

Isolation of *Bartonella quintana* from a Woman and a Cat following Putative Bite Transmission[∇]

Edward B. Breitschwerdt,^{1*} Ricardo G. Maggi,¹ Betsy Sigmon,¹ and William L. Nicholson²

Intracellular Pathogens Research Laboratory and the Center for Comparative Medicine and Translational Research, Department of Clinical Sciences, College of Veterinary Medicine, North Carolina State University, Raleigh, North Carolina,¹ and Viral and Rickettsial Zoonoses Branch, Centers for Disease Control and Prevention, Atlanta, Georgia²

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We report here the detection of *Bartonella quintana*, after putative bite transmission, in pre-enrichment blood cultures from a woman and from two feral barn cats. Prospective molecular epidemiological studies are necessary to characterize the risk of human *Bartonella quintana* infection following cat bites.

CASE REPORT

On 2 October 2004, a 43-year-old female Caucasian riding instructor was bitten on the left hand, adjacent to the thumb, by a feral barn cat at a local riding establishment in central North Carolina. The instructor had daily contact with cats, dogs, and horses for at least 10 years. The cat bite induced a severe, somewhat refractory cellulitis that required surgical debridement and treatment with three antibiotics (amoxicillin trihydrate-clavulanate for 48 h, followed by an injection of ceftriaxone sodium at an emergency care facility and oral cefdinir sodium for 10 days). In addition to near-daily exposure to cats, dogs, and horses, this person also reported frequent exposure (almost daily) to biting flies and mosquitoes and occasional exposure (approximately once a month) to fleas and ticks.

On October 23, the riding instructor's 8-year-old male German Shepherd was accidentally hit by a farm truck. While lifting the dog into the truck, the woman was bitten on the left wrist, resulting in an infection that was treated for 7 days with amoxicillin trihydrate-clavulanate, followed by levofloxacin for 7 days. Due to recent cat and dog bites, the possibility of *Bartonella* sp. transmission was pursued. Using a previously described approach (8, 9), *Bartonella quintana* DNA was detected by real-time PCR targeting the 16S-23S intergenic transcribed sequence (ITS) region from a pre-enrichment (*Bartonella* alpha-proteobacteria growth medium [BAPGM]) blood culture inoculated on 22 November 2004, 2 weeks after the cessation of antibiotic treatment. Subsequently, 16S-23S ITS and RpoB (RNA polymerase beta chain) amplicons were obtained from the BAPGM culture by conventional PCR for DNA sequencing. The *B. quintana* ITS sequence (GenBank accession no. DQ648598) was 99.8% (523 of 524 bp) similar to *B. quintana* strain Fuller (GenBank accession no. L35100). Subsequently, the RpoB gene sequence was found to be 99.2% (650 of 655 bp) similar to *B. quintana* strain Toulouse (GenBank accession no. BX897700). Serum, obtained on 16

December, was reactive to *B. quintana* (1:128), *B. vinsonii* subsp. *berkhoffii* (1:256), and *B. henselae* (1:64) antigens by immunofluorescence antibody (IFA) testing at the Centers for Disease Control and Prevention. Initial attempts to catch the two feral cats were not successful. A pre-enrichment blood culture from the woman's pet dog was negative (by DNA testing and subculture), and serum *Bartonella* antibodies were not detected by IFA testing.

In January 2005, the two feral cats from the riding establishment, including the previously mentioned cat that induced the bite wound, were captured and adopted by the riding instructor. The serum *B. henselae* IFA reciprocal titers were 4,096 and 1,024, respectively. *Bartonella* DNA was not amplified from either cat's blood sample; however, using an identical pre-enrichment culture approach, as was used for the human blood sample, *Bartonella* DNA (genus-specific primers) was amplified from both cat BAPGM culture samples. Using conventional PCR, an ITS amplicon was obtained from one cat and had a DNA sequence 99.8% similar (523 of 524 bp) to *B. quintana* strain Fuller (GenBank accession no. L35100). This ITS DNA sequence was also identical to the *B. quintana* sequence obtained from the riding instructor (GenBank accession no. DQ648598). Subsequently, the RpoB gene was amplified by conventional PCR from the pre-enrichment cultures from both cats. The two cat RpoB gene sequences were 100% homologous (655 of 655 bp) and differed by only 1 bp from the *B. quintana* RpoB sequence obtained from the riding instructor. Both RpoB sequences were 99.4% similar (651 of 655 bp) to *B. quintana* strain Toulouse (GenBank accession no. BX897700).

DNA was not detected in a blood sample from the riding instructor following direct extraction or from a pre-enrichment blood culture inoculated on 31 January. The IFA antibody titers were identical, except for *B. quintana* (1:64). Again, on 24 May 2005, *Bartonella* DNA was not detected in the blood or after pre-enrichment blood culture, and the *B. quintana*, *B. vinsonii* subsp. *berkhoffii*, and *B. henselae* reciprocal titers were 64, 128, and 32, respectively. The dog was retested and remained antibody negative and culture (PCR) negative. After clinical resolution of the cat and dog bite injuries, this individual has not experienced any systemic illnesses during an 18-month follow-up period.

* Corresponding author. Mailing address: Department of Clinical Sciences, College of Veterinary Medicine, North Carolina State University, 4700 Hillsborough St., Raleigh, NC 27606. Phone: (919) 513-8277. Fax: (919) 513-6336. E-mail: ed_breitschwerdt@ncsu.edu.

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The amplification of the RpoB gene was performed using the oligonucleotides RpoB 1615s (5'-ATYACYCATAARCG YCGTCTTTCTGCTCTTGG-3') and RpoB 2267as (5'-GGA TCTAAATCTTCYGTGACGRATAACAATACG-3') as forward and reverse primers, respectively. Amplification was performed in a 25- μ l final volume reaction mixture containing 12.5 μ l of Tak-Ex Premix (Fisher Scientific), 0.25 μ l of 30 μ M concentrations of each forward and reverse primer (IDT DNA Technology), and 12.5 μ l of DNA from each sample tested. Negative controls were prepared with 12.5 μ l of DNA from blood of a healthy dog or 12.5 μ l of DNA extracted from uninoculated BAPGM controls (when testing BAPGM pre-enrichment cultures). Positive controls were prepared by serial dilution (using dog blood DNA) of *B. henselae* DNA down to a 0.001-pg/ μ l final concentration. The PCR conditions for RpoB amplification were as follows: a single hot-start cycle at 95°C for 2 min, followed by 55 cycles of denaturing at 94°C for 15 s, annealing at 62°C for 15 s, and extension at 72°C for 18 s. Amplification was completed by an additional cycle at 72°C for 1 min, and the products were analyzed by 2% agarose gel electrophoresis with detection using ethidium bromide under UV light. Amplicons were sequenced to establish species and strain identification using a previously described methodology (8, 9).

As reviewed in several recent publications, numerous animals, including bovine, canine, feline, human, and rodent species, can serve as chronically infected reservoir hosts for various *Bartonella* species (2, 4). In addition to scratch and bite transmission, an increasing number of arthropod vectors, including biting flies, fleas, keds, lice, sand flies, and potentially ticks, have been implicated in the vector transmission of *Bartonella* species (3, 6).

Dog and cat bites are a frequent cause of emergency room presentations in the United States (14). This case report provides serological and molecular evidence supporting cat bite transmission of *B. quintana* to a human being. The riding instructor in the present study was bitten by both a feral cat and her pet dog within a 3-week period. There was no serological, molecular, or culture evidence to support *Bartonella* infection in the dog. Since this person reported frequent exposure to numerous animals and arthropods, the mode of *Bartonella* transmission was not definitively established. However, since *B. quintana* was subsequently isolated from both feral barn cats, cat bite transmission of *B. quintana* seems to represent the most plausible explanation. Based upon two subsequent negative blood culture results, *B. quintana* infection in the woman appeared to be immunologically self-limiting, and this individual has remained healthy during an 18-month follow-up period. Apparently, *B. quintana* infection was not eliminated by 26 consecutive days of antimicrobial therapy administered for bite-induced cellulitis. Since direct extraction from blood did not result in PCR amplification of *Bartonella* DNA, pre-enrichment culture (1 ml of blood inoculated into 9 ml of liquid medium) of viable organisms was necessary to increase bacterial numbers so as to allow for PCR detection and sequencing of *B. quintana* DNA from both the patient and the two cats. *B. quintana* infection, at least in the cat that induced

the bite, was presumably chronic since the cat isolate was obtained 3 months after the human isolate.

Based upon ITS and RpoB sequences, both the feral cats and the riding instructor were infected with *B. quintana*. Sequence analyses comparing one cat and the human *Bartonella* ITS amplicons obtained in the present study were 100% homologous, and these sequences were 99.8% homologous with *B. quintana* strain Fuller. Compared to *B. quintana* strain Toulouse, the human and cat RpoB gene sequences were 99.2 and 99.4% similar, respectively. Unfortunately, it was not possible to compare the RpoB sequences for the two cats and the riding instructor to that of the Fuller strain of *B. quintana* (which were closely related to Fuller by ITS sequencing) because no RpoB gene sequence is currently available for *B. quintana* strain Fuller in the GenBank database. Interestingly, all attempts to amplify *Bartonella* DNA from cat and human isolates using Pap31 primers were unsuccessful (9). This could suggest that the *B. quintana* strain described here does not contain a Pap31 bacteriophage-associated gene.

Diagnostically, cats are not routinely tested in our laboratory for *B. quintana* antibodies; however, a previous serosurvey of cats from Israel and North Carolina identified individual cats that were seroreactive only to *B. quintana* antigens (1). Prior to this report, four *Bartonella* species were isolated from cats, including *B. henselae*, *B. clarridgeae*, *B. bovis*, and *B. kohlerae* (2). Although *B. henselae* is the predominant cause of cat scratch disease, there is increasing evidence that cats may be involved in the transmission of *B. quintana* to humans. Recently, *B. quintana* DNA was amplified from cat dental pulp (7) and from cat fleas (*Ctenocephalides felis*) in France (12). In addition, there are a limited number of case reports that implicate cat contact and human *B. quintana* infection. Two middle-aged female patients with cat contact and chronic peripheral or mediastinal adenomegaly, respectively, were infected with *B. quintana* (5). Central nervous system infection with *B. quintana* was documented in two males from the southeastern United States, one of whom had received numerous scratches from a kitten several weeks prior to the onset of seizures (11). Based upon serology and PCR, *B. quintana* was implicated as the cause of seizures in an 11-year-old boy who had been scratched by a kitten (13).

Historically, *B. quintana* has been considered a specialist bacterium for which human beings were the only known reservoir host and the human body louse (*Pediculus humanus corporis*) was the only known vector. Recently, our laboratory isolated *B. quintana* from a cynomolgus monkey, and *B. quintana* DNA was amplified from the blood or heart valve of two dogs with endocarditis (10, 13a). It is possible that dogs, non-human primates, and cats may also serve as reservoir hosts for *B. quintana*. In addition, as is true for *B. henselae* transmission among cats, *Ctenocephalides felis* may be an unrecognized vector for *B. quintana* transmission among cats (5). The prior historical failure to isolate *B. quintana* from cats may reflect infrequent bacteremia or a lack of sensitivity of previously available culture techniques. *Bartonella* DNA and *Bartonella* antibodies were not detected on two occasions in the pet dog in the present study following the bite. Other than two dogs with *B. quintana* endocarditis, there are no reports of *B. quintana* infection in dogs, making dog-to-human bite transmission less likely (13a). Additional prospective studies are necessary

to characterize the risk of human *Bartonella* infection after a cat or dog bite and to determine whether these infections are always self-limiting.

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