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# Experimental Infection of Horses with *Bartonella henselae* and *Bartonella bovis*

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**Background:** Experimental infection of horses with *Bartonella* species is not documented.

**Objectives:** Determine clinical signs, hematologic changes, duration of bacteremia, and pattern of seroconversion in *Bartonella henselae* or *Bartonella bovis*-inoculated horses.

**Animals:** Twelve (2 groups of 6) randomly selected healthy adult horses seronegative and culture negative for *Bartonella* spp.

**Methods:** Experimental/observational study: Group I: *B. henselae* or saline control was inoculated intradermally into 4 naïve and 2 sentinel horses, respectively. Group II: same design was followed by means of *B. bovis*. Daily physical examinations, once weekly CBC, immunofluorescent antibody assay serology, real-time polymerase chain reaction (PCR), and twice weekly blood cultures were performed for 6 weeks and at postinoculation day 80 and 139. *Bartonella* alpha-Proteobacteria growth medium (BAPGM) enrichment blood culture was performed for horses that seroconverted to *B. henselae* antigens.

**Results:** Transient clinical signs consistent with bartonellosis occurred in some *Bartonella*-inoculated horses, but hematological alterations did not occur. Three *B. henselae*-inoculated horses seroconverted, whereas 1 *B. bovis*-inoculated horse was weakly seropositive. In Group I, *B. henselae* was amplified and sequenced from BAPGM blood culture as well as a subculture isolate from 1 horse, blood from a 2nd horse, and BAPGM blood culture from a 3rd horse although a subculture isolate was not obtained. All sentinels remained PCR, culture, and serology negative.

**Conclusions:** Detection of *Bartonella* sp. in blood after experimental inoculation supports bacteremia and seroconversion. Culture with BAPGM may be required to detect *Bartonella* sp. Although mild clinical signs followed acute infection, no long-term effects were noted for 2 years postinoculation.

**Key words:** Animal model; Bacterial; Bacterial species; Bartonella; Bartonellosis; Epidemiology; Horse; Infectious diseases; Microbiology; Species Zoonoses.

Bartonellosis refers to a spectrum of emerging zoonotic diseases with clinical manifestations depending on the infecting *Bartonella* species, the host species infected, and host immunosuppression or immunocompetence. In humans, fever, lymphadenopathy, anemia, central nervous system disorders, endocarditis, vasculitis, hepatosplenic disease, and osteomyelitis are associated with *Bartonella henselae* infection.[1,2] In immunocompromised humans and dogs, *B. henselae* causes vasoproliferative bacillary angiomatosis and peliosis hepatis,[2–5] and *Bartonella bovis* is associated with endocarditis in cattle.[6] Although *Bartonella* sp. had been isolated from numerous wildlife species, domestic animals, and humans worldwide,[7] it was not until 2008 that *B. henselae* was isolated or detected by polymerase chain reaction (PCR) amplification in the blood of 2 adult horses from North Carolina (USA), one with vasculitis and the other with chronic intermittent shifting leg lameness.[8] In 2009, *B. henselae* was reported in an aborted equine fetus in Indiana (USA),[9] and in 2011, *B. henselae* DNA was amplified from the bone marrow and spleen of a horse that succumbed to hemolytic anemia in Germany.[10] A group of horses in France was seropositive to *B. bovis* antigens without overt clinical signs (H.J. Boulouis, personal communication). Although the mode or modes of transmission to horses are not established, *Bartonella* DNA has been amplified from biting flies and *Ixodes* ticks in California, indicating possible sources of vector transmission.[11–13]

Although these reports provide preliminary support for natural infection with *Bartonella* in horses, experimental infection of horses with *Bartonella* has not been attempted. In this study, horses were experimentally inoculated with an equine isolate of *B. henselae*[8] and a bovine isolate of *B. bovis*.[14] Clinical and hematological trends, duration of bacteremia and kinetics of seroconversion were characterized. We hypothesized that

**Abbreviations:**

| | |
|---|---|
| BAPGM | *Bartonella* alpha-Proteobacteria growth medium |
| *Bvb* | *Bartonella vinsonii* subsp. *berkhoffii* |
| IFA | immunofluorescent antibody assay |
| PID | postinoculation day |
| PCR | polymerase chain reaction |
| RT-PCR | real-time polymerase chain reaction |

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*B. henselae*-inoculated horses would become bacteremic, would seroconvert, and would develop transient clinical signs, whereas *B. bovis*-inoculated horses would seroconvert without bacteremia or clinical signs.

## Materials and Methods

### *Inoculate Preparation*

*Bartonella henselae* (SA2 ITS strain, isolate designation 2008EO-1) isolated from a bacteremic mare's blood[8] and *B. bovis* (isolate designation 91-4) isolated from a bacteremic cow's blood[14] were grown on heart infusion agar[a] containing 5% fresh rabbit blood. The plates were incubated at 35°C in 5% $CO_2$ for 5 days, and bacterial growth was resuspended in 5 mL sterile saline.[15] Both isolates were low passage and maintained at −80°C until revived for this study.

### *Horse Inoculation*

Twelve horses negative on serology and blood culture for *Bartonella* were divided into 2 groups of 6 animals. Group I (*B. henselae*, horses #1–6) included 6 adult mares (a Quarter Horse, a Selle Français, and 4 Thoroughbreds) aged 4–18 years. Group I horses were housed together in the same outdoor paddock and not allowed direct contact with horses outside the group for the study period. Four horses were injected intradermally with 0.5 mL of $8.4 \times 10^8$ colony forming units (cfu)/mL of *B. henselae* divided equally between 2 sites on the left neck (Fig 1A).[16] Two negative control sentinel horses injected intradermally with 0.5 mL saline divided equally between 2 sites on the left neck were housed in the same paddock.

Group II (*B. bovis*, horses #7–12) included 6 adult geldings (an Arabian, 2 Quarter Horses, and 3 Thoroughbreds) aged 3–17 years. Group II horses were housed together in the same outdoor paddock and not allowed direct contact with other horses for the entire study period. Four horses were injected intradermally with 0.5 mL of $7.0 \times 10^8$ cfu/mL of *B. bovis*[14] divided equally between 2 sites on the left neck. Two negative control sentinel horses injected intradermally with 0.5 mL saline divided equally between 2 sites on the left neck were housed in the same paddock. Mares and geldings were housed separately according to the rules of the research facility. Horses in each group were assigned randomly as they approached or were caught by the researchers, with the first 4 being *bartonella* inoculees and the last 2 being saline controls.

Physical examinations were performed daily for 6 weeks and at postinoculation days (PIDs) 80 and 139 by a veterinarian and veterinary students blinded to the horses' inoculation status. During the first 6 weeks, blood was collected once weekly for complete blood count (CBC), real-time (RT)-PCR, and serology by indirect immunofluorescent antibody assay (IFA), and twice weekly for conventional blood culture. Blood also was drawn on PID 80 and 139 for CBC, RT-PCR, serology, and conventional blood culture. Researchers performing CBC, PCR, and serology were blinded to sample identity.

All horses were treated daily with topical 10% permethrin fly repellent spray. The study protocol was approved by the University of California-Davis Institutional Animal Care and Use Committee.

### *Conventional Blood Culture-UC Davis*

Aseptically obtained whole blood samples collected in 3 mL plastic ethylenediaminetetraacetic acid (EDTA) tubes were frozen at −70°C until used. The tubes were centrifuged at 5,000 × *g* for 30 minutes at room temperature. The supernatant was discarded, and blood pellets were resuspended in 125 μL of M 199 inoculation medium and plated onto 2 heart infusion agar plates containing 5% fresh rabbit blood. The plates were incubated at 35°C in 5% $CO_2$ for 4 weeks and examined at least twice weekly for bacterial growth.[17]

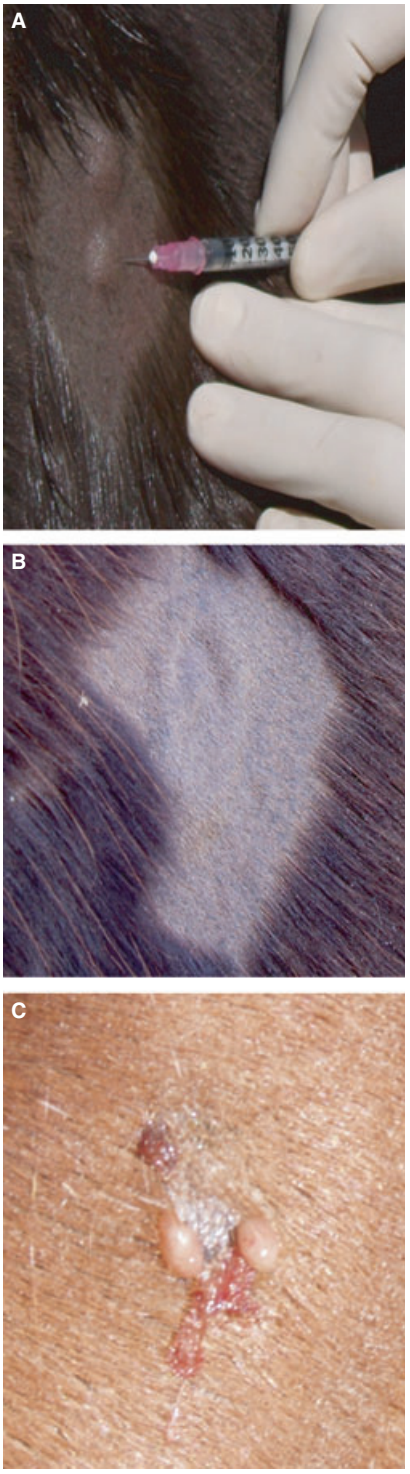


**Fig 1.** (**A**) Inoculation, Horse 3 PID 0; (**B**) Pitting edema, Horse 3 PID 4; (**C**) Purulent drainage, Horse 5 PID 16.

### *Real-Time PCR-UC Davis*

Primers targeting the gltA gene from NCBI (DQ383817.1) region of *Bartonella* were used as previously described.[18]

### *Serology-UC Davis*

Specific IgG antibodies against *B. henselae* and *B. bovis* antigens were detected with an IFA assay similar to the procedure previously described,[19] except Vero cell culture was used for both antigens, and fluorescein-labeled goat antihorse $IgG^b$ at 1 : 200 was the conjugate. The degree of fluorescence was scored based on the intensity of the fluorescence from 0 to 4, each slide having its own positive control at a score of 4. The reading was done by 2 different blinded researchers and then compared. A score $\geq 2$ on a scale of 0–4 at a 1 : 64 titer was considered positive.[15,16] A score of 1 at 1 : 64 was considered suspect or weakly positive, and a score of 0 (no fluorescence observed) was negative.

### *BAPGM Enrichment Blood Culture-North Carolina State University Intracellular Pathogens Research Laboratory*

A quantity of 1–2 mL of EDTA-anticoagulated aseptically obtained whole blood was inoculated into 10 mL of *Bartonella* alpha-Proteobacteria growth medium (BAPGM) and incubated at 35°C in a 5% $CO_2$, water-saturated atmosphere. After 7 and 14 days of incubation, 500 μL of the BAPGM-enriched blood culture media was subinoculated onto 10% sheep blood agar plates and incubated at 35°C and 5% $CO_2$. Plates were examined for colony formation at 7, 14, and 21 days postplating.[20]

### *BAPGM Enrichment Platform-NCSU Intracellular Pathogens Research Laboratory*

Primers targeting the 16S-23S rRNA intergenic spacer (ITS) region of *Bartonella* were used as previously described.[21] Sequences were aligned and compared with GenBank sequences by AlignX7 software.[c,][22] The BAPGM diagnostic platform consisted of PCR after direct extraction of DNA from blood and serum samples, PCR after enrichment culture for 7 and 14 days, and PCR after subculture onto a blood agar plate, if colony growth was visualized. To assess for potential laboratory contamination, an un-inoculated BAPGM culture flask was processed simultaneously and identically with each batch of horse blood and serum samples. Specifically, while establishing cultures in a biosafety hood, the top was removed from the BAPGM un-inoculated control flask until all samples were processed. After Intracellular Pathogens Research Laboratory standard operating procedures, sample preparation including BAPGM cultures and agar plate subinoculation, DNA extraction, PCR preparation and PCR amplification, and analysis were performed in separate laboratory rooms to avoid culture and DNA contamination. In addition, negative and positive *Bartonella* DNA test control samples, consisting of bacteria-free blood DNA and DNA spiked with *B. henselae* genomic DNA at 0.5 genome copies/μL, respectively, were used for each batch of DNA tested. All amplicons were cloned and sequenced to confirm the *Bartonella* species and strain.

## Results

### *Clinical Signs*

Group I: An injection-site reaction, including mild edema, sensitivity, and pruritus, was seen in all 4 *B. henselae*-inoculated horses (Horses 2, 3, 5, 6; (Fig 1B). Two horses (Horses 5, 6) developed spontaneous purulent drainage at the inoculation sites on PID 16 and 17 (Fig 1C). In Horses 2 and 3, cutaneous sensitivity resolved by PID 6, and edema resolved by PID 10. All 4 inoculated horses developed mild, cool, nonpainful left-sided superficial cervical lymph node enlargement (Horse 2, PID 4–6; Horse 3, PID 8; Horse 5, PID 4–14; Horse 6, PID 2–3). Limb edema, mild to moderate in severity and sensitivity, and mild in warmth, was observed in 3 of 4 inoculated horses (Horse 3 right hind, PID 14; Horse 5 all limbs with hind limbs more swollen than fore limbs, PID 4; Horse 6 both hind limbs PID 4, left fore PID 12, right hind PID 30–33). Edema resolved without treatment in all horses.

Group II: Moderate injection-site cutaneous sensitivity and mild edema occurred PID 1 in all 4 *B. bovis*-inoculated horses (Horses 7, 8, 9, 12) and resolved by PID 13. Two horses developed mild, cool, nonpainful left-sided superficial cervical lymph node enlargement (Horse 7, PID 2; Horse 12, PID 1). One horse (Horse 12, PID 7) developed mild colic, which responded to flunixin meglumine (1.1 mg/kg, IV, once) and water, mineral oil and electrolytes via nasogastric tube. This horse subsequently developed mild (in severity, sensitivity, and warmth) bilateral hind limb edema (PID 9–13) and mild diffuse nonpainful, nonpruritic urticaria (PID 10–13); both signs resolved without treatment. Horse 9 also developed mild to moderate nonpainful, nonpruritic diffuse urticaria (PID 10–13). Pyrexia was not observed in any horse in either group at any sampling time point.

### *Clinical Pathology-UC Davis*

Groups I and II: All CBC indices remained within laboratory reference ranges for all horses.

### *Conventional Blood Culture-UC Davis*

Groups I and II: Bacterial growth was not visualized after inoculation of rabbit blood agar plates with blood pellets from *B. henselae* and *B. bovis*-inoculated horses or injection-site drainage (Horses 5 and 6).

### *Real-Time PCR-UC Davis*

Groups I and II: *B. henselae* and *B. bovis* DNA were not amplified from any extracted blood sample or purulent material.

### *Serology-UC Davis*

Group I: Three (Horses 2, 5, 6) of 4 *B. henselae*-inoculated horses seroconverted to *B. henselae* antigens. All horses were antibody negative at the first sampling time point, PID 6. Horse 2 became seropositive (1 : 64, PID 8), and achieved and maintained a peak antibody titer (1 : 1,024, PID 12–42). The titer was 1 : 256 by PID 80, and the horse became seronegative by PID 139.

Horse 5 was weakly seropositive (<2 at 1 : 64, PID 8) and achieved and maintained a peak titer (1 : 1,024, PID 19–42). The titer was 1 : 64 by PID 80, and the horse was seronegative by PID 139. Horse 6 was weakly seropositive (<2 at 1 : 64, PID 8) and achieved a peak titer (1 : 2,048) by PID 19. The titer decreased by 1 dilution (1 : 1,024 by PID 26), remained 1 : 1,024 until PID 42 and was 1 : 256 by PID 80. The horse was seronegative by PID 139.

Group II: One horse (Horse 12) was serologically suspect for *B. bovis* antigen (score <2 at 1 : 64, PID 19), remained borderline positive until at least PID 33 and was seronegative by PID 42.

### *BAPGM Enrichment Blood Culture Platform-NCSU*

Group I: Samples from the 3 *B. henselae* seropositive horses (Horses 2, 5, 6) were further evaluated by conventional PCR before and after BAPGM enrichment blood culture. After extraction of EDTA blood samples, *B. henselae* was amplified from all 3 seropositive horses (Horses 2, 5, 6). Agar plate subculture *B. henselae* isolates were obtained 7 and 14 days after BAPGM enrichment culture of a PID 6 blood sample from Horse 5. Both isolates were confirmed to be *B. henselae* by DNA sequencing. Horse 6 was *B. henselae* PCR positive on a 7-day BAPGM-enriched blood sample at PID 42, but subculture inoculation did not result in visible agar plate growth.

Horse 2 was *B. henselae* PCR positive on a blood sample extract from PID 42, but PCR after BAPGM enrichment culture was negative. *Bartonella vinsonii* subsp. *berkhoffii* (*Bvb*) DNA was amplified and sequenced from a PID 42 blood sample obtained from Horse 5, but the serum sample collected on that day was seronegative for *Bvb*.

### *Results Summary*

In Group I, all horses were seronegative at the 1st sample collection, PID 6. The earliest evidence of *B. henselae* seroconversion occurred at PID 8, peaked by PID 12 in 1 horse and by PID 19 in 2 horses and persisted until at least PID 80. Of the 4 *B. henselae*-inoculated horses, the 3 that seroconverted were all seronegative by PID 139. The earliest and only evidence of bacteremia was detected at PID 42 in Horses 2 and 6 and at PID 6 in Horse 5. In Group II, 1 horse was serologically suspected for *B. bovis* antigen at PID 19–33, and was seronegative by PID 42. None of the other 3 *B. bovis*-inoculated horses seroconverted.

## Discussion

In this study, 3 horses (Horses 2, 5, 6) inoculated intradermally with a low passage *B. henselae* seroconverted and became bacteremic. Weak seropositivity, but not bacteremia, was detected in 1 horse (Horse 12) after inoculation of *B. bovis*. Despite our inability to isolate or amplify *B. bovis* DNA from its blood, Horse 12 sequentially developed cervical lymphadenopathy, colic, mild bilateral hind limb edema, and diffuse urticaria between PIDs 1 and 10 and became weakly seropositive to *B. bovis* antigens by PID 19. Clinical signs analogous to the *B. henselae*-inoculated horses suggested that a mild and transient *B. bovis* bacteremia may have developed.

The *B. henselae*-inoculated horses' antibody kinetics was comparable to antibody kinetics in dogs experimentally infected with *Bvb*. The pattern of bacteremia resembled that found after experimental inoculation in cats with *B. henselae* and in dogs after experimental infection with culture-grown *Bvb* (PID 11–42).[23] In 9 cats experimentally inoculated intradermally with *B. henselae,* 3 patterns of bacteremia occurred: lasting 5–7 weeks, 4 cats; 9–13 weeks, 4 cats; and, relapsing, 1 cat.[24] These findings were in contrast to a study in which 18 cats inoculated by transfusion with blood infected with *B. henselae*, *Bartonella clarridgeiae*, or both became intermittently bacteremic until PID 454.[25] Therefore, when interpreting these results, the route of infection, species of *Bartonella*-inoculated, and the fact that the organisms were not transmitted by their natural vector all should be considered. Infection of horses by blood transfusion or by a competent insect vector would likely result in different clinical, serological, and microbiological patterns considering that inoculation of culture-grown *Borrelia burgdorferi* rarely induces disease in dogs, whereas inoculation via tick attachment induces arthritis in a small percentage and chronic infection in most, if not all, dogs.[26]

We hypothesized that transient clinical signs including fever, malaise, lymphadenopathy, and anemia would occur in horses associated with *B. henselae,* but not *B. bovis* because *B. bovis* seropositive horses lacking clinical signs were previously found in France (H.J. Boulouis, personal communication). *Bartonella bovis* bacteremia is common in cattle worldwide with isolation or PCR prevalence as high as 84% occurring in California.[27–29] Because biting flies are the presumed vector for *B. bovis*, this bacterium is likely inoculated into horses often, but existing data suggest that chronic bacteremia or disease in horses is unlikely. However, urticaria observed in Horses 7 and 12 may represent a clinical manifestation of bartonellosis because this sign occurs in some infected humans (E.B. Breitschwerdt, unpublished data). *Bartonella henselae*-inoculated horses developed injection-site reactions and regional lymphadenopathy, which are signs of *B. henselae* infection in humans[1,2] and experimental *Bvb* infection in dogs.[15] The *B. henselae*-inoculated horses also developed limb edema, a manifestation described in *B. henselae* bacteremic humans[30] and dogs.[22,31] Although direct causality between these signs and *Bartonella* infection cannot be confirmed nor refuted, physical examinations were performed only once daily and signs such as transient pyrexia could have gone undetected. Although all inoculated horses were clinically healthy as of December 2011, testing tissues for *Bartonella* DNA would determine persistent infection status and provide additional information regarding bartonellosis in horses.

Serology data from this study provide evidence for a humoral immune response after needle inoculation of culture-grown organisms. Although IFA testing yielded consistent results for *B. henselae* antibodies, a validated and standardized test for horses is unavailable.[8]

One horse (Horse 5) was sequentially positive for *B. henselae* and *Bvb*. The source and mode of *Bvb* infection was not identified but could have been related to inoculation by an unknown vector. Although the competent vectors for *Bvb* are unknown, *Bvb* DNA has been detected in fleas collected from gray foxes[32] and in questing *Ixodes* ticks.[33] Previous studies documenting natural *Bvb* infection in dogs in California support the plausibility of vector transmission.[34] Preinoculation screening by serology, PCR, and conventional blood culture did not generate evidence of *Bvb* infection. However, as illustrated in this study, enrichment culture may be necessary to increase bacterial numbers and enable PCR detection. Preferential amplification of *B. henselae* with ITS primers in *Bvb* and *B. henselae*-inoculated liquid co-cultures is documented.[35] Although *Bvb* seropositivity was not detected in this horse, discrepant PCR and serological results are documented in dogs[20,35] and in *Bvb* naturally infected horses from North Carolina (N.A. Cherry and E.B. Breitschwerdt, unpublished data). Several studies in humans also report detection or isolation of *Bartonella* or both in seronegative patients.[36–39] Although molecular testing was not performed on blood samples from the seronegative horses, future studies are warranted to determine if *Bartonella* bacteremia could also be identified in seronegative horses as it was in these humans. In humans, host anergy or antigenic variability among *Bartonella* strains could result in false negative serology,[37] providing a possible explanation for negative *Bvb* serology results and failure to detect *B. henselae* seroconversion in Horse 3. Because the inoculation strains used in this study were low passage and appropriately stored and revived, it is unlikely that their antigenicity varied greatly from the original infecting strains from which they were derived. Co-infection with multiple vector-borne pathogens can complicate the clinical presentation and alter hematological patterns of disease.[40] The role co-infection plays in equine bartonellosis is unknown.

In 2 *B. henselae*-inoculated horses, including Horse 5 from which *Bvb* DNA was PCR-amplified and sequenced from blood, *Bartonella* DNA was detected by PCR after direct extraction from blood but not after enrichment culture, a circumstance documented in dogs.[20] As previously suggested, this discrepancy may result from PCR amplification of nonviable *Bartonella* DNA in blood samples or from a dilution effect whereby bacteria are unable to thrive and replicate after inoculation of blood into liquid BAPGM. As illustrated by this study's comparative culture and PCR results derived by testing in 2 different laboratories with different culture approaches and PCR gene targets, the molecular detection and isolation of *Bartonella* sp. from experimentally infected horses is challenging. Regardless of the cause of these discrepant results, diagnostic evaluation of horses suspected of bartonellosis should include PCR after direct extraction from the patient sample, PCR after BAPGM enrichment blood culture, and PCR to identify *Bartonella* sp. DNA in a subculture isolate.

The extent to which *Bartonella* infection contributes to immunosuppression is not reported.[41] Conversely, host immune status, especially immunosuppression, likely influences the clinical presentation and disease severity after *Bartonella* infection.[4,5] In this study, baseline CBC results and normal initial physical examinations suggested that all horses were free from obvious disease before experimental inoculation. Serial serum samples were banked for future biochemical analysis.

Although unknown, natural *B. henselae* infection in horses likely occurs via arthropod vector transmission because biting flies, fleas, and ticks routinely encountered in a horse's environment reportedly harbor *Bartonella* species. Mechanical transmission of *Bartonella* through wounds, bites, scratches, or needle stick also may occur.[42,43]

Study limitations include the relatively small sample size per group and the relatively small aliquots of blood available for BAPGM testing as this modality was an addition to the original objectives. For best BAPGM results, 2–3 mL of blood typically are recommended, but only 1 mL of blood per *B. henselae* horse was available. The decreased volume may have decreased the likelihood of obtaining an isolate. Ideally, samples from all horses would have been subject to BAPGM testing if not for financial limitations. Although the influence of sex was not assessed attributable to inability to house male and female horses together, sex differences are not reported for *Bartonella* infections in many species, including dogs, cats,[44] and foxes.[45] To eliminate the potential for vector-borne transmission, housing the horses in a vector-proof environment would have been ideal but was not possible. The study was performed in early spring before peak vector activity, and topical insect repellent was applied daily to the horses. No ticks and only occasional biting flies (PID 80 and 139) were observed during the study period. The injection-site drainage from Horses 5 and 6 was RT-PCR negative, and rabbit blood agar culture failed to grow bacteria. Perhaps an isolate could have been obtained after enriched BAPGM culture, but a sufficient sample was not available for this test.

The present data support bacteremia and seroconversion in horses after experimental infection with *B. henselae* and the use of BAPGM to confirm *Bartonella* bacteremia. Although the clinical manifestations of equine bartonellosis are not yet fully defined, testing horses displaying clinical signs consistent with *Bartonella* infection in other species should be considered in the interim.

## Footnotes

[a] Difco Laboratories, Detroit, MI

[b] KPL, Gaithersburg, MD
[c] Vector NTI Suite 6.0, InforMax Inc, Rockville, MD

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*Disclosure*: In conjunction with Dr Sushama Sontakke and North Carolina State University, Dr. Breitschwerdt holds US Patent No. 7,115,385; Media and Methods for cultivation of microorganisms, issued October 3, 2006. He is the chief scientific officer for Galaxy Diagnostics, a company that provides diagnostic testing for detection of *Bartonella* species infection in animal and human patient samples.

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## Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Table S1.** Reciprocal titer, clinical signs post *Bartonella henselae* inoculation day (PID) 0, 6, 8, 12, 19, 26, 34, 42, 80, 139 for Horses 2, 3, 5, 6. Initial peak titer denoted by *. Bacteremia denoted by +. Isolate obtained by I. Injection site drainage = D, edema = E, sensitivity = S. L = left-sided superficial cervical lymphadenopathy, LE = limb edema, N = no clinical signs.

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