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RECENT ADVANCES IN THE LABORATORY DIAGNOSIS OF EQUINE PARASITIC DISEASES

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The laboratory diagnosis of most equine parasitic diseases continues to rely on standard methods. These techniques are well documented in numerous current texts. This article focuses on recent advances in diagnostic testing for equine parasitic diseases. These include diagnostic tests for equine protozoal myeloencephalitis, cryptosporidiosis, and giardiasis.

EQUINE PROTOZOAL MYELOENCEPHALITIS

Western Blot Testing

Clinical diagnosis of equine protozoal myeloencephalitis (EPM) is problematic. Multifocal lesions may occur anywhere in the central nervous system, resulting in clinical signs compatible with virtually any equine neurologic disease.[5, 12, 15] Accurate antemortem diagnosis has been enhanced greatly by the recent development of the Western blot and polymerase chain reaction tests (Equine Biodiagnostics, Inc, Lexington, KY) to detect parasite-specific antibodies or parasite DNA in the blood or cerebrospinal fluid (CSF) of affected horses.[7, 11] An immunofluores-

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cence test (Oklahoma State University, Stillwater, OK) based on an antigen extract of *Sarcocystis cruzi* from cattle also has been used to test equine serum. This technique lacks the sensitivity and specificity of Western blot analysis and cannot differentiate exposure to *S. neurona*, the causative agent of EPM, from exposure to *S. fayeri*, a common parasite of equine skeletal muscle.[5] Western blot analysis is based on electrophoretic separation of proteins from cultured *S. neurona* merozoites. Separation of parasite proteins permits evaluation of serum and CSF samples for the presence of antibodies that react with *S. neurona*–specific proteins.

Interpretation

Seroprevalence studies and clinical testing indicate that exposure to *S. neurona* is common throughout North America.[10, 12] Nationally, seroprevalence is estimated to be 30%; however, exposure seems to be at least 10% higher among horses in the Eastern part of the United States. High seroprevalence among clinically normal horses limits the value of serum testing. It is not possible to distinguish between antibody titers resulting from routine exposure and those resulting from active disease. Seroprevalence among horses with neurologic disease, however, has been twice that of the general horse population.[10] Serum from horses with confirmed histologic diagnoses of EPM have tested positive in more than 90% of the cases examined.[12] A negative serum test does have diagnostic value. Although it does not completely rule out a diagnosis of EPM, the likelihood for that individual is very low.

The presence of parasite-specific antibodies in CSF has been highly correlated with clinical disease at postmortem.[12] Approximately 90% of CSF samples tested from histologically confirmed cases of EPM have tested positive. It has been found that peracute cases initially may test negative and that long-term cases with permanent CNS damage also may test negative. Apparently, some horses also respond poorly and do not produce detectable amounts of parasite-specific antibody. False-positive results also may occur. The percentage among horses without detectable histopathologic lesions of EPM, however, has been low. Blood contamination of CSF during collection has been the most common reason for false-positive CSF results. Samples that contain obvious blood contamination may be confounded and should be avoided. New samples should be drawn in a few days. Centrifugation and removal of red blood cells does not correct the problem. Serum antibodies will remain in the CSF supernate. Blood-brain barrier compromise also may allow serum antibodies to pass into CSF. The immunoglobulin G (IgG) index/albumin quotient developed by Dr. Frank Andrews (University of Tennessee, Knoxville, TN) permits accurate evaluation of blood-brain barrier integrity and provides valuable assistance for interpretation of EPM test results.[1]

DNA Diagnostic Testing

Recently, the polymerase chain reaction (PCR) DNA diagnostic test for *S. neurona* was developed to detect the parasite in blood or spinal fluid of affected horses.[7] Amplification primers were designed from the nucleotide sequence of the 18S small ribosomal subunit gene of *S. neurona*.[6] Primers are actually very short pieces of synthetic single-stranded DNA. The nucleotide sequence of each primer was selected to match a small piece of the parasite or target DNA to be amplified. If target DNA is present in the sample to be tested, the primers will attach and allow it to be replicated billions of times. The copies of target DNA or product DNA are of known size and can be separated easily by electrophoresis and stained for visualization.

Interpretation

The PCR test has proved to be most useful for confirmation of suspect and/or negative CSF Western blot test results. The PCR test depends on the integrity of the parasite DNA target sequence. A strong inflammatory response favors enzymatic degradation of parasite DNA in CSF, which may reduce test sensitivity; therefore, the PCR test should be useful early in the disease and again late in the disease process. Long-term, nonprogressive cases may not have eliminated the parasite completely even though the Western blot test result has become suspect or negative.

Generally, it has not been necessary to use the PCR test to confirm positive Western blot results for CSF samples. However, if a Western blot result is suspicious in any way, eg., light reactivity, background reactivity) or if the sample is blood contaminated, then it is advisable to attempt to confirm the result by PCR testing and/or the IgG index/albumin quotient. Tests conducted using spinal fluid samples from histologically confirmed EPM cases demonstrated more than 83% sensitivity and 100% specificity.[7] The use of collection tubes containing edetic acid (EDTA) and keeping samples chilled allows maximal test sensitivity by minimizing further enzymatic activity after collection.

The presence of parasite DNA in an equine blood sample suggests that the horse recently ingested *S. neurona* sporocysts. It is not known how long detectable amounts of parasite DNA remain in the blood. It is assumed that parasites are either eliminated quickly or that they penetrate the blood-brain barrier and are confined to the central nervous system. The Western blot test for parasite-specific antibodies probably provides evidence of exposure for a much greater length of time. Because the incubation period of EPM seems to be quite variable (2 weeks to 2 years), the Western blot is more useful for blood testing under most circumstances.

CRYPTOSPORIDIOSIS

Immunofluorescence Testing

Cryptosporidium parvum infection occasionally may result in foal diarrhea.[2, 21] Immunocompetent and immunocompromised foals are susceptible, but immunocompromised foals are at greater risk.[4, 8, 9, 17, 18] Although *Cryptosporidium* alone may cause foal diarrhea, it also has been associated with concurrent infection with other enteric pathogens (adenovirus, coronavirus, rotavirus, *Giardia*, *Salmonella*).[4, 17, 18, 19] It is important to consider testing for these agents as well.

A recent study by Xiao and Herd demonstrated that the most reliable method for the diagnosis of equine cryptosporidiosis is the direct immunofluorescence assay (Merifluor, Meridian Diagnostics, Inc, Cincinnati, OH).[19] Standard sugar floatation and/or acid-fast staining techniques may detect oocysts, but sensitivity and specificity are much lower.[19, 20] Although direct immunofluorescence requires shipment to a veterinary diagnostic laboratory, the increased reliability of testing is worth the inconvenience. Because oocyst shedding may be intermittent, fecal samples should be collected for at least 3 days, kept refrigerated, and shipped chilled to a diagnostic facility. Alternatively, samples can be stored and shipped in 10% formalin.[21]

Interpretation

It is important to remember that simply finding *Cryptosporidium* oocysts in foal feces does not indicate disease.[19] Xiao and Herd found an average oocyst prevalence of 21% among foals on nine farms in southern Ohio and central Kentucky using the direct immunofluorescence assay. Samples taken from mature horses and yearlings were negative. In southern Ohio, oocysts were detected in the feces of foals from 4 to 23 weeks of age. Peak shedding occurred from 5 to 8 weeks of age. Individual foals shed oocysts for 1 to 14 weeks with a mean of 3.4 weeks. Forty-four percent of the foals shed oocysts multiple times. The longest interval between oocyst shedding was 6 weeks. None of the foals in the study developed diarrhea. The primary source of infection, however, was considered to be via fecal contamination from other foals. The prepatent period for *Cryptosporidium* is only 4 to 5 days.

Cryptosporidial diarrhea in immunocompetent foals generally develops before 4 weeks of age and is usually self-limiting and lasts for 1 to 8 days.[2, 21] Concomitant infections, however, may lead to severe dehydration and death. Diarrhea in older foals has been reported and may be long-term and recurrent until they become yearlings.[21]

GIARDIASIS

Immunofluorescence Testing

Giardia intestinalis (or *lamblia* or *duodenalis*) has been associated with listlessness, diarrhea, and abdominal pain in horses of any age.[2, 14, 16] *Giardia* trophozoites are flagellate protozoans that parasitize the small intestine of many mammals and humans.[13] The organisms are transmitted by the direct fecal-oral route. Characteristic cysts survive for an extended period in the environment.

The direct immunofluorescence test used to diagnose *Cryptosporidium* also contains monoclonal antibodies against *Giardia.* The test provides a much more sensitive method to detect the presence of *Giardia* than traditional methods. Fecal enzyme-linked immunosorbent assay (ELISA) kits (Prospect/*Giardia*, Alexon Biomedical, Inc, Rolling Meadows, IL and GiardEIA, Antibodies Inc, Davis, CA) are available for the detection of *Giardia* antigen in feces. The reliability of antigen ELISA kits for use in horses, however, has not been established. Because shedding is intermittent, fecal samples should be collected for 3 to 5 days. Storage of feces in 10% formalin is satisfactory for immunofluorescent detection, but it may interfere with traditional methods.[22]

Interpretation

It is important to remember that the presence of *Giardia* in feces does not necessarily indicate disease.[19] Xiao and Herd recently found an average *Giardia* infection rate of 12% on eight farms in southern Ohio and central Kentucky. *Giardia* infection was found in all age groups, but was higher (17%–29%) among foals. Infection rates were 5% to 17% in weanlings, 0% to 9% in yearlings, and 2% to 28% in mares (a 28% infection rate was found among nursing mares). Foals on one farm in southern Ohio were tested every 2 weeks from March to October. Shedding of *Giardia* cysts began at 2 weeks of age and continued for 1 to 16 weeks (mean, 5.2 weeks). Because the prepatent period for *Giardia* is 1 to 2 weeks, mares were considered the primary source of infection for foals. Cyst excretion peaked by 9 weeks of age and frequently was intermittent. The longest period between shedding was 8 weeks. None of the horses in any age group of the study developed diarrhea.

SUMMARY

This article reviews recent advances in laboratory diagnosis of equine parasitic diseases. Laboratory diagnosis of most equine parasitic diseases continues to rely on standard methods. Only laboratory diagnostic tests for EPM, cryptosporidiosis, and giardiasis were included. The criteria for testing and interpretation of results for each new diag-

nostic method were explained. Western blot and PCR testing for EPM and immunofluorescent staining with monoclonal antibodies for cryptosporidiosis and giardiasis were reviewed.

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