Diagnosis of *Rhodococcus equi* Pneumonia in Foals: PCR or Culture?

Debra C. Sellon, DVM, PhD, Dipl. ACVIM; Thomas E. Besser, DVM, PhD; Rebecca S. McConnico, DVM, PhD, Dipl. ACVIM; Sally L. Vivrette, DVM, PhD, Dipl. ACVIM

PCR (Polymer Chain Reaction) of tracheal wash fluid is more sensitive than culture techniques (fewer false-negative results) for diagnosis of *R. equi* pneumonia and results are available more rapidly. Culture should be performed at the same time as PCR to identify other potential pathogens. Authors’ addresses: Department of Veterinary Clinical Sciences (Sellon) and Microbiology and Pathology (Besser), College of Veterinary Medicine, Washington State University, Pullman, WA 99164; Department of Veterinary Clinical Sciences, School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA 70803 (McConnico); and Department of Clinical Sciences, 4700 Hillsborough St., College of Veterinary Medicine, North Carolina State University, Raleigh, NC 27606 (Vivrette). © 2000 AAEP.

1. Introduction

*R. equi* is a gram-positive, acid fast, pleomorphic coccobacillus that is associated with severe pneumonia, pulmonary abscessation, and enteritis in foals from 1–6 mo of age. Onset of clinical signs is often insidious, with foals presenting for examination after the disease has progressed to severe pulmonary consolidation with large abscesses. *R. equi* is a normal inhabitant of the soil and manure of horse farms. Isolates that have been associated with pneumonia in foals uniformly contain a virulence-associated plasmid. On endemic farms, clinical signs of pneumonia are often sufficient to prompt a diagnosis of *R. equi* pneumonia in a foal of appropriate age. However, in foals from non-endemic farms, diagnosis often relies on culture of the organism from tracheal wash (TW) fluid samples. Differentiation of *R. equi* pneumonia from other types of bacterial pneumonia in foals is important because of the differences in recommended antimicrobial therapy.

TW fluid cultures may be negative for *R. equi* in infected foals because the organism is a facultative intracellular pathogen, because of prior antibiotic administration, or because of the presence of multiple pathogenic bacterial species in the sample. Hillidge reported that only 7 of 11 foals (62%) with positive *R. equi* cultures at necropsy and 57 of 89 (64%) of foals with radiographic evidence of lung abscessation yielded *R. equi* on culture of TW. We investigated the accuracy of TW fluid culture and PCR for identification of *R. equi* in foals with pneumonia.

2. Materials and Methods

Foals between 2 weeks and 8 months of age that presented for evaluation of pulmonary disease were included in the study. TW fluid samples were obtained by transtracheal or transendoscopic aspiration from all foals and submitted for culture using standard microbiologic techniques. If *R. equi* was isolated from a TW fluid sample, the isolate was assayed by PCR, Western blot, and/or plasmid isolation to determine the presence or absence of a virulence associated plasmid.

DNA was extracted from aliquots of each TW sample and assayed by PCR for the presence of DNA from the *R. equi* bacteria (16S) and its associated virulence plasmid (VP) as previously described. At the conclusion of the study, clinicians were asked to declare if a foal had *R. equi* pneumonia or not based on all the clinical information available, diagnostic test results, response to treatment, and follow-up information. Sensitivity, specificity, positive predictive value, and negative predictive value for PCR and culture were determined using final clinical diagnosis as the reference standard.

3. Results

Samples were collected from 53 foals, ranging in age from 2 weeks to 8 months with a mean of 3.4 ± 1.7 mo. There were 23 Quarter Horse type (Quarter...
Horse, Paint Horse, Appaloosa), 13 Thoroughbred, 5 Tennessee Walking Horse, 3 Arabian, 3 Morgan, and 1 each Warmblood, Draft Horse, Saddlebred, Albino, and mixed light breed foals. There were 27 male and 26 female foals.

*R. equi* was cultured from the TW fluid sample in 17% of foals. TW fluid samples from 34% of foals were PCR positive for *R. equi* bacterial and virulence plasmid DNA. An additional 4 foals were PCR positive for virulence plasmid DNA but negative for bacterial DNA. There were 13 foals with TW fluid samples that were positive by PCR for *R. equi* but negative for virulence plasmid DNA.

Culture of TW fluid was more specific than PCR for diagnosis of *R. equi* pneumonia but produced more false-negative results. There were 8 foals that were culture negative but PCR positive. These included 6 foals that were considered highly likely to have had *R. equi* pneumonia and 2 foals that were considered unlikely to have *R. equi* pneumonia.

The PCR assay was more sensitive than standard culture and produced more false-positive results. PCR was positive for all culture positive foals except one. This foal was PCR negative for *R. equi* virulence associated plasmid. Characterization of the *R. equi* isolated from this foal confirmed that it was an “avirulent” strain, probably representing an environmental contaminant in the TW fluid. PCR results were available approximately 24 h after receipt of the sample, often several days before standard culture results were available.

4. Discussion

The absence of an ante mortem “gold standard” reference test for this population of foals with pneumonia made determination of the accuracy of any individual test difficult. The use of final clinical impression as the reference standard was considered the best method for final comparison of test accuracy. Neither culture nor PCR assay was 100% accurate. PCR had a very high sensitivity and negative predictive value for this population of foals suggesting that negative PCR results are highly reliable for ruling out *R. equi* as a cause of pneumonia in a foal with pulmonary disease.

PCR can identify foals with *R. equi* pneumonia that are culture negative, avoiding prolonged delays in instituting appropriate antimicrobial therapy. In addition, PCR results are usually available much more rapidly than standard culture results, especially if many types of bacteria are present in the tracheal wash fluid. This may facilitate more rapid decisions about optimal antimicrobial therapy in a critically ill foal. However, PCR does not provide any information about concurrent infection with other potential bacterial pathogens. Therefore, PCR should be utilized as an adjunct diagnostic test with simultaneous culture of the same TW fluid sample. PCR is very sensitive and may give some false positive results, probably because of detection of environmental contaminants. Therefore, final diagnosis should always be based on consideration of the history, clinical presentation and results of all available diagnostic information.

This work was supported by USDA Animal Health Formula Funds and funds from the State of Washington.

References