Identification of *Bartonella henselae* in the Liver of a Thoroughbred Foal with Severe Suppurative Cholangiohepatitis

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A 3.5-month-old, 170-kg, Thoroughbred colt was referred to University of Pennsylvania's New Bolton Center for evaluation of dullness and dermatitis of the pasterns and muzzle. The pastern dermatitis affected all 4 limbs, appeared 10 days before presentation, and was treated by the owner with an iodine solution and triple antibiotic ointment, with no improvement. Dermatitis of the muzzle appeared 2 days before presentation and was treated with dexamethasone (0.02 mg/kg IV once). The day before presentation, the colt was noted to be uncharacteristically quiet with a dull demeanor. Before these problems, the foal was reported to have been healthy and thriving. Serum biochemistry analysis (SBA) performed in the morning of presentation identified increased liver enzyme activities, which prompted referral of the foal. The foal was born in Kentucky and had been moved to its current farm in Maryland 1 month earlier. The farm was reported to have problems occasionally with *Rhodoccocus equi* (*R. equi*) pneumonia in its foals. The foal had been routinely vaccinated and dewormed.

On physical examination, the colt had a body condition score of 4/9 and was dull with decreased response to environmental stimuli. Heart rate (60 beats/min) and rectal temperature (100.4F) were normal but tachypnea was present (36 breaths/min). Sclerae and oral mucous membranes were mildly icteric. No heart murmurs or arrhythmias were auscultated. Normal bronchovesicular sounds were audible on rebreathing examination, but the foal coughed several times after removal of the rebreathing bag. The submandibular lymph nodes were considered normal on palpation. No abnormalities were identified on abdominal auscultation. Despite dullness, the foal had a good appetite for hay and was observed to nurse well. Erythematous lesions with crusts were present in regions of unpigmented and pigmented skin, including all 4 pasterns (worst in the hind limbs) and the muzzle (unpigmented). Joint palpation was within normal limits and no

Abbreviations:

BAPGM	*Bartonella* alpha-Proteobacteria growth medium
CSF	cerebrospinal fluid
H&E	hematoxylin and eosin stain
HCT	hematocrit
HGB	hemoglobin
IFA	indirect immunofluorescence antibody
MCV	mean corpuscular volume
RBC	red blood cells
SAMe	S-adenosylmethionine
SBA	serum biochemistry analysis
TMS	trimethoprim-sulfamethoxazole
TTW	transtracheal wash

obvious lameness was seen. Aside from its very dull demeanor, neurologic examination was normal.

Clinical laboratory tests (CBC and SBA) identified the presence of mature neutrophilia (10,200/μL; reference range, 2,200–8,100/μL), hyperproteinemia (8.6 g/dL; reference range, 4.6–6.9 g/dL), hyperfibrinogenemia (679 mg/dL; reference range, 200–400 mg/dL), increases in GGT (184 IU/L; reference range, 12–45 IU/L) and SDH (19.65 IU/L; reference range, 0.30–7.0 IU/L) activities, increased total bilirubin concentration (7.1 mg/dL; reference range, 0.1–1.9 mg/dL), increases in serum lipids (triglycerides and cholesterol, 116 mg/dL; reference range, 11–52 mg/dL and 149 mg/dL; reference range, 51–109 mg/dL, respectively), and increased bile acid concentration (16.9 μmol/L; reference range, 1.0–8.6 μmol/L). Red blood cell indices (HCT, RBC numbers, HGB, MCV) were within normal limits. Direct bilirubin concentration was within the normal reference range. Ammonia concentration was within the normal reference range.

Based on the history, physical examination and abnormal liver function test results, a hepatopathy with secondary photosensitization was suspected. Abdominal ultrasonography was performed to further characterize the hepatopathy, and identified an enlarged liver that displaced peritoneal viscera caudally. The hepatic parenchyma had diffusely increased echogenicity and no biliary distension was present. The remainder of the abdominal ultrasound examination was normal.

Because the foal had signalment (age), history (endemic *R. equi* on the farm), physical examination (cough, tachypnea), and hematologic (inflammatory leukogram and hyperfibrinogenemia) findings that could be consistent with a respiratory tract infection, thoracic ultrasonography and radiography also were performed.

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Thoracic radiography disclosed the presence of an alveolar pattern superimposed on the cardiac silhouette whereas thoracic ultrasonography identified multiple pleural irregularities and regions of pulmonary consolidation associated with the right ventral lung margins; findings from both modalities were consistent with mild focal bronchopneumonia. A transtracheal wash (TTW) was performed and aspirated samples were submitted for cytologic analysis, *R. equi* polymerase chain reaction (PCR)[a] and aerobic and anaerobic bacterial culture and antimicrobial susceptibility testing, in an attempt to identify an underlying infectious etiology for the pneumonia. Cytologic analysis was normal and *R. equi* PCR was negative. Bacterial culture yielded growth of *Pasteurella pneumotropica* and *Streptococcus dysgalactiae ss equisimilis*.

The foal then was placed under general anesthesia to perform additional diagnostic procedures including an ultrasound-guided liver biopsy, skin biopsies and cerebrospinal fluid (CSF) collection. A right-sided ultrasound-guided liver biopsy was performed with a Tru-Cut[b] needle and submitted for histologic analysis as well as aerobic bacterial culture and antimicrobial susceptibility testing. Bacterial culture yielded no aerobic growth. Histologic evaluation of the liver biopsy

specimens (Fig 1A) identified moderate numbers of neutrophils, lymphocytes, plasma cells, and proliferating spindle cells extending from portal to centrilobular zones, dissecting between and often disrupting hepatic cords. Neutrophils were concentrated within portal zones and often surrounded, but rarely infiltrated the bile ductules. Multifocal individual hepatocyte necrosis was present with occasional discrete clusters of neutrophils forming microabscesses. Remaining hepatocytes often contained cytoplasmic gold to brown granular pigment. No etiologic agents were observed on H&E or Gram-stained sections. A Warthin-Starry stained section disclosed occasional pleomorphic, 3–7 µm long, curved, blunt spiral and beaded agyrophilic organisms that had morphology consistent with *Bartonella* spp. spirochetes (Fig 1B). Histologic results of the liver biopsy specimen were consistent with severe subacute neutrophilic cholangiohepatitis with bridging fibrosis. Skin biopsy specimens of the muzzle and pasterns were obtained for histologic analysis, aerobic and anaerobic bacterial culture and antimicrobial susceptibility testing. Photosensitization vasculopathy and secondary pyoderma were diagnosed, and growth of *Staphylococcus aureus ss aureus* was obtained. To eliminate central nervous system disease as a cause of
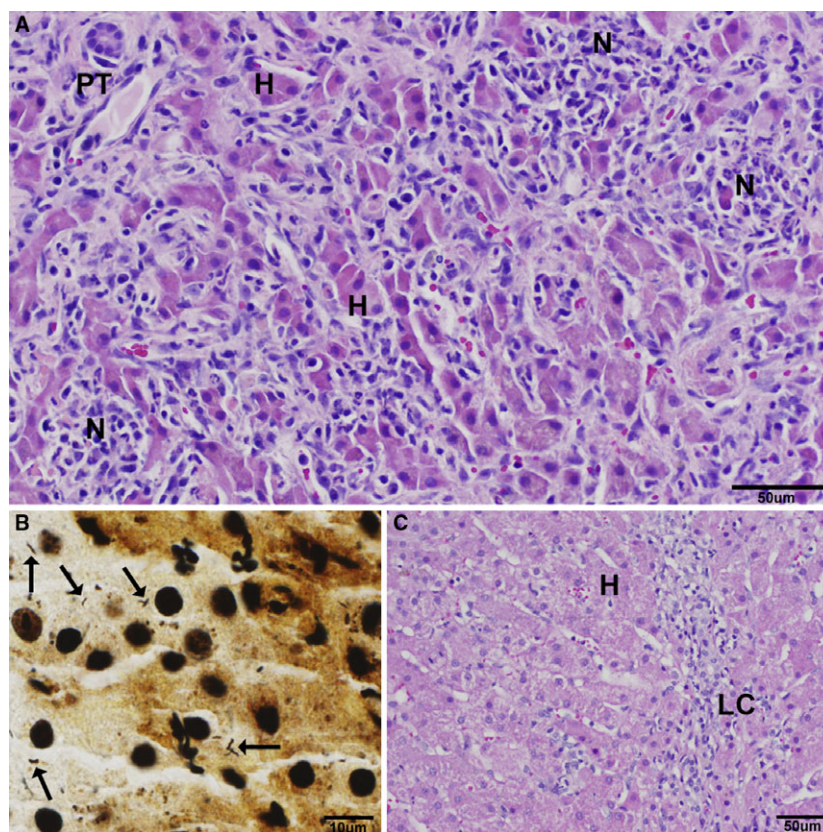
Fig 1. Photomicrographs of initial (**A**, **B**) and follow-up (**C**) Tru-Cut[b] liver biopsies from the foal. H&E stain of the initial biopsy specimen (A) shows severe neutrophilic inflammation (N) admixed with proliferating spindle cells effacing hepatic cords, and dissecting among remaining hepatocytes (H), bridging portal tracts (PT) and extending to centrilobular zones. A Warthin-Starry stain of the initial biopsy specimen (B) reveals 3–5 µm long curved to spiral organisms (arrows) with morphology consistent with *Bartonella* spp. An H&E stain of the follow-up biopsy specimen (C) shows mild lymphocytic inflammation (LC) within portal zones but restoration of the hepatocytes (H) and hepatic cords.

the markedly dull demeanor, atlanto-occipital puncture was performed and CSF submitted for cytologic analysis, which was normal.

After discharge of the foal, additional investigation of the spirochete structures seen on histologic evaluation of the liver biopsy specimen was pursued. Paraffin-embedded liver tissue was submitted for *Bartonella* spp. PCR[c] and was negative. Unfortunately, at the time, fresh-frozen tissue was no longer available for enrichment PCR.

The foal was hospitalized for 12 days and initially treated with clarithromycin[d] (7.5 mg/kg PO q12 h) and rifampin[e] (5 mg/kg PO q12 h) targeting potential *R. equi* pneumonia (days 2–7) and before knowledge of the etiology of the hepatopathy. Flunixin meglumine[f] (1.1 mg/kg IV q12 h) also was administered (days 2–4). Once liver histology results were available and TTW *R. equi* PCR was confirmed to be negative (day 7), the foal was switched to trimethoprim-sulfamethoxazole (TMS[g], 30 mg/kg PO q12 h) and maintained on rifampin (5 mg/kg PO q12 h) as an empirical broad-spectrum antibiotic regimen for treatment of the cholangiohepatitis, targeting enteric bacteria. TMS and rifampin also were considered reasonable antibiotic choices should *Bartonella* spp. be implicated in the disease process. Pentoxifylline[h] (7.5 mg/kg PO q12 h) and S-adenosylmethionine (SAMe,[i] 12 mg/kg PO q24 h) were used for their potential hepatic antifibrotic and antioxidant effects, respectively. The skin lesions were treated conservatively by cleaning with a povidione-iodine solution[j] q48 h, topical application of silver sulfadiazine[k] ointment and bandaging. The bacterial organisms cultured from the TTW and skin biopsy specimens were sensitive to all antimicrobial drugs that the foal had received. Fibrinogen and total bilirubin concentrations steadily decreased over the course of hospitalization. On day 12, fibrinogen and total bilirubin concentrations were 593 mg/dL and 3.6 mg/dL, respectively. However, GGT activity increased during treatment in hospital, and was 339 IU/L on day 12, despite antimicrobial changes and the foal showing steady clinical improvement. By discharge (day 13), the foal had a bright demeanor, normal vital parameters and the skin lesions showed considerable improvement. The foal was discharged with instructions for continued treatment with TMS (30 mg/kg PO q12 h) and liver protectants (SAMe 12 mg/kg PO q24 h and pentoxifylline 7.5 mg/kg PO q12 h) until normalization of the liver enzyme activities. Adjustments to treatment would be made based on clinical response and results of weekly hematology and SBA. Recommendations were made to keep the foal away from direct sunlight until resolution of the liver disease.

A 4-month follow-up visit was scheduled because GGT activities remained increased despite the foal having normal physical examination findings with complete resolution of the photosensitization dermatitis. A liver ultrasound examination was repeated and showed sharp liver margins that did not extend past the costochondral junctions. The hepatic parenchymal echogenicity still was mildly diffusely increased. Compared to the previous ultrasound examination, the liver was considerably decreased in size, possibly indicating improving or resolving chronic liver disease. An ultrasound-guided right-sided liver biopsy was repeated under standing sedation. Histologic analysis identified the presence of variable numbers of lymphocytes admixed with few spindle cells and minimal fibrous connective tissue surrounding and infiltrating portal and a few centrilobular zones (Fig 1C). Portal zones typically had increased bile duct profiles and occasionally adjacent hepatocytes were entrapped by proliferating fibroblasts, but inflammatory cells were not observed within bile ducts. A Warthin-Starry stain did not identify structures with bacterial morphology. Histologic analysis confirmed mild multifocal chronic lymphocytic hepatitis. Liver tissue obtained from the biopsy was submitted for aerobic bacterial culture and antimicrobial susceptibility, which yielded no growth. Enrichment PCR for *Bartonella* spp.[c] was performed on the fresh-frozen liver specimen and was positive after 14 days and sequence verification confirmed the PCR product as *Bartonella henselae*. Despite clinical improvement and improved ultrasonographic and histologic appearance of the liver, the foal's GGT activity had continued to increase and was 542 IU/L at the 4-month recheck. At this time, antimicrobial treatment was switched to minocycline[l] (4 mg/kg PO q12 h). Repeat assessment of GGT activities at 5 and 6 months identified decreasing activity, until normalization at 7 months (37 IU/L). Two weeks after normalization of GGT activity, all treatments were discontinued and the foal was reported to be thriving.

To the authors' knowledge, this is the first reported case of cholangiohepatitis in a foal associated with identification of *B. henselae* in the liver. Natural infection in sick and healthy horses with *B. henselae* and other *Bartonella* species has been reported recently, with amplification of the organism from blood or enrichment cultures.[1] Also, experimental infection of healthy horses with *B. henselae* produced detectable bacteremia and seroconversion in naïve horses.[2] A few reports of *B. henselae*-associated infections in horses have been reported previously, but none have included enrichment culture identification of *B. henselae* in the liver of a foal or adult horse. Bacteremia in a horse caused by *B. henselae* was first reported in 2008; the organism was identified in a mare with presumptive vasculitis and in a gelding with chronic arthropathy, respectively.[3] Johnson et al reported abortion of an equine fetus because of infection with *B. henselae*, with presence of the organism confirmed in the lung, liver and kidney of the fetus.[4] *B. henselae* also was identified recently in a 2-year-old mare in Germany that succumbed to hemolytic anemia; the organism was identified in the blood, spleen, and bone marrow.[5]

Although this report is the first to describe *Bartonella*-associated liver disease in an equid, bartonellosis has a wide range of clinical manifestations in other species, including hepatosplenic disease. In people, the typical acute *B. henselae* infection, known as cat scratch disease, most commonly results in fever and

regional lymphadenopathy.[6,7] An atypical form of *B. henselae* infection resulting in granulomatous hepatitis has been reported in humans, most commonly in children and in liver transplant recipients.[8,9] A recent report by Venderhayden et al reported necrotizing granulomatous hepatitis in an immunocompetent woman, and *B. henselae* was amplified by PCR using blood and surgically removed liver tissue.[10] Gillespie et al. also described 2 dogs with granulomatous hepatitis in which *B. henselae* and *B. clarridgeiae* DNA was amplified from the liver.[11] In dogs and people, *B. henselae* also has been associated with peliosis hepatis, a vasculoproliferative disorder resulting in formation of multiple blood-filled cavities in the liver.[12,13]

Bartonella henselae is a gram-negative facultative intracellular, fastidious organism that is particularly difficult to culture, making diagnosis challenging.[6] With the advent of enrichment PCR techniques, the organism may be more readily identifiable. *B. henselae* transmission usually occurs through arthropod vectors, such as biting fleas, flies, and ticks.[7] As evidenced by cat scratch disease, *Bartonella* spp. also can be transmitted mechanically, through scratches, bites, and wounds.[7] Although not known in horses, it is probable that transmission occurs through these same mechanisms. In this foal, one can only speculate about how *Bartonella* spp. infection occurred, but transmission by an arthropod or mechanical vector seems possible, with subsequent hematogenous spread and colonization of the liver.

Cholangiohepatitis in horses is associated with biliary stasis and usually occurs after ascending infection of the biliary tree from the small intestine or by hematogenous spread of bacteria through the portal circulation.[14] Enteric bacteria such as *E. coli* and *Salmonella* spp. have been cultured from the liver of cholangiohepatitis cases, which further supports the hypothesis of ascending infection.[14] In contrast, hepatic bartonellosis likely occurred via the hematogenous route, which is the suspected pathogenesis in other species.[7] Hepatogenic photosensitization likely occurred as a result of cholestasis, causing the photodynamic agent phylloerythrin to accumulate in the skin, where it then reacted to sunlight.[15]

In people, antimicrobial treatment of typical cat scratch disease is not usually recommended and does not appear to alter the clinical outcome.[16] However, treatment of other clinical syndromes, for example, hepatosplenic or vascular manifestations, in both immunocompetent and immunosuppressed individuals is recommended, but conclusive evidence as to the best treatment is lacking. Unfortunately, whereas *Bartonella* spp. are susceptible to many antibiotics in vitro, this susceptibility often does not correlate well with in vivo susceptibility and the ability to treat clinically.[6,7,17,18] In humans, doxycycline, erythromycin and rifampin are commonly recommended antibiotics for treatment of various *Bartonella* infections, but clinical amelioration also has been documented with other classes of drugs.[7] For hepatosplenic syndromes in people, there is anecdotal evidence that treatment with gentamicin,

TMS, rifampin and ciprofloxacin, used alone or in combination, has been effective.[6,8] In the foal of this report, clinical response was observed during treatment with TMS and rifampin, but *B. henselae* still was identified in the liver by enrichment PCR at a 4-month re-evaluation. A decrease and normalization in GGT activity was only observed once the foal had been switched to minocycline. Minocycline may have had better intracellular penetration and thus was more successful in eliminating the underlying infection, but this remains speculative.

Additional diagnostic tests could have been considered in this case including serologic testing for *Bartonella* spp. However, it was recently shown that IFA methods were not helpful in identifying prior exposure or current infection in foals and mature horses in which *Bartonella* spp. bacteremia had been confirmed by enrichment PCR.[1] In people and dogs *with B. henselae* bacteremia, poor correlation between IFA seroreactivity and PCR has been documented.[19] Investigating the presence of bacteremia with BAPGM blood enrichment culture techniques, as described by Duncan et al, also could have been considered.[20]

The findings in this case should prompt clinicians to consider *B. henselae* in the differential diagnosis of hepatitis and other hepatic manifestations in horses. Furthermore, enrichment PCR on fresh-frozen tissue should be prioritized, to increase likelihood of diagnosis, considering the difficulties in identifying the organism.

Footnotes

Acknowledgment

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