

Research Article

Volume 4 Issue 5 – July 2017
DOI: 10.19080/AIBM.2017.04.555646

Adv Biotech & Micro

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Enrichment Blood Culture Isolation of *Bartonella henselae* from Horses with Chronic Circulatory, Musculoskeletal and/or Neurologic Deficits



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Submission: April 13, 2017; Published: July 27, 2017

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Abstract

Background: Because published case-based evidence is limited and only one experimental equine infection study has been reported to date, bartonellosis, a recently recognized equine disease, is rarely considered as a differential diagnosis by equine clinicians. Microbiological documentation of *Bartonella* spp. infections has been difficult to achieve in the laboratory; therefore, elucidation of the potential clinical and pathological aspects of chronic bartonellosis in horses has not been thoroughly investigated.

Objective: To summarize the historical, clinical and laboratory findings in horses with microbiologically confirmed *Bartonella* spp. bacteremia.

Study Design: During medical consultations, testing for *Bartonella* spp. bacteremia was recommended for horses with histories of chronic circulatory, musculoskeletal and/or neurologic deficits.

Methods: *Bartonella* ePCR™ (an adaptation of the *Bartonella* alpha Proteobacteria growth medium [BAPGM] enrichment blood culture platform) was performed by Galaxy Diagnostics, Inc. to establish intravascular infection (bacteremia) in each horse. *Bartonella henselae* indirect fluorescent antibody testing was performed for horses with documented bacteremia.

Results: Within a 14 month time frame, the authors identified nine *B. henselae* bacteremic horses out of the total number (n=22) tested. Historical abnormalities included exercise intolerance, shifting leg lameness, circulatory deficits, cranial nerve dysfunction, endocrine imbalances and neurologic impairment. Serum samples were available from five of the nine bacteremic horses, and one horse was sero-reactive with antibody titer of 1:128 to *Bartonella henselae* antigens.

Main limitations: Variability of disease manifestations in these horses may decrease the clinical applicability of these findings. All horses had received prior therapeutic efforts while this study focused upon bartonellosis, therefore, the presence and effect of other infectious or non-infectious diseases was not evaluated in the context of this study.

Conclusion: Prospective controlled studies are needed to define the duration of bacteremia, diagnostic testing criteria, and treatment outcomes for horses infected with *B. henselae*.

Keywords: Bartonellosis; Horses; BAPGM; *Bartonella henselae*

Supplementary Material

Indirect fluorescent antibody testing using a panel of *Bartonella* species antigens and the BAPGM enrichment blood culture/PCR platform, with DNA sequence confirmation of the infecting *Bartonella* species is available for clinical diagnostic and research study purposes through Galaxy Diagnostics, Inc.

Introduction

Genus *Bartonella* is currently comprised of at least 34 species or subspecies of fastidious, intracellular, Gram-negative aerobic bacteria [1-3]. *Bartonella* spp. have been increasingly recognized as pathogens of substantial medical importance in

cats, dogs and humans [1-3]. However, equine bartonellosis remains largely undefined as a disease of horses. These highly adaptive, intravascular and endotheliotropic bacteria are transmitted among domestic and wild animals throughout the world, primarily by blood-feeding arthropod vectors, including bedbugs, biting flies, fleas, lice, mites, sand flies, ticks and potentially spiders [4-6]. In addition, bites, scratches, needle sticks, and for some species, transplacental, have all been implicated as modes of disease transmission [1-4]. Persistent intravascular infection with different *Bartonella* spp. has been reported in numerous pet, production and wildlife animals [1-3]. Following transmission in nature, duration of bacteremia in horses is unknown, whereas following experimental intradermal inoculation of four horses with culture-grown *Bartonella henselae*, three sero-converted and developed bacteremia and mild cutaneous disease manifestations [7]. It was not until 2008 that *B. henselae* was isolated or detected for the first time by polymerase chain reaction (PCR) amplification in the blood of two adult horses from North Carolina (USA), one with vasculitis and the other with chronic intermittent shifting leg lameness [8]. In 2009, *B. henselae* infection was documented 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]. More recently, *B. henselae* was isolated using the BAPGM enrichment blood culture approach from a Thoroughbred foal with granulomatous hepatitis, pathology that had been previously reported in dogs and humans [11].

Serology, PCR amplification of organism-specific DNA sequences from blood, and conventional blood culture isolation techniques have not proven useful for confirming a diagnosis of canine, equine or human *Bartonella* bacteremia [12-15]. Relatively recent advances in microbiological culture technology have enabled more sensitive documentation of *B. henselae* bacteremia in foals and in horses with presumptive vasculitis and chronic arthropathy [13]. The purpose of this study was to summarize historical, clinical and laboratory findings in horses with laboratory confirmation of *Bartonella* spp. bacteremia using *Bartonella* ePCR™ (Galaxy Diagnostics, Inc. Morrisville, NC USA) platform.

Materials and Methods

Horses included in this study had a history of chronic symptoms in conjunction with poor skeletal muscle exercise tolerance, microcirculatory deficits, and/or neurologic dysfunction. Chronicity was based upon owner reported symptoms extending over a period of at least two years. Poor exercise tolerance was defined as delayed forward phase of stride and/or muscle weakness (with or without muscle spasms) and/or shifting leg lameness. Microcirculatory involvement was defined in terms of sequelae indicative of poor arterial oxygenation of soft tissue(s) and/or chronic inflammation in a wide range of tissues and/or evidence of impaired venous and lymphatic drainage. Neurologic dysfunction was defined as

unexplained cranial nerve deficits and/or symptoms consistent with neurotransmitter imbalances. Additional historical abnormalities involving endocrine system impairment, a finding reported in other mammalian species with confirmed bartonellosis, were considered as adjunct criteria for *Bartonella* bacteremia testing.

Historical, Physical Examination and Blood Specimen Collection

Study population: Each owner provided historical and demographic data for their horses' travel and arthropod exposure history. Physical examinations were concurrently performed by the respective primary care veterinarians in conjunction with blood specimen collection from each horse on three alternate days. Ethylene diamine tetraacetic acid (EDTA)-anti-coagulated blood and serum samples from horses were shipped to Galaxy Diagnostics, Inc. (Research Triangle Park, NC, USA) from Australia and the United States for *Bartonella* ePCR™ testing.

Bartonella IFA serology

As previously described by Cherry NA et al. [14], IFA was performed to document detection of *Bartonella* antibodies using *B. henselae* (San Antonio-2, and Houston-1 strains), *B. vinsonii* subsp. *berkhoffii* (genotypes I and II), *B. koehlerae* and *B. quintana* organisms grown in mammalian cells as antigens. Serum was available from 10 of the 22 horses described in this study. Sera (kindly provided by Dr. Bruno Chomel, University of California, Davis) from three horses experimentally-infected with *B. henselae* (SA2 strain, isolate designation NCSU 2008-EO-1) were used as IFA positive controls. In our assay, endpoint titers for these three horse sera were 1:512, 1:512, and 1:2048. The starting dilution was 1:16, with endpoint titers defined as the last dilution at which brightly-stained organisms could be detected by fluorescence. A cut-off titer defining sero-reactivity was arbitrarily defined as 1:64.

Bartonella ePCR™

Bartonella ePCR™, an adaptation of a previously-described approach that combines PCR amplification of *Bartonella* spp. DNA from blood before and after inoculation into BAPGM, was performed [12,13,15,16]. For each blood sample, 2mL of EDTA blood was spiked into 10mL BAPGM and incubated at 35-37 °C with 5% CO₂. DNA was extracted directly from 200µl of EDTA blood and from 200µl of BAPGM enrichment blood cultures after three incubation time points (8, 14 or 15, and 21 or 22 days) using an automated QIA Symphony Workstation and DSP DNA blood mini kit (Qiagen, Valencia, CA). As extraction controls, a positive culture control consisting of BAPGM spiked with *B. henselae* and a negative culture control containing only BAPGM were processed simultaneously with each sample set to ensure extraction was completed successfully and that extraction buffers and reagents were not contaminated with *Bartonella* DNA. *Bartonella* DNA was amplified using conventional *Bartonella* genus primers targeting the 16S-23S intergenic spacer region

(ITS) as previously described [14,17].

In addition, *Bartonella* genus qualitative real-time PCR (qPCR) was performed targeting the *Bartonella* 16S-23S ITS gene using oligonucleotides (5' CTTAGATGATGATCCCAAGCCTTYTGGC3') and (5' GCCCTCCGGRTAAAYCGGAAACC 3') as forward and reverse primers, respectively. Amplification was performed in a 25µl final volume reaction containing 12.5µL of MyTaq Premix (Bioline USA, Inc., Taunton, MA) for conventional PCR or SensiFAST™ SYBR No-ROX kit (Bioline USA, Inc., Taunton, MA) for qPCR, 0.3µL of 100µmol/L of each forward and reverse primer (IDT@DNA Technology, Coralville, IA), 6.9µL molecular grade water, and 5µL DNA from each sample tested. Amplification was performed using an Eppendorf Mastercycler EPgradient® (Eppendorf, Hauppauge, NY) for conventional PCR and the Bio-Rad CFX96 Touch™ Real Time PCR Detection System (Bio-Rad, Inc., Hercules, CA) for qPCR with 0.01 and 0.001 pg/µL *B. henselae* SA2 genomic DNA as PCR positive controls. Negative controls included BAPGM and molecular-grade water. For conventional PCR, thermocycling conditions consisted of initial denaturation at 95 °C for 2 minutes, followed by 55 cycles with denaturation at 94 °C for 15 seconds (s), annealing at 68 °C for 15s, and extension at 72 °C for 18s. PCR products were analyzed by 2% agarose gel electrophoresis. For qPCR, thermocycling conditions consisted of initial denaturation at 95 °C for 2 minutes, followed by 45 cycles with denaturation at 94 °C for 10s, annealing at 66 °C for 10s, and extension at 72 °C for 10s. Positive amplicons were sequenced to identify *Bartonella* sp. and ITS genotype. Bacterial species and genotype were defined by comparing similarities with other sequences deposited in the GenBank database using the Basic Local Alignment Search Tool (BLAST version 2.0).

Results

Bartonella pre and post-enrichment PCR and IFA serology results for the nine bacteremic horses are summarized in Table 1. Demographic findings for the *B. henselae* bacteremic horses are summarized in Table 2. Each of these horses had histories of poor exercise tolerance, microcirculation deficits, neurological abnormalities, neuroses, and/or evidence of endocrine system impairment. All nine horses had histories indicative of varying degrees of poor exercise tolerance. Delayed forward phase of stride was manifested as a tendency to land toe first (cases 1, 2, 3 and 9), toes worn flat as a result of toe dragging (cases 1, 3, 5 and 9), reluctance to move forward under saddle (cases 1, 3, 5 and 6), refusal to move at all with or without rearing (cases 5 and 6), subluxating patellas (locking stifles) (cases 1 and 4) and pastern and fetlock subluxation (case 2). Four horses (cases 4, 7, 8 and 9) were prone to stumbling on level as well as uneven surfaces. Skeletal muscle abnormalities included a wide range of symptoms: total body soreness (cases 1, 2, 5, 6, 7, 8 and 9), back soreness with top line muscle atrophy (cases 1, 4, 5, 6, 7 and 9), rigid muscles (cording) between hips and stifles (cases 1, 3, 4, 5 and 6), cross firing (disunited cantering, skipping) at the canter (cases 1, 3, 4, 6 and 7), chronic inter costal muscle soreness

(cases 1, 4, 6 and 8), tendency to travel hollow backed and high headed (cases 5 and 8), grunting under saddle (cases 6 and 7), shivers (case 2), flaring nostrils at rest (case 2), esophageal muscle spasms (choke, swallowing problems) (case 9) and dropped head syndrome (case 1). Smooth muscle abnormalities included dribbling urine (with odd urination habits) (cases 1, 4 and 6), constipation (scant dry manure) (cases 4 and 9) and bloated abdomen (cases 2 and 5). Two horses (cases 2 and 4) exhibited shifting leg lameness (front to back and/or side to side) that would wax and wane over time.

Microcirculation deficits were reported in terms of hypersensitivity to cold weather, particularly cold wet weather (cases 3, 5, 6 and 8), absence of dapples (cases 1, 5, 6, 7 and 8), pale mucous membranes including the tongue (cases 3 and 5) and increased central arterial and venous pressures (case 1). Complications associated with poor tissue oxygenation included recurring scratch-like lesions (cases 1, 2, 7 and 9), fungal skin conditions resistant to treatment (streptothricosis) (cases 1, 2 and 4), yeast-like secretions in the sheath of geldings (cases 1 and 4), swollen joints (knees, hocks, stifles) (cases 2, 5 and 6), cording of lymphatic vessels (cases 1 and 5), chronic exertional rhabdomyolysis (case 4), recurring cellulitis (case 1), chronic thrush in and around the frogs (cases 1 and 9), chronic bacterial foot abscesses (case 1), chronically swollen eyelids (cases 5 and 9), chronically swollen parotid salivary glands (case 3) and chronic bacterial corneal infections with blepharospasm (cases 1, 4 and 9).

Neurologic abnormalities involved both central and peripheral nervous systems. Head and neck findings included tongue laxity (case 7) and odd compulsive habits involving the tongue (case 4) (hypoglossal nerve), mouthiness (nipping, biting, licking) (cases 4 and 6) and Bell's Palsy (case 7) (facial nerve), hypersensitivity to sound (cases 2 and 6) and balance problems in a moving horse trailer (cases 4 and 7) (vestibulocochlear nerve), intense cranial pruritis during and after exercise (case 6) (trigeminal nerve), repetitive flehmen response (case 3) (olfactory nerve), and in 2 horses (cases 2 and 6) optic nerve related issues: recurrent uveitis (ERU), depth perception deficits (altered spatial behavior), photic headshaking and preference for shade vs. bright light. Three horses (cases 4, 7 and 8) had generalized proprioceptive deficits. Peripheral integument hyper-reactivity was recorded as hypersensitivity to a variety of triggers: rain or bathing (cases 4 and 6), blankets (cases 2, 4 and 6), girthing or cinching (cases 1, 4, 5 and 6), grooming tools (cases 2, 4 and 6), insects or insect bites (cases 4 and 7), strong sunlight or sunburn (case 5), and fly spray (case 9). Additional dermal abnormalities included chronic capped elbows (shoe boils) (case 1), callouses on one or both elbows (cases 1 and 9), and sweet-itch of the mane and/or tail with intense pruritis of ventral midline and sheath area (case 4).

All nine horses displayed neuroses, consistent with neurotransmitter imbalances. Most common were depression (horses 1, 2, 5 and 7), moodiness, grumpiness, an acquired

argumentative attitude (cases 5, 6, 7 and 9), and changes in temperament from mild mannered to belligerent (cases 1, 2, 5 and 6). Three horses (cases 5, 6 and 7) displayed anger, aggression and “temper tantrums”. Similar behavior patterns included explosive outbursts such as bolting (cases 5 and 6), panic attacks (cases 2 and 3), running backwards during panic attack (cases 2 and 6), violently pulling back while tied (cases 2 and 6), violent kicking episodes (cases 7 and 9), and bizarre fearful hallucinogenic-like behavior (cases 2 and 6). Two horses (cases 4 and 7) displayed frequent yawning. Narcolepsy (falling asleep randomly and/or collapsing) (case 8), random seizures (case 7) and combined attention or hyperactivity deficits (case 6) were reported in 1 horse each.

individual horses with goiter (case 5), anhydrosis (case 5), Cushing’s syndrome (case 9), irregular estrus cycles (case 6) and chronic intermittent lactation (over a period of five years) (case 9) respectively. Aberrations with immune system components were recorded in terms of allergic reactions following routine vaccination (cases 3, 4 and 6), equine sarcoid lesions (cases 2 and 5) and alopecia areata (cases 4 and 5). An abundance of miscellaneous performance limiting issues included shortened stride length going downhill (cases 2, 3, 4, 5, 6 and 9), inconsistent behavior from day to day (cases 2, 3, 5, 6 and 7), and extreme separation anxiety (cases 5 and 6). Inability to relax (muscle twitching, trembling, prancing), repetitive behavior (pacing), obsessive compulsive behavior patterns and inability to tolerate changes in routines were common to all nine horses.

Evidence of endocrine system impairment occurred in

Table 1: *Bartonella* pre- and post-enrichment PCR and IFA serology results for nine *Bartonella henselae* bacteremic horses.

Case #	Date	PCR (Blood)	PCR (8 day Blood Culture)	PCR (14/15 day Blood Culture)	PCR (21/22 day Blood Culture)	<i>B. henselae</i> IFA titer
1	11/4/2014	Negative	Positive (2 nd)	Negative	Positive (3 rd)	N/A
1	6/16/2015	Negative	Negative	Positive (2 nd)	Negative	<1:16
2	11/10/2014	Negative	Positive (1 st)	Negative	Negative	N/A
3	11/10/2014	Negative	Negative	Positive (2 nd)	Negative	N/A
4	4/21/2015	Negative	Positive (2 nd)	Positive (3 rd)	Positive (3 rd)	<1:16
4	7/28/2015	Negative	Negative	Negative	Negative	<1:16
5	8/6/2014	Negative	Negative	Negative	Positive (3 rd)	N/A
5	12/8/2014	Negative	Negative	Negative	Negative	<1:16
6	4/21/2015	Negative	Positive (1 st)	Negative	Negative	<1:16
6	6/12/2015	Negative	Negative	Negative	Negative	<1:16
7	12/16/2014	Negative	Negative	Negative	Positive (1 st & 2 nd)	N/A
8	8/12/2015	Negative	Negative	Positive (3 rd)	Negative	N/A
9	8/18/2015	Negative	Negative	Positive (2 nd)	Negative	1:128

N/A: Serum not available for testing; “1st”, “2nd” or “3rd” indicates which blood draw was *B. henselae* PCR positive.

Table 2: Demographic Findings in Nine *Bartonella henselae* bacteremic horses.

Case #	Breed	Sex	Age at testing (years symptomatic)	Location	Travel history	Arthropod exposure
1	Holsteiner	Gelding	8yr (4)	US (California)	Czech Republic	Ticks, mosquitos
2	Holsteiner	Mare	6yr (4)	Australia	(New South Wales)	Ticks
3	Thoroughbred cross warmblood	Mare	10yr (2)	Australia (New South Wales)	Sydney, Singapore	Ticks
4	Andalusian	Gelding	7yr (5)	US (New Hampshire)	US (Vermont)	Ticks, deer flies, black flies
5	Quarter Horse cross	Gelding	9yr (5)	Australia (Victoria)	Queensland, Victoria	Ticks, mosquitos, bot flies
6	Quarter Horse	Mare	11yr (6)	US (North Carolina)	US (Georgia)	Ticks, mosquitos, deer flies
7	Quarter Horse	Mare	13yr (10)	US (Montana)	US (Oklahoma)	Mosquitos, deer flies
8	Arabian	Gelding	20yr (8)	US (Montana)	US (Florida)	Mosquitos, deer flies
9	Appaloosa	Mare	29 (11)	US (North Carolina)	US (North Carolina)	Ticks, mosquitos, deer flies

Discussion

Bartonella henselae bacteremia was documented by BAPGM enrichment blood culture in nine horses with chronic, intermittent, relapsing, and at times progressive symptoms that spanned a several year time frame. Importantly, all of these horses had overlapping symptoms involving the musculoskeletal, nervous, and endocrine and circulatory systems. The extent to which *B. henselae* bacteremia contributed to individual or the collective symptoms reported in this case series could not be determined. Previously, Merrell and Falkow described the challenges associated with establishing disease causation for stealth pathogens, including members of the genus *Bartonella* [18]. As vector-transmitted, intracellular, organisms, the evolutionary lifestyle of this genus is characterized by erythrocyte and endothelial cell tropisms. Based upon *in vitro* studies, *B. henselae* can also invade monocytes, dendritic cells, CD34+ progenitor cells, pericytes, and synoviocytes (unpublished data) [19-22]. Thus, once bacteremic infection is established, the organism is able to circulate throughout the microvasculature of the host, and presumably localize in microcapillaries within various tissues, thereby involving organs throughout the body. Chronic micro vascular infection, in conjunction with interactions associated with the host immune response, presumably results in a diverse and fluctuating spectrum of symptoms that may span years in duration. Satisfying Koch's postulates has represented the historical benchmark for establishing infectious disease causation; however, the original postulates have substantial limitations when attempting to support disease causation associated with stealth pathogens. Because infections with various *Bartonella* species induce similar or identical pathology in different animal hosts, including humans, the Postulate of Comparative Infectious Disease Causation was proposed as an additional fifth Koch's postulate [23].

This postulate states that microbiological documentation of the same infectious agent(s) in three different animal species with identical pathology supports stealth pathogen infectious disease causation. This postulate has medical utility when assessments are based upon common histopathological and microbiological findings in three different animal hosts. It is also possible that the comparative documentation of similar, subtle and non-specific symptoms among different companion animal species may further facilitate our understanding of the biological consequences of long standing *B. henselae* bacteremia in horses and other non-reservoir-adapted animals. Because animals share the same environment and because their behaviors are generally carefully monitored by their owners, symptoms and behavioral patterns observed in pets may have comparative medical implications for their human counterparts.

Diagnostic confirmation of *B. henselae* bacteremia in horses remains challenging. For reasons that remain unclear, serology in naturally-infected horses is insensitive. Similar to previous reports [13], only one horse in this study was *B. henselae* seroreactive, despite a history of chronic symptoms

and concurrent diagnostic documentation of bacteremia. When using BAPGM enrichment blood culture to test dogs, cats and humans, shorter incubation periods are often sufficient for growth and detection of *Bartonella* in bacteremic individuals, whereas incubation for 21 or 22 days was necessary to document bacteremia in all but two of these nine horses. This observation could reflect the possibility that bacteremic horses maintain lower levels of bacteremia compared to most infected cats, dogs or humans, as comparable amounts of blood are inoculated into the liquid medium, regardless of the animal species being tested. When testing humans for evidence of *Bartonella* spp. bacteremia using BAPGM enrichment blood culture, obtaining specimens from three time points within a seven day period increased diagnostic sensitivity [24]. This approach also appears to have microbiological utility when testing horses, as no horse was PCR positive in all three blood culture specimens. Thus, until a more sensitive microbiological method becomes available, the *Bartonella* ePCR™ triple draw approach is recommended when assessing *Bartonella* spp. bacteremia in horses.

There are several limitations inherent in this study. The duration and types of symptoms varied widely among horses that were tested, thereby decreasing the clinical utility of the reported findings. Case controlled studies will be necessary to define specific symptoms or symptom patterns that would support the need for *Bartonella* spp. diagnostic testing. Clinical evaluations were performed by different equine clinicians, in different countries, all dealing with a broad range of economic, logistical and technical limitations related to specimen collection and the provided medical care. For all horses in this study, there had been extended, prior diagnostic and therapeutic efforts to manage their symptoms. Depending upon geographic location and clinician preference, some horses were tested for other infections contributing evidence of exposure to *Borrelia* and *Toxoplasma* spp. Testing performed by the authors was directed solely at determining if a horse was *Bartonella* spp. bacteremic. Thus, other infectious or non-infectious diseases were not systematically evaluated. Historically, use of the BAPGM blood culture platform has documented bacteremic co-infections with *B. henselae* and other bacteria in dogs [25] and humans [26]. Thus, there appears to be a complex pathobiome of blood that potentially changes with age, breed (genetics), nutrition, toxin exposure and interactions with a spectrum of bacteria, viruses and protozoa in the environment. Subcultures were not performed on samples from horses in this study to assess co-infections. Based upon evolving comparative clinical, microbiological and pathological data, tick borne pathogens, including *Bartonella* species, may constitute an important component of the pathobiome of blood in horses and other animals [27].

Within the context of differential diagnoses, the parameters described within this group of bacteremic horses could be incorporated by equine practitioners into a wide range of routine clinical evaluations such as lameness exams and pre-purchase

exams. As part of a complete physical examination the examiner might review any and all presenting complaints and if necessary engage the horse owner in a series of specific questions for the express purpose of collecting complete historical data including history of exposure to biting insect or arthropod vectors and travel history. For horses presenting with history of chronic and/or sporadic exercise intolerance, fluctuating degrees of unsoundness, deficits in neurotransmission and/or chronic circulation deficits and/or relapsing symptoms such as those reported herein, testing for bartonellosis should be considered. The extent to which *Bartonella* bacteremia might contribute to behavioral abnormalities is beyond the scope of this report, however histories of neurotic, argumentative, unruly and/or aggressive behavior patterns as seen in other species with chronic bartonellosis [28] lend support to a higher index of suspicion when considering testing options.

We conclude that the extent to which bartonellosis causes chronic disease manifestations in horses deserves additional research consideration. In-depth, sequential “lifetime” studies that span years in duration may be required to establish whether some or all of the symptoms described in the horses in this case series are causally associated with bartonellosis. It is possible that persistent intravascular infection with *Bartonella* spp. contributes to complex disease expression in horses, as seemingly occurs in humans and other animal species.

Competing Interest

In conjunction with Dr. Sushama Sontakke and North Carolina State University, Dr. Breitschwerdt holds U.S. Patent No. 7,115,385; Media and Methods for cultivation of microorganisms, which was issued October 3, 2006. He is the chief scientific officer for Galaxy Diagnostics, Inc. a company that provides diagnostic testing for the detection of *Bartonella* species infection in animals and human patients. Dr. Natalie Cherry is the Laboratory Supervisor and Research Specialist for Galaxy Diagnostics Inc. The remaining authors have no potential conflicts.

Source of Funding

This research received no specific grant or funding from any agency in the public, commercial, or not-for-profit sectors. This research was supported in part by Galaxy Diagnostics, Inc. and donations to the North Carolina State Veterinary Medical Foundation Vector Borne Diseases Research Fund.

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DOI: [10.19080/AIBM.2017.04.555646](https://doi.org/10.19080/AIBM.2017.04.555646)

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