Bartonella Species in Blood of Immunocompetent Persons with Animal and Arthropod Contact

Edward B. Breitschwerdt,* Ricardo G. Maggi,* Ashlee W. Duncan,* William L. Nicholson,† Barbara C. Hegarty,* and Christopher W. Woods‡

Using PCR in conjunction with pre-enrichment culture, we detected Bartonella henselae and B. vinsonii subsp. berkhoffii in the blood of 14 immunocompetent persons who had frequent animal contact and arthropod exposure.

Attempts to isolate Bartonella sp. from immunocompetent persons with serologic, pathologic, or molecular evidence of infection are often unsuccessful; several investigators have indicated that Bartonella isolation methods need to be improved (1–4). By combining PCR and pre-enrichment culture, we detected B. henselae and B. vinsonii subsp. berkhoffii infection in the blood of immunocompetent persons who had arthropod and occupational animal exposure.

The Study

From November 2004 through June 2005, blood and serum samples from 42 persons were tested, and 14 completed a questionnaire, approved by the North Carolina State University Institutional Review Board. Age, sex, animal contact, history of bites, environment, outdoor activity, arthropod contact, travel, and medical history were surveyed. Bacterial isolation, PCR amplification, and cloning were performed by using previously described methods (5–7). Each blood sample was tested by PCR after direct DNA extraction, pre-enrichment culture for at least 7 days, and subculture onto a blood agar plate (Figure). An un inoculated, pre-enrichment culture was processed simultaneously as a control. Methods used for DNA extraction and conventional and real-time PCR targeting of the Bartonella 16S-23S intergenic spacer (ITS) region and heme-binding protein (Pap31) gene have been described (7,8). Conventional PCR amplicons were cloned with the pGEM-T Easy Vector System (Promega, Madison, WI, USA); sequencing was performed by Davis Sequencing, Inc. (Davis, CA, USA). Sequences were aligned and compared with GenBank sequences with AlignX software (Vector NTI Suite 6.0 (InforMax, Inc., Bethesda, MD, USA) (7,8). B. vinsonii subsp. berkhoffii, B. henselae, and B. quintana antibodies were determined by using a modification of a previously described immunofluorescence antibody assay (IFA) procedure (9).

Study participants included 12 women and 2 men, ranging in age from 30 to 53 years; all of them reported occupational animal contact for >10 years (Table). Most had daily contact with cats (13 persons) and dogs (12 persons). All participants reported animal bites or scratches (primarily from cats) and arthropod exposure, including fleas, ticks, biting flies, mosquitoes, lice, mites, or chiggers. All participants reported intermittent or chronic clinical symptoms, including fatigue, arthralgia, myalgia, headache, memory loss, ataxia, and paresthesia (Table). Illness was most frequently mild to moderate in severity, with a waxing and waning course, and all but 2 persons could perform occupational activities. Of the 14 participants, 9 had been evaluated by a cardiologist, 8 each by an infectious disease physician or a neurologist, and 5 each by an internist or a rheumatologist. Eleven participants had received antimicrobial drugs.

When reciprocal titers of ≥64 were used, 8 persons were seroreactive to Bartonella antigens (online Appendix Table, available from www.cdc.gov/eid/content/13/6/938-appT.htm). B. henselae or B. vinsonii subsp. berkhoffii was detected or isolated from all 14 participants. At the time of initial testing, Bartonella DNA was amplified directly from 3 blood samples, from 7 pre-enrichment liquid cultures, and from 4 subculture isolates (Table). For 5 persons, results of PCR and culture of initial samples were negative. Overall, Bartonella DNA was amplified from 11 (28%) of 40 extracted blood samples, 13 (33%) of 40 pre-enrichment cultures, and 5 isolates. For 7 persons, B. henselae DNA was amplified at multiple time points. Bartonella DNA was never amplified from any PCR control or uninoculated culture control.

By using the ITS target region, 2 distinct B. henselae ITS and Pap31 strains were sequenced, B. henselae Hous-

![Diagram of sample processing and testing.](Image)
Table. Selected demographic, epidemiologic, and medical information reported by 14 immunocompetent persons infected with *Bartonella henselae* or *B. vinsonii* subsp. *berkhoffii*.

| Characteristic/symptom | Study participant no. | Total, \( N = 14 \) |
|------------------------|------------------------|----------------------|
|                         | 1 2 3 4 5 6 7 8 9 10 11 12 13 14 |                      |
| Sex                    | F F F M F F F M F F F M F M F |                      |
| Age, y                 | 51 30 48 44 53 50 32 33 48 53 52 39 52 44 32 |                      |
| State of residence     | NC NC NC CO VA CA NC VA CA CA CA CA VA MN |                      |
| Occupational exposure  | V VIA AHR V V CR VIA VIA VIA VIA VIA V WB WB |                      |
| Daily contact          | Y/Y Y/N N/Y Y/Y Y/Y Y/Y Y/Y Y/Y Y/Y Y/Y Y/Y Y/Y Y/Y Y/Y |                      |
| Contact with           | 2/1 3/3 4/4 4/4 3/3 2/3 3/3 2/2 4/4 2/4 3/1 3/2 NA/3 4/3 |                      |
| fleas/ticks†           | Cl Cl II II II Cl Cl Cl Cl II II Cl II Cl |                      |
| Self-health assessment‡|                       |                      |
| Fatigue                | + + - + + + + + + + + + + + | 13                    |
| Joint pain             | + + - + + + + + + + + + U | 10                    |
| Difficulty sleeping    | + + - - + - + + + + + - | 9                     |
| (insomnia)             |                       |                      |
| Muscle pain            | + + - - - U + + - + U + + + | 8                     |
| Difficulty remembering| + + - - + - + + + + - U | 8                     |
| Loss of               | + + + - + - + + + - U | 7                     |
| sensation or numbness  |                       |                      |
| Balance problems       | + + - + + + + + + + | 7                     |
| Headache               | + + - - + + + + + + U | 7                     |
| Tremors                | + + - - + + + + + + - - | 6                     |
| Irritability           | + - - - + + + + + + - | 6                     |
| Bowel or bladder       | + + - - + + + + + + + - | 6                     |
| dysfunction            |                       |                      |
| Eye pain               | + + - + + + + + + - - - | 5                     |
| Blurred vision         | + - - - + + + + + + - | 5                     |
| Sleepiness             | + - - - + + + + - - + | 5                     |
| Syncope or            | + + + - + + - - - - - | 5                     |
| fainting episodes      |                       |                      |
| Shortness of           | + + - + - + + + - - U | 5                     |
| breath                 |                       |                      |
| Muscle weakness        | + - - + + + + + + U | 5                     |

*F, female; M, male; NC, North Carolina; CO, Colorado; VA, Virginia; CA, California; MN, Minnesota; V, veterinarian; VIA, veterinary assistant; AHR, animal health researcher; CR, cattle rancher; WB, wildlife biologist; Y, yes; N, no, with respect to the study participant’s daily contact with dogs/cats; Cl, chronically ill; II, infrequently ill; +, yes; - , no; blank, no answer reported; U, unknown.
†Reported as frequencies and defined as follows: 1, daily; 2, infrequently (weekly); 3, occasionally (monthly); 4, almost never (yearly).
‡Self-health assessment: As part of the questionnaire, study participants were asked to rate their own health status: healthy, infrequently ill, or chronically ill.

Conclusions

Persistent human infection with *B. bacilliformis* and *B. quintana* has been previously documented, whereas infection with *B. henselae* (cat-scratch disease [CSD]) is generally considered self-limiting (1,2,10). Recently, *B. henselae* DNA was amplified from the blood of a child 4 months after CSD diagnosis (11). Our study indicates that *B. henselae* and *B. vinsonii* subsp. *berkhoffii* can induce occult infection in immunocompetent persons and that detection can be enhanced by combining PCR with pre-enrichment culture. Considering only the results from initial blood samples, PCR detected *Bartonella* DNA in 3 samples, all of which were subsequently PCR positive by subculture or enrich-

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ment culture. In samples from 5 persons, pre-enrichment was necessary, and in 5 other persons, sequential sampling was necessary to detect *Bartonella* infection. Intermittent bacteremia, as occurs in *B. henselae*–infected cats (12), antimicrobial drug administration, low bacterial copy numbers, and low inoculum volume (1 mL) may have contributed to intermittent detection or inability to isolate *Bartonella* spp. from some participant samples. Although our approach is an improvement over historical isolation approaches, our results emphasize ongoing limitations associated with the detection of *Bartonella* infection. Obtaining stable *Bartonella* subcultures (n = 5 in this study) has proven problematic for other specialized laboratories that routinely culture for *Bartonella* spp. (3,4). To our knowledge, the *B. vinsonii* subsp. *berkhoffii* type II isolate described in our study is the only type II human isolate reported to date (8). Various combinations of *B. henselae* and *B. vinsonii* subsp. *berkhoffii* strain types were detected in the same blood sample or sequential blood samples. The coexistence of *B. henselae* genetic variants has been described among primary patient isolates, which suggests that multiple genotypes may emerge within the same person (13).

Overall, 57% of persons tested were seroreactive to 1 or all 3 *Bartonella* test antigens. Previous reports from the United States identified a *B. henselae* seroprevalence of 3% in healthy blood donors and a cumulative seroprevalence of 7.1% to both *B. henselae* and *B. quintana* antigens in veterinary professionals (1). In this and other studies, serologic test results did not correlate with PCR amplification or isolation results. Antigenic variability among *B. henselae* test strains can cause false-negative IFA results in persons with suspected CSD. Also *B. henselae*, *B. quintana*, or *B. elizabethae* antibodies were not detected in some persons with DNA evidence of active infection (1,3,4).

Animal contact, often to a wide spectrum of domestic and wild animal species, is an obvious consequence of the daily activities of the study population, which is biased by veterinary occupational exposure and by self-selection (volunteer bias). Cats are considered the primary reservoir host for *B. henselae*, whereas coyotes and foxes are considered reservoir hosts for *B. vinsonii* subsp. *berkhoffii* (1,2,8). Detection of *B. vinsonii* subsp. *berkhoffii* in 4 of 5 Californian participants could be related to the high prevalence of bacteremic coyotes in this region as well as to the potential transmission by a tick vector (1,2). All 14 participants reported frequent arthropod exposure. Although *Bartonella* spp. transmission by ticks has not been proven, several recent studies have identified *Bartonella* DNA in questing ticks, ticks attached to animals, and ticks attached to humans (1,2,14).

Despite reporting chronic or episodic illness, most participants continued to effectively maintain daily professional and personal activities. The symptoms described in the study patients are very similar to those described in a community and hospital-based surveillance study of CSD patients, in whom CSD-associated arthropathy was an uncommon chronic syndrome affecting mostly young and middle-age women (15). Our study was initiated to investigate the feasibility of combining PCR with pre-enrichment culture. Prospective studies, with appropriate controls, are needed to characterize the prevalence and clinical relevance of persistent *Bartonella* infection in immunocompetent persons.

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Dr Breitschwerdt is a professor of medicine and infectious diseases at the College of Veterinary Medicine, North Carolina State University. He is also adjunct associate professor of medicine at Duke University Medical Center. His research focuses on comparative medical aspects of zoonotic vectorborne infections in cats, dogs, and humans.

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Address for correspondence: Edward B. Breitschwerdt, North Carolina State University College of Veterinary Medicine, 4700 Hillsborough St, Raleigh, NC 27606, USA; email: ed_breitschwerdt@ncsu.edu
### Appendix Table. Serologic and PCR results from blood collected at multiple time points from 14 persons with frequent animal and arthropod contact

| Participant no./day sample collected | Direct extraction from blood/serum | After 7-d pre-enrichment culture | Blood agar plate isolate | Bartonella PCR result | Bartonella IFA reciprocal titers |
|-------------------------------------|-----------------------------------|---------------------------------|--------------------------|----------------------|-------------------------------|
|                                     |    |                                | B. henselae H1-like‡†     |                      | N/A N/A N/A                |
| Participant 1                       | 0  | Neg                            | Neg                       | B. henselae           | N/A N/A N/A                |
|                                     | 50 | B. henselae H1-like‡†          | Neg                       | Neg                   | 32 <32 <32                 |
|                                     | 67 | Neg                            | Neg                       | B. henselae           | 128 32 64                  |
|                                     | 165| Neg B. henselae                | Neg                       | <32 <32 <32           |
|                                     | 239| Neg                            | Neg                       | N/A N/A N/A           |
|                                     | 299| Neg                            | Neg                       | <32 <32 <32           |
|                                     | 351| Neg                            | Neg                       | 256 64 32             |
| Participant 2                       | 0  | Neg                            | Neg                       | <32 <32 <32           |
|                                     | 72 | Neg                            | Neg                       | <32 <32 <32           |
|                                     | 89 | B. henselae SA2-like‡†         | Neg                       | <32 <32 <32           |
|                                     | 106| Neg B. henselae SA2-like‡†      | Neg                       | 128 64 64             |
| Participant 3                       | 0  | Neg                            | B. henselae SA2-like‡†     |                      | 128 128 128                |
|                                     | 44 | Neg                            | B. henselae SA2-like‡†     |                      | 1024 256 256               |
|                                     | 105| Neg                            | B. henselae SA2-like‡†     |                      | 512 256 256                |
| Participant 4                       | 0  | Neg                            | B. henselae SA2-like‡†     |                      | 64 <32 64                  |
|                                     | 33 | Neg                            | Neg                       | N/A N/A N/A           |
| Participant 5                       | 0  | B. vinsonii subsp. berkoffii (type II)‡† | Neg | B. vinsonii subsp. berkoffii (type II)‡† | <32 <32 <32 |
|                                     | 26 | Bh (SA2-like)‡§¶               | Neg                       | <32 <32 <32           |
| Participant 6                       | 0  | Neg                            | B. henselae SA2-like‡†     |                      | <32 <32 <32                |
|                                     | 35 | Neg                            | B. henselae SA2-like‡†     |                      | <32 <32 <32                |
|                                     | 147| B. henselae SA2-like‡†         | Neg                       | <32 <32 <32           |
| Participant 7                       | 0  | Neg                            | B. henselae SA2-like‡†, B. vinsonii subsp. berkoffii (type II)‡† | Neg | 32 <32 <32 |
| Participant 8                       | 0  | Neg                            | B. henselae SA2-like‡†     |                      | <32 <32 <32                |
|                                     | 25 | Neg                            | B. henselae H1-like‡¶     |                      | <32 <32 <32                |
|                                     | 183| Neg                            | B. henselae H1-like‡¶     |                      | <32 <32 <32                |
|                                     | 215| Neg                            | Neg                       | 32 <32 <32            |
| Participant 9                       | 0  | B. vinsonii subsp. berkoffii   | B. vinsonii subsp. berkoffii | Neg | N/A N/A N/A |
| Participant | Specimen | PCR Result | IFA | Serum | PCR Result | PCR Result |
|-------------|----------|------------|-----|-------|------------|------------|
| 10          | B. vinsonii subsp. berkoffii (type I)†‡ | Neg | 82   | Neg   | 256        | 128        | 128        |
| 11          | B. vinsonii subsp. berkoffii (Type II)†‡§ | Neg | 92   | Neg   | 128        | 64         | 128        |
| 12          | B. vinsonii subsp. berkoffii (type II)†‡ | Neg | 0    | Neg   | 32         | <32        | 32         |
| 13          | B. vinsonii subsp. berkoffii (type II)†‡ | Neg | 103  | Neg   | 64         | 32         | 32         |
| 14          | B. henselae | Neg | 193  | Neg   | <32        | <32        | <32        |
| 15          | B. henselae | Neg | 0    | Neg   | <32        | <32        | <32        |
| 16          | B. henselae | Neg | 62   | Neg   | <32        | <32        | <32        |

*IFA, immunofluorescence antibody assay; Neg, negative for Bartonella spp. by PCR; NA, serum not available for testing; H1, B. henselae Houston 1; SA2, B. henselae San Antonio 2.
†Independent PCR from blood and serum identified a Bartonella spp.
‡Identified by DNA sequencing.
§Blood source for PCR or sequencing result.
¶Serum source for PCR or sequencing result.