species tested by immunofluorescent assay. Western blot results were positive and characteristic of *Bartonella* infection (Figure 1, panel A). Results of PCR (*Bartonella* genus Lightcycler assay and standard PCR for cardiac valve) and cell culture were positive, and amplification products of the expected size were obtained. Among known validated species, sequences obtained shared 99.1% (1,438/1,445 bp) homology with *B. tribocorum*, *B. henselae*, and *B. vinsonii* for the 16S rRNA gene, 89.5% homology with *B. grahamii* for the ITS gene, 93.4% homology with *B. vinsonii* subsp. *berkhoffii* for *rpoB*, 91.7% homology with *B. vinsonii* subsp. *berkhoffii* for *ftsZ*, and 92.5% homology with *B. vinsonii* strain Baker for *gltA*. The phylogenetic position of *Candidatus* *B. mayotimonensis* among members of the genus *Bartonella* based on comparisons of concatenated sequences of the 5 genes is shown in Figure 2. Sequences of *gltA*, 16S rDNA, *ftsZ*, ITS, and *rpoB* were deposited in GenBank under accession nos. FJ376732–FJ376736.

Histologic analysis of resected aortic valve showed infective endocarditis with vegetation containing microorganisms that stained with Warthin-Starry and Giemsa. Warthin-Starry staining showed darkly stained bacilli consistent with *Bartonella* species (Figure 1, panel B). Results of staining with periodic acid–Schiff, Gram, and Grocott-
method for diagnosis of bacterium. To determine the reservoir(s) and vector(s) for this novel visiting his daughter. Additional investigations are needed his farm and also had intermittent contact with cats while (502 Emerging Infectious Diseases • www.cdc.gov/eid • Vol. 16, No. 3, March 2010

voles (5). Other species have been found in mam-
malian hosts, including rats (5), dogs and coyotes (5), cats (5), humans (5), moles (5), voles (5), cows (5), deer (5), and rabbits (5) (3–6,12,14,15). Our patient had direct exposure to mice on his farm and also had intermittent contact with cats while visiting his daughter. Additional investigations are needed to determine the reservoir(s) and vector(s) for this novel bacterium.

The immunofluorescent assay, the current serologic method for diagnosis of Bartonella infection, does not distinguish among Bartonella species. Only Western blot analysis and cross-adsorption enable serologic identification of species. PCR and culture are critical when a Bartonella species is identified for the first time as a human pathogen. Newly encountered Bartonella strains should be considered a new species if a 327-bp gltA fragment shares <96.0% sequence similarity with those of validated species, and if an 825-bp rpoB fragment shares <95.4% sequence similarity with those of validated species as reported in the current case (8).

This case reinforces the hypothesis that any Bartonella species can cause human infection, including culture-negative endocarditis. Candidatus B. mayotimonensis should be added to the list of human pathogens that can cause culture-negative endocarditis.

Dr. Lin was an internal medicine resident at the Mayo Clinic in Rochester, Minnesota, when the patient’s condition was investigated. She is currently an endocrinology fellow at Massachusetts General Hospital in Boston, Massachusetts. Her research interest is neuroendocrinology.

References
1. Drancourt M, Mainardi JL, Brouqui P, Vandenesch F, Carta A, Leh-
nert F, et al. Bartonella (Rochalimaea) quintana endocarditis in three homeless men. N Engl J Med. 1995;332:419–23. DOI: 10.1056/NEJM199502163320702
2. Holmes AH, Greenough TC, Balady GJ, Regnery RL, Anderson BE, O’Keane JC, et al. Bartonella henselae endocarditis in an immunocompetent adult. Clin Infect Dis. 1995;21:1004–7.
3. Daly JS, Worthington MG, Brenner DJ, Moss CW, Hollis DG, Wey-
ant RS, et al. Rochalimaea riceae sp. nov. isolated from a patient with endocarditis. J Clin Microbiol. 1993;31:872–81.
4. Roux V, Eykyn SJ, Wylie S, Raoult D. Bartonella vinsonii subsp. berkoffi as an agent of afebrile blood culture-negative endocarditis in a human. J Clin Microbiol. 2000;38:1698–700.
5. Avidor B, Graidy M, Efrat G, Leibowitz C, Shapira G, Schattner A, et al. Bartonella koehlerae, a new cat-associated agent of culture-negative human endocarditis. J Clin Microbiol. 2004;42:3462–8. DOI: 10.1128/JCM.42.8.3462-3468.2004
6. Raoult D, Roblot F, Rolain JM, Besnier JM, Loulergue J, Bastides F, et al. First isolation of Bartonella alstacca from a valve of a patient with endocarditis. J Clin Microbiol. 2006;44:278–9. DOI: 10.1128/JCM.44.1.278-279.2006
7. Vikram HR, Bacani AK, DeValeria PA, Cunningham SA, Cockerill FR III. Bivalvulur Bartonella henselae prosthetic valve endocarditis. J Clin Microbiol. 2007;45:4081–4. DOI: 10.1128/JCM.01095-07
8. La Scola B, Zaiter Z, Khamis A, Raoult D. Gene-sequence-based criteria for species definition in bacteriology: the Bartonella para-
digm. Trends Microbiol. 2003;11:318–21. DOI: 10.1016/S0966-
842X(03)00143-4
9. Kumar S, Tamura K, Jakobsen IB, Nei M. MEGAZ2: molecular evolu-
tionary genetics analysis software. Bioinformatics. 2001;17:1244–5. DOI: 10.1093/bioinformatics/17.12.1244
10. Lepidi H, Fournier PE, Raoult D. Quantitative analysis of val-
ular lesions during Bartonella endocarditis. Am J Clin Pathol. 2006;114:880–9. DOI: 10.1309/R0KQ-823A-BTC7-MUUJ

Figure 2. Phylogenetic tree showing the position of Candidatus Bartonella mayotimonensis among members of the genus Bartonella based on comparisons of concatenated sequences of the 16S rRNA gene, the citrate synthase gene gltA, the RNA polymerase β-subunit gene rpoB, the cell division gene ftsZ, and the 16S–23S rRNA internal transcribed spacer region sequences. The tree was constructed by using the neighbor-joining method and a maximum-likelihood–based distance algorithm. Numbers on branches indicate bootstrap values derived from 500 replications.
11. La VD, Tran-Hung L, Aboudharam G, Raoult D, Drancourt M. *Bartonella* quinquana in domestic cat. Emerg Infect Dis. 2005;11:1287–9.

12. Rolain JM, Brouqui P, Koehler JE, Maguina C, Dolan MJ, Raoult D. Recommendations for treatment of human infections caused by *Bartonella* species. Antimicrob Agents Chemother. 2004;48:1921–33. DOI: 10.1128/AAC.48.6.1921-1933.2004

13. Eremeeva ME, Gerns HL, Lydy SL, Goo JS, Ryan ET, Mathew SS, et al. Bacteremia, fever, and splenomegaly caused by a newly recognized *Bartonella* species. N Engl J Med. 2007;356:2381–7. DOI: 10.1056/NEJMoa065987

14. Breitschwerdt EB, Kordick DL. *Bartonella* infection in animals: carriership, reservoir potential, pathogenicity, and zoonotic potential for human infection. Clin Microbiol Rev. 2000;13:428–38. DOI: 10.1128/CMR.13.3.428-438.2000

15. Chomel BB, Boulouis HJ, Maruyama S, Breitschwerdt EB. *Bartonella* spp. in pets and effect on human health. Emerg Infect Dis. 2006;12:389–94.

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