

RESOLUTION NUMBER: 14 **Approved**

SOURCE: **COMMITTEE ON EQUINE**

SUBJECT MATTER: **Advancing Equine Diagnostics at the United States Department of Agriculture, Animal and Plant Health Inspection Service, Veterinary Services, National Veterinary Service Laboratory**

BACKGROUND INFORMATION:

The United States Department of Agriculture (USDA), Animal and Plant Health (APHIS), Veterinary Services (VS), National Veterinary Services Laboratories (NVSL) plays a critical role in protecting equine health in the United States (US) through timely reporting of results from validated diagnostic tests for equids tested at import or those tested during an epidemiologic equine disease investigation.

The foreign animal disease testing performed by USDA-APHIS-VS-NVSL is critical to minimize the risk of introduction or spread of equine diseases such as dourine, glanders, contagious equine metritis and equine piroplasmiasis (EP). Science has shown the impact of stress and transport on the equine immune system can infrequently cause a non-specific immune response and trigger a non-negative diagnostic test result. Additionally, the import disease testing methods utilized in the US have recognized limitations, which result in the non-negative test result classification. Between 2011 and 2021, USDA-APHIS-VS-NVSL diagnostic testing results have identified 20 non-negative glanders testing imported equids and 24 non-negative dourine testing imported equids. Regardless of the reason for the non-negative test result, equids with the non-negative classification are quarantined for an extended period of time, until a negative test result is obtained. There is great impact on the health and wellbeing of the individual non-negative equids while in quarantine; especially if it is a fit performance or breeding horse.

The current gold standard of culturing for the diagnosis of contagious equine metritis poses challenges to timely release of equids from quarantine. Although *Taylorella (T.) equigenitalis* colonies are typically visible 72 hours after plating of a positive sample, in some situations it may take up to a week for colonies to appear. Thus, tests are not confirmed negative until day 7. International research has shown, a rapid, robust confirmatory test, such as the polymerase chain reaction (PCR) test, that does not have as stringent sample transport requirements as when submitting swabs for culture, would be highly beneficial to state animal health officials and diagnosticians. A validated PCR test would be a more economical, quicker means of screening stallions for the carrier

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state than conventional culture. The PCR assay for *T. equigenitalis* requires additional research to ensure that it is fully validated for the determination of the status of stallions, mares and geldings based on screening swabs and perhaps other

USDA-APHIS-VS-NVSL has advanced the diagnostic testing capabilities for equine piroplasmiasis over the years to enable the identification of acute recent infection and chronic infection. However, the identification of EP-positive horses has increased the need to identify the genotypic strains of organisms in positive EP equids detected in the US. While years of surveillance has shown that natural, endemic transmission of EP is not currently known to be occurring in the US, a small number of EP positive horses are found every year in two main high-risk groups: 1) Quarter Horse racehorses infected by iatrogenic transmission, many with ties to unsanctioned racing, and 2) horses illegally moved into the US from EP-endemic countries via Mexico. Strain-typing of EP organisms in these cases would identify and confirm epidemiological links between cases to be detected. Currently, however, there is no validated method to determine different strains of each EP organism (*Theileria equi* and *Babesia caballi*) complicating the epidemiological and trace back investigations.

Thus, advances in diagnostic testing for equine foreign animal diseases at the USDA-APHIS-VS-NVSL is essential to protecting and promoting the health of the nation's equine population.

RESOLUTION:

The United States Animal Health Association (USAHA) urges the United States Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS), Veterinary Services (VS), National Veterinary Services Laboratory (NVSL) to devote the resources necessary to further pursue advancing the diagnostic tests for dourine and glanders. Furthermore, USAHA urges USDA-APHIS-VS-NVSL to continue to pursue the development and validation of the polymerase chain reaction test for contagious equine metritis and the genotyping capabilities for equine piroplasmiasis organisms.

INTERIM RESPONSE:

The United States Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS), Veterinary Services (VS) recognizes the concerns of the United States Animal Health Association (USAHA) and appreciates the opportunity to respond.

Throughout fiscal years 2022 and 2023, NVSL evaluated several potential adjustments to the equine import testing algorithm.

- Glanders: NVSL collaborated with the Friedrich-Loeffler Institute (FLI) in Germany to evaluate the current complement fixation test (CFT) - with both commercially available and NVSL-produced antigens, a new commercial double-antigen ELISA (daELISA), and existing western blots (with an FLI- and NVSL-developed protocol). That evaluation found the daELISA showed improved sensitivity and specificity over both versions of the CFT and could be a candidate as the primary

glanders screening test for equine import. The glanders western blot performed acceptably as a subsequent confirmatory test for any non-negative results from the daELISA. In light of these results, APHIS is considering an update to the equine import testing guidelines for glanders.

- **Dourine:** In 2021, NVSL improved the protocol for qualifying lots of CFT antigen, which are used throughout the world. Last November, NVSL participated in the European Union Reference Laboratory for Equine Diseases Workshop on Equine Infectious Anemia and Dourine/Surra (VETQAS), which reaffirmed the CFT as the primary prescribed screening test for dourine. VETQAS will continue to provide a proficiency test, whose serum samples are provided by the World Organisation for Animal Health (WOAH) reference laboratory for dourine, the French Agency for Food, Environmental and Occupational Health & Safety (ANSES). NVSL participates in this proficiency test annually and continues collaborating with ANSES and other international partners toward advancing dourine diagnostics.
- **Genotyping equine piroplasmosis organisms:** In January 2023, NVSL published a [complete genome assembly of *Theileria equi*](#) in *Microbiology Resource Announcements*, providing a comparison reference for isolates encountered in diagnostic testing. NVSL has engaged both Mexico and Puerto Rico to provide blood from naturally infected horses (and cattle for bovine piroplasmosis) in those geographic locations; and will continue to attempt to isolate organisms from imported horses on the rare occasions when such infected animals are identified. Analysis of a reference genome for *Babesia caballi* is nearly complete, and it will be published in the coming months.
- **PCR for contagious equine metritis:** NVSL collaborated with other WOAH Reference Laboratories in 2020-2021 to complete an interlaboratory comparison of PCR methods for *Taylorella equigenitalis*, leading to an updated chapter in the Manual of Diagnostic Tests and Vaccines for Terrestrial Animals adopted in May of 2022, recommending PCR as a diagnostic tool for this disease. Because of the low prevalence of this disease worldwide, obtaining sufficient positive samples from naturally infected animals for validation studies is challenging. NVSL will develop duplicate sample collection protocols for both culture and PCR to contribute to the validation of PCR. APHIS-VS is committed to evaluating options for our import policy once we have the opportunity to evaluate the risk, science, and make sound policy recommendations.