

# *Babesia equi*-induced anemia in a Quarter Horse and subsequent regulatory response

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**Case Description**—A 7-year-old Quarter Horse gelding used for unsanctioned racing was examined because of fever and anorexia.

**Clinical Findings**—Physical examination revealed fever, tachycardia, and tachypnea. Results of a CBC indicated anemia and mild thrombocytopenia. Results of microscopic examination of a blood smear indicated piroplasms in erythrocytes, consistent with *Babesia* spp. Regulatory authorities were contacted, and results of serologic testing at the National Veterinary Services Laboratories confirmed acute *Babesia equi* infection.

**Treatment and Outcome**—Equids on the home premises of the index horse were placed under quarantine. Those equids were tested for piroplasmiasis, and 6 of 63 horses had positive results for *B. equi*. Another horse that had previously been housed on the index premises also had positive results for *B. equi*. Competent tick vectors for piroplasmiasis organisms were not identified. All 8 horses with piroplasmiasis were Quarter Horses that participated in unsanctioned racing and were trained by the same person. Two of the horses were illegally removed from the index premises; these 2 horses and the other horse with piroplasmiasis that was previously housed on the index premises could not be found. The other 5 horses with piroplasmiasis were euthanized. Investigators concluded that transmission of *B. equi* among horses was most likely iatrogenic.

**Clinical Relevance**—The United States has been considered piroplasmiasis free. However, veterinarians should consider piroplasmiasis in horses with signalments and clinical signs similar to those of the index horse of this report. Regulatory authorities should be contacted regarding horses in which piroplasmiasis is suspected. (*J Am Vet Med Assoc* 2013;242:992–996)

On June 1, 2009, a 7-year-old Quarter Horse gelding that was being used for unsanctioned horse racing was examined at Kansas State University Veterinary Medical Teaching Hospital because of fever, anorexia, and lethargy of 48 hours' duration. The horse had raced 8 days previously and had received an unknown amount of dexamethasone and furosemide the day prior to the initial examination. Communication with this

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## ABBREVIATIONS

CFT	Complement fixation test
IFA	Indirect immunofluorescent antibody assay
IMHA	Immune-mediated hemolytic anemia

owner was difficult, and a complete history was likely not obtained.

On day 1 (the day of hospital admission), physical examination revealed fever (39.7°C [103.5°F]), tachycardia (76 beats/min), and tachypnea (72 breaths/min). Mucous membranes were mildly hyperemic, and gastrointestinal tract sounds were increased in frequency. Results of rectal examination were unremarkable, and nonmalodorous, soft feces were present in the rectum. A CBC revealed mild anemia (PCV, 26%; reference interval, 31% to 47%), monocytosis (1,800 cells/μL; reference interval, 100 to 1,000 cells/μL), thrombocytopenia (64,000 platelets/μL; reference interval, 100,000 to 350,000 platelets/μL), and hyperfibrinogenemia (700 mg/dL; reference interval, 100 to 400 mg/dL). Serum biochemical analyses revealed azotemia (creatinine concentration, 1.9 mg/dL [reference interval, 0.8 to 1.8 mg/dL]; BUN concentration, 28 mg/dL [reference interval, 9 to 22 mg/dL]); hyperbilirubinemia (8.0 mg/dL; reference interval, 0.5 to 2.7 mg/dL) with an increased indirect bilirubin concentration (7.7 mg/dL; reference interval, 0.3 to 2.6 mg/dL), and sodium, chloride, and potassium concentrations that were slightly lower than

the lower limits of the reference intervals. Urinalysis revealed highly concentrated urine (urine specific gravity, 1.037) and 1+ blood and 2+ protein reactions on the assay reagent strip. Results of thoracic and abdominal ultrasonographic examinations were unremarkable. The primary differential diagnosis at the time of the initial examination was colitis; this differential diagnosis was determined on the basis of fever, soft feces, anorexia, dehydration, serum electrolyte abnormalities, and hyperemic mucous membranes. The horse was moved to an isolation stall. Treatments included lactated Ringer's solution (120 mL/kg/d [55 mL/lb/d], IV), potassium penicillin (20,000 U/kg [9,091 U/lb], IV, q 6 h), gentamicin (6.6 mg/kg [3.0 mg/lb], IV, q 24 h), flunixin meglumine (1.1 mg/kg [0.5 mg/lb], IV q 12 h), and omeprazole (1 mg/kg [0.45 mg/lb], PO, q 24 h).

A CBC and serum biochemical analyses were repeated on day 2, results of which revealed increased severity of anemia (PCV, 15%), a plasma protein concentration within the reference interval, and mild thrombocytopenia (91,000 platelets/ $\mu$ L); azotemia and serum electrolyte abnormalities had resolved. Results of an ELISA for detection of equine infectious anemia were negative. Results of a direct immunofluorescence assay were positive for erythrocyte membrane-bound IgM (percentage of erythrocytes with positive results, 16%) and IgG (6%); results were negative for IgA (< 1%; reference intervals for IgG, IgM, and IgA, < 3%). Because the horse was anemic and had a history of participation in unsanctioned Quarter Horse racing, Wright-Giemsa staining and microscopic examination of a blood smear were requested; results indicated rare ringed piroplasms (diameter, 0.5 to 1  $\mu$ m) in erythrocytes, which were considered consistent with *Babesia* spp (Figure 1). Erythrocyte autoagglutination was detected during preparation of the blood smear and confirmed with a 1:10 saline (0.9% NaCl) solution dispersion test. A tentative diagnosis of piroplasmosis and IMHA was made. The office of the Kansas State Veterinarian was contacted, and the federal Area Veterinarian-in-Charge sent a foreign animal disease diagnostician to collect a blood sample for submission to the National Veterinary Services Laboratories, Ames, Iowa, for fur-

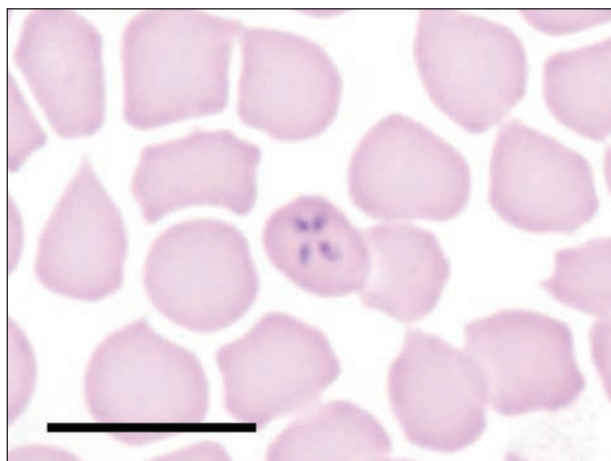


Figure 1—Photomicrographic image of a blood smear of a 7-year-old Quarter Horse gelding (index horse) with acute *Babesia equi* infection. Notice the intraerythrocytic *B. equi* merozoites in a Maltese cross formation. Modified Wright stain; bar = 10  $\mu$ m.

ther testing. No ticks were found on the horse during a thorough examination. The horse was photographed for identification purposes.

Antimicrobial treatments were changed before results of further tests were available. Penicillin and gentamicin were discontinued, and oxytetracycline was administered (6.6 mg/kg, IV, q 12 h). The rate of IV administration of fluids was decreased (60 mL/kg/d [27 mL/lb/d]) for 12 hours, then administration was discontinued. Administration of flunixin meglumine and omeprazole was continued at the same doses that were used initially. The horse remained lethargic, tachycardic, and febrile during the next 48 hours, despite treatments. Therefore, on day 3, imidocarb dipropionate (2.2 mg/kg [1 mg/lb], IM, q 24 h for 2 days) was administered on the basis of the presumptive diagnosis of piroplasmosis and the need to provide supportive care during the regulatory investigation. Modest improvements in clinical signs and laboratory test results were detected during the subsequent 48 hours. Fever and anorexia resolved, and the heart rate of the horse decreased to 56 beats/min. Results of a CBC indicated a PCV of 22% and a platelet count (161,000 platelets/ $\mu$ L) within the reference interval. Intraerythrocytic piroplasms were not detected during examination of a blood smear. Local signs of pain and edema developed at the imidocarb injection site within 24 hours after administration of the first dose. No other signs of imidocarb toxicosis were detected.

The results of tests performed at the National Veterinary Services Laboratories were available on day 5. Results of a CFT were positive for *Babesia equi* (at a 1:5 dilution on a microtiter plate) and negative for *Babesia caballi*. However, results of a CFT performed via titration in tubes at an endpoint dilution of 1:5 were negative. Results of a competitive ELISA and an IFA were negative for both *B. equi* and *B. caballi*. Results of real-time and conventional nested PCR assays were positive for *B. equi* and negative for *B. caballi*. Examination of a blood smear revealed intraerythrocytic structures consistent with *Babesia* spp. Results of assays were interpreted by personnel at the National Veterinary Services Laboratories and indicated that the test results were consistent with acute infection of the horse with *B. equi*.

Because of the lack of concordance in results of the 2 CFTs, serologic testing was repeated on day 8. A blood sample was collected from the horse by the foreign animal disease diagnostician, and results of repeated analysis were available on day 10. Results of a CFT and an IFA were positive for *B. equi* at dilutions of 1:10 and 1:80, respectively. Results of PCR (real-time and conventional nested) assays were positive for *B. equi*. Results of a competitive ELISA and examination of a blood smear were negative for *B. equi* and *B. caballi*. A blood sample was resubmitted on day 11 for performance of a direct immunofluorescence assay to detect erythrocyte surface antibodies; results were negative. On day 12, findings of a physical examination were unremarkable (other than imidocarb injection-site reactions) and the PCV had increased to 41%. Previously administered medications were discontinued, and doxycycline (10 mg/kg [4.5 mg/lb], PO, q 12 h) and phenylbutazone (2 mg/kg [0.9 mg/lb], PO, q 24 h) were

administered to the horse for 7 days (to reduce inflammation at imidocarb injection sites).

After confirmation of a diagnosis of *B equi* infection in the index horse of the present report, Missouri state and federal animal health personnel instituted a quarantine for the home premises of the index horse (index premises) located in Jackson County, Mo, on June 6, 2009. The index premises, where the index horse had been housed since January 2009, comprised a boarding stable that housed 63 other equids (59 horses, 3 ponies, and 1 mule). All equids at the facility were tested for piroplasmiasis by personnel at the National Veterinary Services Laboratories. Results reported for blood samples collected on June 9 and 12, 2009, indicated an additional 6 horses on the premises had positive results for *B equi*. On June 9, 5 of these horses had positive results for a competitive ELISA, 3 had positive results for a CFT, 5 had positive results for an IFA, and 4 had positive results for a PCR assay. On June 12, 5 of these horses had positive results for a competitive ELISA, 2 had positive results for a CFT, 6 had positive results for an IFA, and 2 had positive results for a PCR assay. All 6 of these horses with positive results for *B equi* were Quarter Horses participating in unsanctioned or bush track racing. The other 57 equids at the facility were tested multiple times for detection of *B equi* and *B caballi* infection during a 30-day surveillance period; results for these horses were negative.

Fourteen other horses had recently been moved from the index premises in Missouri prior to detection of piroplasmiasis in the index horse; these horses were located and tested to detect *B equi* and *B caballi* infection. Thirteen of these horses had negative results for piroplasmiasis, and one of these horses (a Quarter Horse participating in unsanctioned racing in Kansas) had positive results for *B equi* infection via competitive ELISA, IFA, and PCR assay but had negative results for a CFT. Thus, 8 Quarter Horses with a history of participation in unsanctioned racing under the management of the same trainer were identified that had positive results for *B equi* infection.

A complete examination of all equids on the index premises was performed for detection of ticks as a part of the regulatory response. No ticks were found on any of the horses. Additionally, a complete tick survey of the index premises was conducted from June 23 to 29, 2009, by personnel of the Southeast Cooperative Wildlife Disease Study, which included tick dragging and wildlife capture techniques. During this 7-day period, only 4 ticks were collected from the index premises, none of which were identified as competent tick vectors for the organisms that cause piroplasmiasis in equids.

The index horse was discharged from the Kansas State University Veterinary Medical Teaching Hospital on day 12 under state and federal regulatory supervision. The federal Area Veterinarian-in-Charge and the Kansas state livestock commissioner were present when the owner came to the hospital for discharge of the horse. The horse was loaded on a trailer, the trailer was officially sealed, and the Kansas livestock commissioner followed the trailer back to the farm of origin in Missouri, which was already under quarantine. The 7 horses on the index premises in Missouri that had

positive results for *B equi* infection (including the index horse) were housed together in a group of stalls away from the other horses on the premises. These horses received radiofrequency identification implants, and their stalls were padlocked. The affected horses, their stalls, and the interior and perimeter of the barn where they were housed were sprayed with an acaricide.

After in-depth discussions with the owners and trainer of the horses with piroplasmiasis, the decision was made on June 17 that the infected horses would be euthanized under the supervision of Missouri state and federal animal health personnel the next day. When Missouri state and federal officials arrived on June 18 to euthanize the horses, they found that 2 of the horses with piroplasmiasis had been illegally removed from the premises. Removal of horses had entailed cutting of paddocks on stalls and the gate of the premises during the previous night. The 2 missing horses belonged to 1 owner who was not present at the facility with the other owners on the scheduled euthanasia day. The 5 horses with piroplasmiasis that remained on the premises were euthanized on June 18 (necropsies were not performed).

Kansas state and federal officials were unable to locate the horse with *B equi* infection that had been participating in unsanctioned racing in that state and were unable to locate the owner of that horse. Law enforcement officials were enlisted to assist in locating the 3 missing horses with positive results for *B equi* infection, but after months of investigation, the missing horses were not found. During the investigation, it was suggested that all 3 of the missing horses were likely moved illegally to Mexico within a few days after they were reported missing, but no official record of the horses entering Mexico could be found. Quarantine of the index premises in Missouri was discontinued on July 9, 2009, after all requirements were met (including repeated testing of horses with negative results for *B equi* infection).

## Discussion

Piroplasmiasis is endemic in horses in many parts of the world. The United States has been considered free of piroplasmiasis since 1988<sup>1</sup>; however, outbreaks of the disease in horses in the United States have recently been reported.<sup>2,3</sup> In 2008, 20 horses with piroplasmiasis were identified during an outbreak of *B equi* infection in Florida.<sup>2,3</sup> Potential tick vectors were identified via tick surveillance during that disease outbreak, but all ticks collected had negative PCR assay results for *Babesia* spp. Results of comprehensive tick surveillance performed at the index premises in Missouri of the present report indicated few ticks were present on the premises and no competent tick vectors for *B equi* were identified. As was found during the piroplasmiasis outbreak in Florida in 2008, no evidence of involvement of ticks in the spread of *B equi* among horses on the index premises was identified during the investigation of the present report.

The epidemiological investigation of horses of the present report was challenging. The owners of the horses that had positive results for *B equi* only spoke Span-

ish. Despite the use of Spanish-speaking translators, the owners were reluctant to speak with the state and federal officials. Additionally, the person determined to be the owner of the index horse provided conflicting information regarding his identity, involvement in ownership of the horse, and knowledge regarding locations of the horses with piroplasmiasis that had been illegally removed from the quarantined index premises. That same owner threatened hospital personnel when his horse was placed under quarantine at the Kansas State University facility, which prompted implementation of police oversight of the horse and its caretakers. Several other horse owners were evasive during the interview process.

The only useful epidemiological facts identified regarding the 8 horses of the present report that had piroplasmiasis included the routine involvement of the horses in unsanctioned racing. Several owners and the trainer admitted during interviews that the horses were raced during weekends at local, unsanctioned, privately owned exercise tracks that had been rented for that purpose. All affected horses had been trained by a single trainer while they were stabled on the index premises in Missouri. At least one of the affected horses had likely been in Mexico previously. The most recent race that the index horse participated in before developing clinical signs was at an unsanctioned racetrack during May 23 to 25, 2009.

Personnel involved in unsanctioned racing often use unsanitary methods that can spread blood-borne pathogens, such as *Babesia* spp, among horses. Administration of large volumes of blood from a donor horse to a racing participant prior to a race day (ie, blood doping) has a high risk for transmission of disease among horses. Reuse of needles or syringes for IV administration of drugs or vitamins to horses at a racetrack or barn just prior to a race is another common unsanitary practice of personnel at unsanctioned racing venues.<sup>2,a</sup>

Some horses that are imported (either legally or illegally) to the United States from Mexico regularly participate in unsanctioned racing events. Horses from Mexico are a source of *Babesia* spp infection for horses participating in unsanctioned racing, as determined in an epidemiological investigation conducted during the 2008 equine piroplasmiasis outbreak in Florida.<sup>2,a</sup> Because horses infected with *Babesia* spp, especially *B equi*, can be lifelong carriers of the organisms, participation of horses infected with *Babesia* spp in unsanctioned racing and the high-risk practices of personnel associated with that activity increase the potential for transmission of *Babesia* spp to other horses at unsanctioned racing venues.

Results of the epidemiological investigation of the present report indicated that the spread of *B equi* among horses was most likely attributable to unsanitary practices of personnel (ie, blood doping and reuse of needles and syringes) involved in unsanctioned racing activities. The source of *Babesia* spp infection was likely a horse that had spent a portion of its life in Mexico. Transmission of *Babesia* spp organisms to the index horse of the present report likely occurred via administration of blood or prerace medications (with a contaminated needle or syringe) just prior to the unsanctioned races conducted from May 23 to 25, 2009.

A diagnosis of IMHA was made for the index horse of this report on the basis of positive results of direct immunofluorescence erythrocyte antibody tests and detection of autoagglutination. The cause of IMHA in this horse was unknown, but could have been attributable to *B equi* infection or exposure to erythrocytes of other horses. The mechanism of hemolysis during piroplasmiasis is unknown, to the authors' knowledge; proposed mechanisms include mechanical lysis of RBCs, production of hemolytic factors by *Babesia* spp organisms, and immune-mediated mechanisms. Results of another study<sup>4</sup> indicate 10% of *Babesia*-infected dogs in Hungary have IMHA. Other investigators<sup>5-7</sup> identified antierythrocyte membrane antibodies in dogs with *Babesia gibsoni* infection via ELISA and immunoblotting analyses. The strain of *Babesia* sp infecting an animal may affect the pathogenesis of erythrocyte destruction. Results of another study<sup>8</sup> indicate 4 of 6 dogs infected with *Babesia canis vogeli* had immunoglobulins on the surfaces of erythrocytes; however, the 24 dogs in that study that were infected with *Babesia canis canis* had negative results for erythrocyte surface immunoglobulins. Iatrogenic exposure to RBCs of other animals may also have caused IMHA in the index horse. A diagnosis of idiopathic IMHA without piroplasmiasis may have been made if the blood smear had not been carefully examined. Characteristics of horses involved in the piroplasmiasis outbreak in Florida in 2008 alerted clinicians evaluating horses of the present report to the possibility of *Babesia* spp infection. Prior to the report<sup>2</sup> of the outbreak in Florida, it was considered unlikely that a horse in the geographic center of the United States would have piroplasmiasis. Findings of the investigation of this report and those of other recent reports<sup>2,3,9</sup> of piroplasmiasis in the United States indicate it is important to consider piroplasmiasis as a differential diagnosis for horses with evidence of IMHA.

The goal of treatment of the index horse with imidocarb was to resolve clinical signs of piroplasmiasis during the regulatory investigation, not to eliminate infection. The horse had minimal improvement in clinical signs after 3 days of other treatments, and results of serologic testing conducted by personnel at the National Veterinary Services Laboratories were pending. Repeated serologic testing was necessary for the index horse because of the lack of concordance in results of the 2 CFTs. The effects of imidocarb treatment on serologic tests and persistent infection of horses with piroplasmiasis have not been determined, although studies<sup>9,b</sup> to determine this information are being conducted. Results of another study<sup>10</sup> indicate that imidocarb administration results in seroconversion of horses from CFT seropositivity to CFT seronegativity for antibodies against *Babesia* spp. However, chronicity of infection affects serologic test results because horses can seroconvert from CFT seropositivity to CFT seronegativity for antibodies against *Babesia* spp, even without treatment.<sup>11,b</sup> Clinical signs of imidocarb toxicosis are common in horses and include salivation, colic, injection-site reactions, diarrhea, liver and renal disease, and, for some horses, death.<sup>1</sup> Piroplasmiasis is considered a foreign animal disease in the United States; therefore, veterinarians should contact animal

health regulatory authorities when they identify horses in which piroplasmiasis is suspected. Use of imidocarb should be limited to horses with confirmed piroplasmiasis, and such treatment should only be administered with oversight of regulatory personnel.

Confirmatory tests for piroplasmiasis (for clinically affected or chronically infected horses that may seem to be healthy) are serologic assays including CFTs, IFAs, and competitive ELISAs. Previously, CFTs were the standard tests for diagnosis of piroplasmiasis in horses in the United States. However, for chronically infected horses, CFTs are less sensitive than competitive ELISAs for determination of a diagnosis of piroplasmiasis.<sup>11–14</sup> The sensitivity and specificity of CFTs for diagnosis of piroplasmiasis in horses are 47% and 94%, respectively, whereas the sensitivity and specificity of competitive ELISAs for determination of that diagnosis are 96% and 95%, respectively.<sup>11–13</sup> Competitive ELISAs and IFAs are the tests recommended by the World Organization for Animal Health for detection of piroplasmiasis in horses traveling internationally.<sup>1</sup> However, as for the index horse of the present report, results of CFTs are typically positive and results of competitive ELISAs and IFAs are typically negative during acute infection of horses with *Babesia* spp. The sensitivity and specificity of IFAs for detection of piroplasmiasis in horses are unknown, to the authors' knowledge, but IFA tests are considered more sensitive than CFTs for detection of piroplasmiasis in chronically infected horses.<sup>14–16</sup> Nested PCR assays for *Babesia* spp are currently being used only for research purposes and not for regulatory purposes. The nested PCR assay can be used to detect a positive result for a percentage of parasitemia equivalent to 0.000006%.<sup>17</sup> Several laboratories in the United States have recently been approved for performance of competitive ELISAs to identify piroplasmiasis in horses being transported within or between states.<sup>18</sup> However, approved testing for horses in which piroplasmiasis is suspected can be conducted only at the National Veterinary Services Laboratories, and regulatory authorities must be involved in such testing. As was performed for the horses of the present report, multiple serologic tests (including CFTs) may be needed to confirm a diagnosis of piroplasmiasis during a disease outbreak.<sup>3</sup>

Horses participating in unsanctioned racing in the United States may be at high risk for exposure to *Babesia* spp. Veterinarians should consider piroplasmiasis as a differential diagnosis for horses with signalments and clinical signs similar to those of the index horse of the present report. A diagnosis of IMHA does not exclude the possibility of piroplasmiasis in horses. Diagnosis of piroplasmiasis requires performance of multiple diagnostic tests, and veterinarians must contact regulatory authorities regarding horses in which piroplasmiasis is suspected.

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