Equine Piroplasmosis: Detection of Carriers Prior to Importation into the United States

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Equine piroplasmosis is a tick-borne hemoparasitic disease of horses caused by Babesia equi, B. caballi or both. Piroplasmosis is known to be endemic in every country except the USA, Canada, England, Ireland, Japan and Australia. Thus, in these countries movement of horses serologically positive to either organism is restricted. For many years the complement fixation (CF) test was the Office International des Epizooties (OIE) prescribed test for piroplasmosis serology. However, as indicated in another article in this newsletter, the CF test has well-documented shortcomings that produce false negative results. It is for this reason that a plethora of horses carrying piroplasmosis have been imported into the USA in spite of statutory requirements for negative serology for B. equi and caballi on entry.

In 1959 piroplasmosis was introduced into Florida and was not eliminated from the native horse population until 1982. Cases from new introductions of CF test negative carriers continue to occur in Florida. Several other states have also found seropositive carriers that were initially CF negative on entry to the USA. There are tick populations known to be capable of transmitting B. equi and caballi in large geographic areas of the USA outside of Florida. For these reasons in the late 1980s and early 1990s USDA Agricultural Research Service (ARS) scientists at Washington State University (WSU) began work based on recombinant antigens and monoclonal antibodies aimed at development of better diagnostic tests for equine piroplasmosis. The cELISAs developed by them have been adopted by OIE as the prescribed tests for international trade. VMRD, Inc. has acquired intellectual property licenses for these technologies and further developed them into uniform commercial diagnostic kits: Babesia equi Antibody Test Kit, cELISA and Babesia caballi Antibody Test Kit, cELISA. Each has undergone rigorous testing to insure high levels of sensitivity and specificity so that carriers of Equine piroplasmosis can be detected prior to importation into the USA.

Specificity of these kits was established by testing 2395 and 1924 samples of USA origin by the B. equi and the B. caballi cELISAs, respectively (Figures 1 and 2). All samples were negative for antibody to B. equi and only one sample (0.05%) was found positive by the B. caballi cELISA. Specificity for each assay with these large sample sets was 100% and 99.95%, respectively. It is reasonable to assume that the single B. caballi positive sample was in fact a true positive, perhaps an imported horse. Both kits were tested with check sets from NVSL and compared to results from the APHIS-OIE cELISA protocol (Figures 3 and 4). Results with the two kits correlated 100% with results from the APHIS-OIE protocol. The B. equi kit was further tested against a panel of 78 samples supplied by the USDA ARS Animal Disease Research Unit (ADRU) at WSU. Results compared to the APHIS-OIE cELISA protocol were identical (Figure 5). The B. caballi kit was tested against a panel of 106 samples supplied by ADRU at WSU. Four more positives were detected by the kit than by the APHIS-OIE cELISA protocol (Figure 6). However, these four samples were all very high negatives in the APHIS-OIE cELISA protocol, just below the cut-off, and thus quite possibly false negatives by the APHIS-OIE cELISA protocol. A panel of 158 samples from Thailand (a B. equi endemic country) were tested with the B. equi kit and by the CF test. Eighty-four samples were negative with both assays. However, only 17 of 74 (23%) cELISA positive samples were positive with the CF test. Twenty-three percent is remarkably similar to published findings in which the CF test detected only about 20% of Anaplasma cELISA / PCR positives.3 Clearly, we need to detect horses carrying B. equi and B. caballi PRIOR to their entry into the United States. The CF test is grossly inadequate for the task. Now that cELISA kits with high specificity and sensitivity far in excess of the CF test are available, importation of equids infected with B. equi and B. caballi is inexcusable.

