APHIS Info Sheet

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Seroprevalence of Equine Piroplasmosis Disease Agents in the United States

Background

Equine piroplasmosis (EP) or babesiosis is a disease caused by a blood-borne parasitic infection and affects horses, donkeys, mules, and zebras. The disease agents of EP are *Theileria equi* (also known as *Babesia equi*) and *Babesia caballi*. For this report, *Theileria equi* will be referred to as *B. equi*. Infected animals can remain carriers of the disease agent for long periods and can act as a source of infection to naïve equids. The disease agents are spread by competent ticks or by the transfer of blood from infected to naïve equids through shared needles, improperly shared equipment, and blood or serum transfusions. *Dermacentor nitens*, the tropical horse tick, is currently the only known natural vector of EP agents in the United States. *B. caballi* and *B. equi* have been shown to be experimentally transmitted by three additional U.S. tick species—*D. albipictus*, the winter tick; *D. variabilis*, the American dog tick; and *Boophilus microplus*, the southern or tropical cattle tick. There is also evidence of vertical transmission of the EP disease agent, e.g., dam to foal.

Clinical signs

The clinical signs demonstrated by equids infected with the EP disease agent vary from mild to severe. The mild form of the disease can cause equids to appear weak or show lack of appetite, while more severe cases may have fever, anemia, yellow mucous membranes (jaundice), swollen abdomen, and labored breathing. Other signs include a rough hair coat, red color to the urine, colic, and altered gait or mentation. Carriers of the infection in the chronic phase of the disease can appear normal and might even perform their usual roles, but the infectious agent persists. Chronic infection is best diagnosed by testing the equid's serum for the presence of specific antibodies.

History of EP in the United States

A joint Veterinary Services and State of Florida control program for *B. caballi* began in 1962 in south Florida with the goal of eradicating the disease. The program included quarantine and drug treatment of infected equids, spray treatment for infected and exposed animals, and movement controls to prevent disease spread. As a result of this program, the continental United States was declared free of EP in 1988. In addition, the United States recognizes Iceland and Canada to be EP free. The disease is still found in Africa, the Caribbean, Central and South America, Mexico, the Middle East, and Eastern and Southern Europe.

Required testing for importing equids

The increasingly global nature of the equine industry presents the potential for the reintroduction of EP into the United States. To reduce the risk of importing infected equids from areas in which EP is endemic, it is required that blood from these equids be tested for the presence of antibodies to *B. caballi* and *B. equi* before importation. This U.S. import testing is conducted by the National Veterinary Services Laboratories (NVSL).

Prior to August 22, 2005, the official U.S. import test used for detecting antibodies to EP disease agents was the complement fixation test (CFT). Based on evidence that the CFT has relatively low sensitivity for detecting chronically infected equids, the official testing method was changed to a competitive enzyme-linked immunosorbent assay (cELISA). Thus, because of the low sensitivity of the CFT, it is possible that equids chronically infected with *B. caballi* or *B. equi* were imported into the United States, making it possible that infection from either of these two parasites exists today in equids in the United States.

The specificity of the cELISA for detecting antibodies to *B. equi* and *B. caballi* reported in the World Organization for Animal Health (OIE) Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (2005) is 99.2 percent for *B. equi* and 99.5 percent for

B. caballi. The cELISA has been shown to be more sensitive than the CFT in detecting chronically infected equids (Knowles et al., 1991; Kappmeyer et al., 1999). Less is known about the true sensitivity, but Katz et al. (2000) tested eight known reference-positive control samples and all were positive on the cELISA.

Recent EP cases in the United States

Periodic cases in which equids were serologically positive for EP disease agents yet showed no clinical signs of disease have occurred in the United States. These infected animals have been linked to previous imports that originated from areas in which EP is endemic.

In the past 12 months, there have been two outbreaks of disease—one in Missouri and one in Florida—that included horses with confirmed clinical disease due to infection with *B. equi*. Epidemiological investigations indicated that the disease agents in the outbreaks were transmitted through the sharing of needles as well as blood transfusions among horses involved in unsanctioned racing. In both States, tick surveys utilizing tick dragging and wildlife trapping conducted by the Southeastern Cooperative Wildlife Disease Study ruled out the presence of exotic and competent tick vectors for *B. equi*. It appeared that transmission via tick vectors had not occurred in either outbreak, based on the tick studies and the distribution of infection among the horses on the premises with disease (only horses involved in unsanctioned racing in which needles were shared and blood transfusions occurred were infected). There was a regulatory response to both outbreaks, and the outbreaks are considered resolved.

Equine piroplasmosis serosurvey

This EP serosurvey was undertaken to address the 2007 United States Animal Health Association (USAHA) resolution number 19[1] from the Infectious Diseases of Horses Committee (IDOHC), EP subcommittee with a goal of reporting a national seroprevalence of antibodies to *B. equi* and *B. caballi* among U.S. equids. The serosurvey was funded by USDA-APHIS-VS, with significant contributions from members of the USAHA-IDOHC-EP subcommittee.

Materials and methods

Input on the design and implementation of the EP serosurvey was provided by members of the USAHA-IDOHC-EP subcommittee in tandem with members of the USDA-APHIS-VS staff, NVSL, Agricultural Research Service (ARS), and the USDA-APHIS-VS Centers for Epidemiology and Animal Health. Funding for laboratory materials was provided through APHIS-ARS.

Source and selection criteria for sera used in the survey

To ensure broad geographic representation among animals tested, the chair of the USAHA-IDOHC-EP subcommittee asked 36 National Animal Health Laboratory Network laboratories testing for equine infectious anemia (EIA) and 2 additional laboratories to participate in the EP serosurvey by submitting sera remaining after EIA testing. Of these, 35 laboratories located in 34 States contributed serum samples for the survey. Laboratory directors were assured that no link between contributing laboratories and test results would occur and that only national seroprevalence estimates would be reported. A sample size for the number of sera to be tested was allocated to each laboratory, proportional to the number of EIA tests performed annually.

The population contributing sera for this survey consisted of equids being tested for EIA. Based on the National Animal Health Monitoring System's Equine 2005 study, 37.6 percent of horses on operations with five or more horses are tested for EIA each year. The highest percentage of these operations reported that the primary reason for testing for EIA was show or event requirements within the State, followed by interstate movement and personal knowledge. As the number of equids residing on an operation increased so did the likelihood that one or more horses would be tested for EIA. Thus, the horses that contributed sera for the EP serosurvey were likely to be from operations with multiple horses, some of which moved both intrastate and interstate for purposes of competition or breeding.

No link to the identification of contributing laboratories or sampled horses was provided when results of testing were reported; thus, no traceback to the source of the sera is possible. Without information about the horses' herd of origin, the proposed analysis could not account for the lack of independence of horses originating from the same premises. To compensate for the potential lack of independence, a systematic random sample was taken such that approximately every third to fifth sample contributed by a given laboratory was selected for testing.

In 2007, 2 million EIA tests were performed on U.S. horses. The laboratories supplying samples for the current serosurvey conduct approximately 630,000 (31.5 percent) of the national EIA tests performed annually.

The current serosurvey was designed to allow for expansion of the test results to estimate and report the national prevalence of antibodies to *B. equi* and *B. caballi* in U.S. equids tested for EIA by the selected laboratories. A total of 15,300 samples were tested, which was considered an adequate sample size to estimate a prevalence of 0.1 +/- 0.05 percent for *B. equi* or *B. caballi*.

[1] http://www.usaha.org/committees/resolutions/2007/resolution19-2007.pdf

Testing of sera for this 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 (VRMD)[2] and licensed by USDA-APHIS-VS Centers for Veterinary Biologics. If a test sample produced ≥ 40 percent inhibition, it was considered positive for antibodies to the respective organism. Subsequently, all sera that tested positive on the VMRD cELISA at NVSL as well as borderline samples (those just below the cutoff) underwent further testing via Western Blot test and repeat testing by the VMRD cELISA at the ARS laboratory in Pullman, Washington. Western Blots were performed as described in previous publications (Schwint et al., 2009).

Data analysis

The repeat testing on available positive and borderline sera from NVSL was conducted at the ARS laboratory using the VMRD kit. Results, in terms of the number of positive samples, were similar to those reported by NVSL. The confirmatory testing of VMRD cELISA-positive samples via Western Blot did not lead to usable results due to several factors, including inadequate remaining sera from some of the samples, poor sera quality, and unexpected results (banding patterns) when Western Blot testing these field samples. Although Western Blots had provided a clear indication for positive and negative horses under limited experimental use (Schwint et al., 2009), the testing of greater numbers of sera revealed results (banding patterns) not previously encountered. This made the Western Blot gels uninterpretable without extensive additional analysis. Thus, all prevalence estimates are based on results from testing conducted at NVSL using the VMRD kits. Data were weighted to represent the population of U.S. horses tested for EIA by the 38 selected laboratories, with samples representing the time period of October 2007 through June 2008.

A Bayesian analytical method was used to determine a seroprevalence estimate because no confirmatory tests were available. This analytical method allows the incorporation of reported test characteristics for specificity and sensitivity. The test characteristics used in the Bayesian analysis were derived from Katz et al. (2000). Conservatively, the distribution for prevalence that was used as prior information for the Bayesian model was considered uniform (equal chance of being between 0 and 100 percent). Weighted seroprevalence estimates were used to adjust the number of positives used in the model. The results of the Bayesian analysis are probability distributions for the characteristics of interest. Thus, results for estimated seroprevalence using the Bayesian analysis will be reported as adjusted medians which incorporate the impact of the survey design and 95-percent prediction intervals.

Results

The number of positive tests on the VMRD kit for *B. caballi* and *B. equi* was below the number of false-positive test results that would have been expected based on the previously described test specificity. The Bayesian analysis suggested that the actual specificity of this test, given the circumstances of this survey, is higher than previously described—closer to 99.8 percent for *B. caballi* and 99.9 percent for *B. equi*.

The prevalence reported here is the estimated 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 is 0.054 percent (54 horses per 100,000) [95-percent prediction interval 0.002- 0.21 percent]. The estimate for the adjusted weighted median for the seroprevalence for *B. equi* for this survey is 0.007 percent (7 horses per 100,000) [95-percent prediction interval 0.0003-0.036 percent].

Interpretation of results

This survey indicates that there are likely horses in the United States truly seropositive for *B. caballi* or *B. equi,* but at a very low prevalence. It is important when interpreting the results of this survey to acknowledge that the prevalence reported in this survey is based on serologic testing. Although serology for these disease agents parallels infection status, based on the survey design there is no information to suggest that these animals were clinically affected.

To put the seroprevalence estimates for *B. caballi* and *B. equi* into perspective, these results can be compared with the current EIA reactor seroprevalence based on regulatory testing. About 6 horses out of 100,000 test positive for EIA annually in the United States.

A potential explanation for the presence of horses seropositive for *B. caballi* and *B. equi* in the United States is that horses imported to the United States prior to 2005 were tested by the CFT, which has been shown to have a lower sensitivity for detecting chronically infected animals than the currently used cELISA. The low prevalence of seropositive horses described in this survey could reflect the horses imported prior to 2005 that have remained in the U.S. equine population. It is also feasible that horses tested for EIA, the reference population for the current survey, would likely contain a high percentage of previously imported horses. For example, horses are often imported for competition, and these same horses would likely be part of the population tested for EIA.

Based on the findings in this survey, the prevalence of horses seropositive for *B. caballi* and *B. equi* is very

[2]http://www.vmrd.com/docs/catalogs/2006_Int.pdf

low, even among an equine population within the United States that might be expected to have the highest prevalence.

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