Equine Piroplasmosis

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Equine piroplasmosis (EP) is the disease caused by protozoan hemoparasites *Babesia caballi* and/or *B. (Theileria) equi*. The natural transmission of these parasites is through competent tick vectors. These blood parasites can also be transmitted by transfer of blood from infected to naïve horses through iatrogenic means. American Association of Equine Practitioners members played a key role in the identification of clinical cases of EP in the United States in 2008 and 2009. Through regulatory response and epidemiologic investigation, all known infected horses in the United States are under quarantine as of the spring of 2010. Interim guidance on management of positive and exposed horses has been developed. These guidelines are available through State Animal Health Officials and Federal Area Veterinarians in Charge in each state. Long-term guidelines for management of infected and exposed horses are under development. Equine practitioners can play a role in the identification and work-up of suspect cases and the education of their clients about EP. Authors' addresses: Department of Clinical Sciences, Colorado State University, College of Veterinary Medicine and Biomedical Sciences, Fort Collins, Colorado 80523 (Traub-Dargatz); Equine Program Manager, Division of Animal Industry, Florida Department of Agriculture and Consumer Services, 407 South Calhoun Street, Mayo Building Room 329B, Tallahassee, Florida 32399 (Short); United States Department of Agriculture:Animal and Plant Health Inspection Services Veterinary Services Western Region, 2150 Centre Avenue, Fort Collins, Colorado 80526 (Pelzel); Large Animal Clinical Sciences, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, Texas 77843-4475 (Norman); and United States Department of Agriculture Animal Disease Research Unit, Agricultural Research Services and Department of Veterinary Microbiology and Pathology, Washington State University, Pullman, Washington 99164 (Knowles); e-mail: Josie.Traub-Dargatz@colostate.edu. © 2010 AAEP.

1. Introduction

The goal of this article is to provide background on the causative agents, methods of transmission, clinical signs of disease, diagnostic testing, management of infected horses in the United States, treatment challenges, and history of equine piroplasmosis (EP) in the United States. In addition, short updates on recent outbreaks in the United States and research is provided.

2. Background on EP

Causative Agents

EP is a tick-borne disease that affects horses, donkeys, mules, and zebras. There are two distinctive EP causative agents, *Babesia caballi* and *B. (Theileria) equi*. It has been proposed that *B. equi* be given a taxonomic classification of *T. equi*,[1] whereas other researchers have suggested that a more accu-

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rate classification for this parasite falls between *Babesia* and *Theileria* based on genomic analysis.[2a] Until additional studies are available that determine a final taxonomic classification, for this paper, we will refer to the parasite as *B. (T.) equi* or *B. equi*.

Methods of Transmission

B. caballi and *B. equi* are transmitted by many of the same tick vectors, with multiple types of ticks implicated in the transmission of EP agents on a global perspective. Ticks serve as a reservoir of *B. caballi*, because the organism persists in the ticks through several generations, with transtadial and transovarial transmission occurring in some types of ticks.[3] In contrast, horses are the primary reservoir of *B. equi*, with no transovarial transmission shown to date.[3] Few countries are considered free from native transmission of EP agents through ticks, and the prevalence of EP infections is consistent with the distribution of known competent tick vectors.[3]

Dermacentor (Anocentor) nitens, the tropical horse tick, was reported to be the primary vector of *B. caballi* transmission during the 1960s outbreak of EP in Florida. A joint United States Department of Agriculture-Animal and Plant Health Inspection Services (USDA:APHIS) and State of Florida eradication program for *B. caballi* brought the outbreak under control through a multifaceted approach that emphasized tick control on horses and equine premises.[4] Prior to 2009, no tick transmission of *B. equi* was recognized in the United States.

The EP agents can also be transmitted by iatrogenic means. Procedures that move blood from an infected horse to a naïve horse through the reuse of equipment such as needles and syringes have been implicated in transmission of EP agents.[5] In addition, the use of horses that are carriers of the EP agent as a source of blood for transfusion could result in transmission.

Clinical Signs

Horses infected with either agent have similar clinical signs. Clinical signs of EP can include fever, anemia, icterus, and anorexia.[3] Digestive tract signs can occur, including colic, constipation, or diarrhea.[3] It has been reported that *B. equi* can be transmitted by intrauterine infection, leading to abortion or neonatal infection, but how often this occurs is not well-documented.[6]

In countries where EP is endemic, foals born to infected mares may be protected from clinical disease through ingestion of protective colostral antibodies; this is called premunition.[3] Thus, in some regions of the world where infection is common, little or no clinical disease may be observed in native horses. However, disease is frequently observed in adult horses suddenly introduced into areas with large numbers of infected ticks.

It is important to recognize that unapparent carriers represent the majority of infected horses.

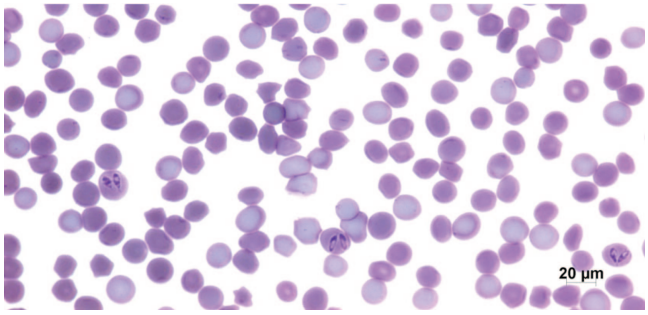

Fig. 1. Intraeyrthrocytic merozoite form of *B. caballi*. (Photo is courtesy of D. Knowles)

Because these horses appear clinically normal, diagnosis of infection relies on laboratory testing that will be described in a separate section.

Diagnosis

Clinical signs can alert the veterinarian to the possibility of EP. However, the clinical signs of EP are consistent with other diseases including equine infectious anemia (EIA), purpura hemorrhagica, idiopathic immune mediated anemia, and intoxications. In cases having clinical signs consistent with EP, examination of blood smears can assist in diagnosis. Giemsa staining of blood smears followed by careful microscopic examination can reveal the intraerythrocytic parasites in acute cases. *B. caballi* can appear pyriform-shaped and occurs in pairs (Fig. 1), whereas *B. equi* appears as four pyriform parasites in a Maltese-cross formation (Fig. 2).[3] Because the parasitemia can be very low in horses that have recovered from clinical disease, the examination of direct blood smears may not allow for detection of the infection in chronic carriers.

To confirm the diagnosis in clinical cases and detect infection in unapparent chronic carriers of EP agents, serologic tests have been used. There are three types of serologic tests that are used to detect antibodies to *B. caballi* and *B. equi*. These include the complement fixation test (CFT), the competitive inhibition enzyme-linked immunoabsorbant assay (cELISA) test, and the indirect immunofluorescent

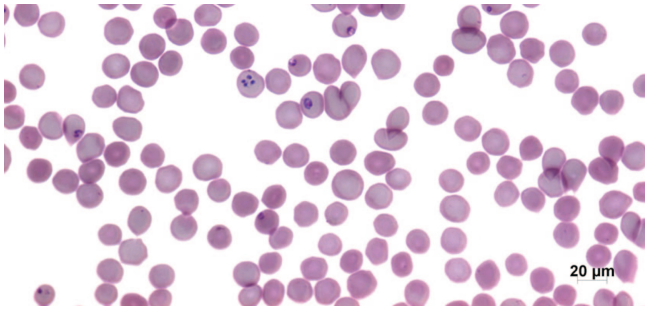

Fig. 2. Intraerythrocytic of trophozoite and merozoite stages of *B. equi*. (Photo is courtesy of D. Knowles)

antibody (IFA) test. The cELISA tests are available as kits,[b] one test kit for detection of antibodies to *B. caballi* and a second kit for detection of antibodies to *B. equi*, and are licensed by the USDA: APHIS:VS Centers for Veterinary Biologics. Currently, the recognized tests for purposes of equine importation by the World Organization for Animal Health (OIE) are the IFA and cELISA tests. Through comparison testing of sera using multiple test methods, it has been determined that the cELISA test is more sensitive at detection of the specific antibody in carrier horses than is the CFT; however, recent clinical cases of EP have illustrated that, in acute infection, the CFT may be positive while the cELISA test is negative until several weeks after clinical disease. Thus, within an outbreak, multiple tests may be necessary to accurately determine a horse's infection status.

Several types of polymerase chain reaction (PCR) tests have been developed and are used for research purposes. What role the PCR test will play in the future for determining a horse's infection status is still being determined.

As of the spring of 2010, all testing for EP has been conducted only at the USDA:APHIS:VS National Veterinary Services Laboratories (NVSL). A plan to approve additional diagnostic laboratories in the United States has been developed, and the approval of additional laboratories will be overseen by NVSL.

Examination of Horse for Ticks and Tick Treatment

Certain types of ticks may have favored sites for attachment on the horse. For example, the tropical horse tick may be found in the ears and false nostril area as well as other locations on the body, such as the axilla or groin area. When performing the examination, it is important to keep in mind the safety of the examiner and the horse. Thorough examination includes the following anatomical areas:

- Beginning at the horse's head, examine the false nostrils visually and palpate with the forefinger.
- Move to the forelock and with thumb opposed to fingers, examine the forelock; while examining the area near the forelock, examine inside and outside of each ear, and continue down the mane from the forelock to the withers.
- Examine the submandibular/intermandibular space with the fingers of the flattened hand, feeling for any unevenness of the skin.
- Examine visually and palpate the axilla of the left side.
- Examine and palpate the posterior area of the fetlock to the coronet of the left front foot.
- Visually examine the udder/scrotum area on the left side.
- Examine visually and palpate the tail and perineum, paying special attention to the ventral aspect of the tail head and area around the anus.
- Examine and palpate the posterior fetlock to the coronet of the left hindfoot.
- Examine the udder/scrotum of the right side.
- Examine and palpate the posterior aspect of the fetlock to the coronet of the right hindfoot.
- Examine and palpate the posterior fetlock to the coronet of the right front foot.
- Examine visually and palpate the axilla of the right side.
- After completing the examination on each horse, hand hygiene should be performed.

Ticks should be documented as to location on the animal where discovered, collected, and preserved in alcohol or formalin, and then, they should be submitted to NVSL for identification.

Permethrin-based acaricides have good efficacy for most types of ticks, both as a toxin and a repellant. There are several brand-name products that have a label indication for use on horses for control of ticks. It is very important that the label of the product to be used includes an indication for efficacy in control of ticks and its safety for use on horses. The user should follow all label instructions and take indicated precautions about the storage, application, and disposal of the product.

U. S. History

In 1960, USDA:APHIS Veterinary Services and the State of Florida began a disease investigation after backyard horses in south Florida became sick with progressive anemia, jaundice, and fever. The investigation determined that the cause was babesia infection and that the agent was carried by tropical horse ticks. A State-Federal EP control program was initiated in 1962 in south Florida to eradicate the disease. The program used quarantine and drug treatment for infected equines, spray treatment for infected and exposed animals, and movement controls to prevent disease spread of EP. As a result of the eradication campaign, the United States was declared EP-free in 1988.[5]

EP is considered a Foreign Animal Disease (FAD) and is a reportable disease.[3] Veterinarians and laboratories that identify suspect cases are required to report to their State or Federal Animal Health Official, which will initiate a regulatory response. The United States reports any identified cases to the OIE. Currently, all identified infected horses are under quarantine, and guidelines for management of infected and exposed horses have been developed and are available from the State or Federal Animal Health Official in each state.[7]

The increasingly global nature of the equine industry presents the potential for the reintroduction of EP into the United States. Since 1970, to reduce the risk of importing infected equids from areas in which EP is endemic, the United States requires that blood from these equids be tested for the pres-

ence of antibodies to *B. caballi* and *B. equi* before importation. Import testing is conducted by the NVSL. Before August 22, 2005, the official U. S. import test used for detecting antibodies to EP disease agents was the CFT. Based on evidence that the CFT has relatively low sensitivity for detecting chronically infected equids, the official testing method was changed to a cELISA. Thus, because of the low sensitivity of the CFT, it is possible that equids chronically infected with *B. caballi* or *B. equi* were previously imported into the United States, making it possible that infection from either of these two parasites exists today in equids in the United States.

Based on a request from the United States Animal Health Association, Infectious Diseases of Horses Committee (USAHA-IDOHC), an EP serosurvey was undertaken, with a goal of reporting a national seroprevalence of antibodies to *B. equi* and *B. caballi* among equids in the United States.[8] The serosurvey was funded by USDA-APHIS-VS, with significant contributions of their time and expertise from members of the USAHA-IDOHC-EP subcommittee.[8] The population contributing sera for this survey consisted of equids being tested for EIA. National Animal Health Laboratory Network (NAHLN) laboratories in 34 states contributed serum samples for the survey. All serum samples were tested at NVSL according to the manufacturer's instructions using two ELISA kits (one for *B. equi* and one for *B. caballi*) manufactured by Veterinary Medical Research Diagnostics[b] (VMRD) and licensed by USDA-APHIS-VS Centers for Veterinary Biologics. If a test sample produced ≥40% inhibition, it was considered positive for antibodies to the respective organism. The prevalence estimated was the median true serologic positive prevalence based on the inclusion of the test characteristics into the analytical method. The estimate for the adjusted, weighted median for seroprevalence for *B. caballi* from this survey was 0.054% (54 per 100,000 horses; 95% prediction interval = 0.002–0.210%). The estimate for the adjusted weighted median for the seroprevalence for *B. equi* for this survey was 0.007% (7 per 100,000 horses; 95% prediction interval = 0.0003–0.0360%). The survey indicates that there are likely horses in the United States that are truly seropositive for *B. caballi* or *B. equi* but at a very low prevalence.[8]

Treatment

It is important when discussing the treatment of infected horses to clarify the goal of such treatment. Treatment options and likelihood of success vary based on whether the treatment is being given to a horse to resolve the clinical signs of disease or completely clear (sterilize) the horse of all the parasites in the body.[3] The goal of clearing the infection is to remove the transmission risk the horse might pose. As mentioned earlier, horses reared in EP endemic areas of the world may never show signs of clinical disease because of premunition. Attempts to clear horses of infection in these endemic areas are not often recommended, because complete clearance would result in the horse becoming susceptible to reinfection and potentially, developing clinical disease.

In contrast, in areas considered free of EP, treatment that resulted in clearance of parasites would be desirable. The challenge has been determining the criteria necessary to prove that complete clearance has occurred with treatment.

There are multiple drugs that have been reported to have an antimicrobial effect on EP agents. However, there are only a few that have been evaluated in vivo for safety and efficacy. Imidocarb dipropionate (ID)[c] has been considered effective in the treatment of clinical signs caused by *B. caballi* infection. A recent report indicates that this drug also resulted in clearance of *B. caballi* from experimentally infected horses based on both tick and blood-transfusion transmission trials.[9] The clinical signs caused by *B. equi* infection can be treated with ID.[3] However, in general, the clearance or sterilization of *B. equi* infection is considered more difficult than the clearance of *B. caballi*. The ability of ID treatment in the clearance of *B. equi* infection is under investigation at the USDA:APHIS:VS.[10] Imidocarb causes a dose-dependant hepatotoxicity and nephrotoxicity.[6] Further information on treatment of EP will be given in the research update.

3. 2008 Florida Outbreak of EP

On August 11, 2008, a horse with clinical disease was hospitalized in Ocala, Florida. A tentative diagnosis of EP was made by the attending veterinarian in consultation with the University of Florida. The attending veterinarian then reported the case to the Florida State Veterinarian's Office. An important aspect of the investigation was the epidemiologic investigation and tick surveillance to determine the likely route of introduction and spread of the EP organism.

As part of the epidemiologic investigation, horses with potential exposure to EP pathogens were quarantined and tested for both *B. equi* and *B. caballi*. Two of six horses having entered the United States from Mexico in 2004 and 2005 tested positive for *B. equi* and were presumed to be the source of the organism for this outbreak

Tick surveillance produced several species of ticks and a potential vector for *B. equi* in *D. variabilis*.[10] All *D. variabilis* ticks were tested for Babesia organisms through PCR and were found to be negative. After an extensive investigation, the EP organism was believed to have been spread by iatrogenic means and not by ticks. All of the horses that were infected with *B. equi* were involved in non-sanctioned Quarter Horse racing. No infections were detected in non-racing horses, including the many

foals, yearlings, broodmares, and ponies that were tested.

On February 12, 2009, the last premises was released from quarantine, with 0 of 20 positive horses remaining in Florida. Final disposition of the test-positive horses was euthanasia or shipment to a U.S. federal facility for research purposes.

4. 2009 Outbreak of EP in Missouri

In June 2009, a Quarter Horse gelding from Missouri presented to a veterinary teaching hospital with clinical signs of EP and was confirmed by laboratory testing to be acutely infected with *B. equi*. Testing of horses epidemiologically linked to the index case led to the identification of seven additional *B. equi* infected horses in Missouri and Kansas. All eight of the infected horses were participating in unsanctioned horse racing and were connected with the same trainer. A total of 67 exposed horses were tested during the incident, with no further disease spread identified. There were no ticks found on any of the horses being investigated. A complete tick survey performed on the index premises in Missouri resulted in the collection of only four ticks, none of which were considered competent vectors for EP agents. It was determined that the most likely cause of EP infection was not by natural tick vector transmission but rather by management practices such as the use of shared needles or transfusion of horses with infected blood or blood products by non-veterinarians.

5. 2009–2010 Outbreak of EP in Texas

In October 2009, a Quarter Horse from a ranch in south Texas was confirmed by laboratory testing to be acutely infected with *B. equi*. Testing of the 360 horses from the index mare's home ranch in south Texas identified a total of 292 *B. equi*-positive horses. Testing of all horses sold from the index ranch between 2004 and 2009 identified 68 additional EP-positive horses located on premises in 14 states, including Texas. As of April 2010, more than 2,100 horses have been tested in connection with the incident, with 376 horses identified as *B. equi*-positive. Continued tracing with subsequent testing of potentially exposed horses is ongoing as of spring 2010. All known infected horses are under strict quarantine in their respective states, with control measures in place to mitigate further spread of infection between horses. Epidemiologic investigation and tick-transmission studies indicate that spread of the disease on the index ranch in Texas was primarily through natural tick transmission by at least two species of competent tick vectors on the premises.

6. Detection of EP Infection in Horses Through Interstate Movement and Enhanced Surveillance Testing in 2009–2010

In response to the identification of *B. equi* infection on a ranch in Texas in October 2009, several states implemented interstate-movement testing requirements for EP on horses coming from Texas. In November 2009, the state of New Mexico began an active EP surveillance program for all horses racing on sanctioned tracks in New Mexico. Additionally, other states, racing commissions, and equine-event organizers began considering the implementation of EP-testing requirements for entry into specific events or venues. Through this increase in EP surveillance and movement testing, additional EP-positive horses unrelated to the Texas, Missouri, or Florida outbreaks have been identified. As of April 2010, these newly identified non-clinical cases have been horses either imported to the United States before 2005 or individual EP-positives found within the sanctioned Quarter Horse racing industry. Epidemiological investigation into these cases is ongoing, and it is expected that continued surveillance and movement testing will identify additional EP-positive horses in these sectors.

7. EP: The Texas Perspective

Based on their investigation, the Texas Animal Health Commission (TAHC) has concluded from the available data that EP is not a widespread geographic phenomenon. The index ranch remains under quarantine and will remain so indefinitely.

The TAHC invited veterinarians from the Texas Veterinary Medical Association, Texas Equine Veterinary Association, Texas A&M University, and several other large equine practices, along with representatives from the Texas equine industry, government agencies, and affiliated stakeholders, to form an EP Working Group in January 2010. The purpose of the initial meeting was to update participants on the current EP situation in Texas and the United States. In addition, the TAHC has requested feedback from the working group concerning moving forward with policy changes related to the EP outbreak. The group has been in regular contact regarding issues and policy and has met through conference call since the initial meeting as needed to discuss matters requiring a consensus of opinion.

The working group meetings have highlighted differences that exist among disciplines regarding how best to mitigate exposure risk posed by known infected horses and those of unknown infection status. Some sanctioned racetracks in Texas are requiring proof of veterinary inspection for ticks and certification that horses have been examined for signs of piroplasmosis or alternatively, a negative cELISA test result for *B. equi*, whereas most county fairs and rodeo events have not changed entrance requirements from previous years. The TAHC has drafted policies regarding quarantine and movement of positive and exposed horses. Long-term policy is under development. The TAHC is continuing to monitor this situation to allow flexibility as new information becomes available. Traceback data strongly suggest that EP existed in Texas long be-

fore the index case was identified, and practitioners should be alert to clinical signs of EP in their patients.

8. Research Updates

Emergence of infection and disease caused by *B. equi* in U.S. horses has led to enhanced research efforts in several areas. These areas are diagnostic test validation, particularly PCR, sequencing and annotation of parasite genomes, testing of chemotherapeutics to determine efficacy in removing transmission risk from infected horses, testing certain types of ticks for their capacity to transmit among equids, and determining the efficiency of in utero (transplacental) transmission.

Diagnostic Testing and Genomic Sequencing Research

One theory for the emergence of *B. equi* infection in horses in the United States is that the use of the CFT to screen imported horses from 1970 to 2005 allowed the entry of chronically infected horses. The cELISA was developed and validated based on a need to have a sensitive test for detection of infection in chronic carriers of EP pathogens. The cELISA became the official test method used by the United States for importation purposes in 2005. Validation of the PCR test (nested and real-time) in field situations is underway. It is important to note that, because the CFT detects immunoglobulin M (IgM) antibody, it remains an important tool for the detection of acute infection.

Sequencing and annotation of the *B. equi* genome has recently been completed, and it should allow for more accurate taxonomic classification and aid in determination of strain differences for susceptibility of the parasite to chemotherapeutic agents.

Treatment

A number of chemotherapeutics have been tested for removing transmission risk from infected horses. Much of the research concerning ID has focused on the treatment of acute parasitemia and clinical disease. There remains controversy concerning the efficacy of ID in its ability to completely remove (clear) infection and transmission risk from equids. Part of the controversy is likely because of the previous use of the CFT to define clearance of infection. Because of the lack of sensitivity of the CFT, negative test results after ID treatment, in some cases, may have been caused by failure of this test to detect persisting infection. Second, it has been suggested that various doses and treatment regimens with ID may have led to ID-resistant strains of both *B. equi* and *B. caballi*. The assessment of ID resistance among EP pathogens is an important current goal of chemotherapeutic research. High-dose ID removed transmission risk from two *B. caballi* infected horses.[8] However, the strain of *B. caballi* used in this study likely had never been exposed to ID and represented a single strain. Literature also suggests that there may be strain variability (*B. caballi* and *B. equi*) in susceptibility to ID. Whether strain variability in susceptibility to ID is the result of inaccurate diagnostics, selection of genetic resistance to ID because of inappropriate treatment protocols, or parasite genetics is not known. The recent completion of the *B. equi* genome may help clarify these questions. The current urgent need for research related to treatment includes the following questions. (1) Is ID treatment capable of broadly removing transmission risk of infected equids? (2) Are there parasite-strain differences in ID susceptibility to clearance? (3) What do post-treatment serum antibody and PCR testing of blood samples predict concerning transmission risk? A dose-dependant toxicity has been reported with the treatment of horses with imidocarb.[6]

Tick-Competency Research

A critical aspect of testing ticks for transmission competency is delineating a given tick's ability to transovarially transmit to the next generation of ticks. An ecologically important difference between *B. caballi* and *B. equi* is that, to date, only *B. caballi* is known to be transovarially transmitted. However, as additional ticks capable of transmitting *B. equi* are discovered,[11] it is important to determine if these ticks transovarially transmit *B. equi*. Ticks capable of transovarial transmission represent an additional parasite reservoir and significantly complicate control measures.

Research Related to in Utero Transmission of *B. equi*

In addition to tick and iatrogenic transmission, a number of reports indicate that *B. equi* can be transmitted in utero (transplacentally or by vertical transmission). However, neither the mechanism nor efficiency of this route of transmission is known. Current research efforts are directed at defining the efficiency of this route of transmission and testing for markers in the infected mare, which predict her risk for in utero transmission of *B. equi*.

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