Clinical Manifestations, Pathogenesis, and Diagnosis of Infections Caused by Rhodococcus equi in Foals

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Rhodococcus equi is a common cause of pneumonia in foals, but extrapulmonary manifestations are common. The virulence of R. equi for foals depends on the presence of a large plasmid expressing the genes for multiple virulence-associated proteins (Vap). The plasmid-encoded VapA is essential for virulence, but expression of VapA alone without any other plasmid-encoded genes is not sufficient to restore virulence. Bacteriologic culture and polymerase chain reaction (PCR) amplification of the vapA gene combined with cytological examination of a tracheobronchial aspirate are necessary to make a definitive diagnosis of pneumonia caused by R. equi. Author’s address: College of Veterinary Medicine, University of Georgia, Athens, Georgia 30602; e-mail: gigueres@uga.edu. © 2010 AAEP.

1. Introduction

Rhodococcus equi, a gram-positive facultative intracellular pathogen, is one of the most common causes of pneumonia in foals between 3 wk and 5 mo of age. Although R. equi can be cultured from the environment of virtually all horse farms, the clinical disease in foals is endemic and devastating on some farms, sporadic on others, and unrecognized on most. On farms where the disease is endemic, costs associated with veterinary care, long-term therapy, and mortality of some foals may be very high. This text reviews the clinical manifestations, pathogenesis, and diagnosis of R. equi infections in foals.

2. Clinical Manifestations

The most common manifestation of R. equi infections in foals is a chronic suppurative bronchopneumonia with extensive abscessation. The slow spread of the lung infection combined with the remarkable ability of foals to compensate for the progressive loss of functional lung makes early clinical diagnosis difficult. Early clinical signs often only consist of a mild fever or a slight increase in respiratory rate that may not be apparent unless foals are exercised or stressed by handling. As the disease progresses, clinical signs may include decreased appetite, lethargy, fever, tachypnea, and labored breathing. Cough and bilateral nasal discharge are inconsistent findings.

Extrapulmonary manifestations of rhodococcal infections are common. In a retrospective study of 150 foals with R. equi infections, 111 (74%) had at least 1 of 39 extrapulmonary disorders. Survival was significantly higher among foals without extrapulmonary disorders (32/39; 82%) than among foals with extrapulmonary disorders (48/111; 43%), but many such disorders were only recognized after death. Intestinal lesions are present in ~50% of
foals with *R. equi* pneumonia presented for necropsy. However, the majority of foals with *R. equi* pneumonia do not show clinical signs of intestinal disease. Abdominal lesions may include ulcerative enterocolitis and typhilitis over the area of the Peyer’s patches, granulomatous or suppurative inflammation of the mesenteric and/or colonic lymph nodes, or, in some cases, a single large abdominal abscess may be the only lesion. Polysynovitis is present in ~25–30% of cases with *R. equi* infections. The degree of joint effusion is variable and, in most cases, lameness is mild or absent. Cytological examination of the synovial fluid usually shows a non-septic monocytosis, and bacteriologic culture of the synovial fluid is negative. Histologic examination showed lymphoplasmacytic synovitis, suggesting an immune-mediated process. Immune-mediated processes may also contribute to the development of uveitis, anemia, and thrombocytopenia in some foals infected with *R. equi*.

Bacteremic spread of the organism from the lungs or gastrointestinal tract may occasionally result in septic arthritis and, more commonly, osteomyelitis. However, foals can occasionally develop *R. equi* septic arthritis or osteomyelitis without apparent lung involvement or other source of infection. *R. equi* vertebral osteomyelitis or disk spondylitis resulting in spinal cord compression has also been reported. Other rare extrapulmonary manifestations of *R. equi* infections in foals include panophthalmitis, guttural pouch empyema, sinusitis, pericarditis, nephritis, and hepatic, renal, and intracranial abscessation.

### 3. Virulence and Pathogenesis

The ability of *R. equi* to induce disease in foals likely depends on both host and microbial factors. *R. equi* is a facultative intracellular pathogen, and its ability to persist in, and eventually destroy, alveolar macrophages seems to be the basis of its pathogenicity. Knowledge of the virulence mechanisms of *R. equi* were largely speculative until the discovery of a virulence plasmid.

Unlike most environmental *R. equi*, isolates from pneumonic foals typically contain an 80- to 90-kb plasmid. Plasmid-cured derivatives of virulent *R. equi* strains lose their ability to replicate and survive in macrophages. Plasmid-cured derivatives also fail to induce pneumonia and are completely cleared from the lungs of foals within 2 wk after heavy intrabronchial challenge, confirming the absolute necessity of the large plasmid for the virulence of *R. equi*.

Nucleotide sequencing of the large plasmid of two foal isolates showed the presence of 69 open reading frames (ORFs). Comparisons of the plasmid sequence with genes previously identified in other microorganisms has identified three functional regions. Two of these regions contain genes encoding proteins involved in conjugation and in plasmid replication stability and segregation. The third region of 27.5 kb bears the hallmark of a pathogenicity island and contains the genes for a family of eight closely related virulence-associated proteins designated VapA and VapC–VapI. VapA is expressed on the bacterial surface, and its expression is temperature regulated, occurring between 34°C and 41°C. VapC, -D, and -E are secreted proteins concomitantly regulated by temperature with VapA. In a recent study, a *R. equi* mutant lacking a 7.9-kb DNA region spanning 6 *vap* genes (*vapA*, -C, -D, -E, -F, -I) was attenuated for virulence in mice and failed to replicate in macrophages. Complementation with *vapA* alone could restore full virulence, whereas complementation with *vapC, vapD, or vapE* could not. Conversely, a recombinant plasmid-cured derivative expressing wild-type levels of VapA failed to survive and replicate in macrophages and remained avirulent for foals, showing that expression of VapA alone is not sufficient to restore the virulence phenotype. These findings show that, although VapA is essential for virulence, other plasmid-encoded products also contribute to the ability of *R. equi* to cause disease. All *vap* genes, as well as five other ORFs within the pathogenicity island, are upregulated when *R. equi* is grown in macrophage monolayers. Of all the *vap* genes, *vapA, vapD, and vapG* are preferentially expressed in the lungs of infected foals, and expression of these genes in the lungs is significantly higher than that achieved during in vitro growth. Regulation of expression of the genes of the pathogenicity island is complex and depends on at least five environmental signals including temperature, pH, oxidative stress, magnesium, and iron. The precise role of each of these genes in the pathogenesis of *R. equi* infections remains to be determined.

*R. equi* isolates are often classified as virulent, avirulent, or intermediately virulent based on their ability to induce disease or death in mice. Virulent *R. equi* isolates contain the large plasmid described above and express VapA. Immediately virulent *R. equi* isolates contain one of four distinct large plasmids encoding a 20-kDa antigen (VapB) that is related to, but distinct from, VapA. In contrast, avirulent *R. equi* isolates do not express Vap antigens. All three categories of *R. equi* have the ability to cause disease in immunocompromised human patients with and without AIDS showed that only ~20% of isolates contain an 80- to 90-kb plasmid and express VapA. Therefore, the pathogenesis of *R. equi* infection in immunocompromised human patients seems to be different from the pathogenesis in foals, in which the virulence plasmid is always found. To the author’s knowledge, immediately virulent isolates (expressing VapB) have never been isolated from foals with naturally acquired *R. equi* infections. Experimentally, heavy intrabronchial challenge of foals with intermediately virulent *R. equi* results in pneumonia but at a dose much higher than that required for induc-
tion of pneumonia with VapA-expressing strains.\textsuperscript{24} Almost all isolates from the submaxillary lymph nodes of pigs produce VapB and are immediately virulent to mice, suggesting that pigs or their environment may be the source of infection for some human cases.\textsuperscript{25} Most isolates from cattle, goats, and dogs are avirulent and do not contain plasmids encoding \textit{vapA} or \textit{vapB}.\textsuperscript{26–28} In contrast, most isolates from cats contain a large plasmid and express VapA.\textsuperscript{27}

Although it has been firmly established that the virulence plasmid is essential for infection of foals, it is also clear that chromosomally encoded factors, such as regulatory genes and metabolic pathways, are also important in allowing the pathogen to thrive within the host.\textsuperscript{29–32} \textit{R. equi} is closely related to \textit{Mycobacterium tuberculosis}, as evidenced by a partial genome sequence of \textit{R. equi} that showed that the majority of \textit{R. equi} genes are largely homologous with \textit{M. tuberculosis}.\textsuperscript{33}

Inhalation of virulent \textit{R. equi} is the major route of pneumatic infection. The incubation period after experimental intrabronchial challenge varies from \textasciitilde9 days after administration of a heavy inoculum to \textasciitilde2–4 wk when a lower inoculum is administered.\textsuperscript{13,34} Lung consolidation can be detected as early as 3 days after heavy intrabronchial challenge.\textsuperscript{13} The incubation period under field conditions is unknown but likely depends on the number of virulent bacteria inhaled and various host-related factors. Ingestion of the organism is a significant route of exposure, and likely also of immunization, but rarely leads to hematogenously acquired pneumonia unless the foal has multiple exposures to large numbers of bacteria.\textsuperscript{35}

4. Diagnosis

The distinction between lower respiratory tract infections caused by \textit{R. equi} and that caused by other pathogens is problematic, especially on farms with no previous history of \textit{R. equi} infections. Many diagnostic tests including complete blood count (CBC), measurement of fibrinogen concentrations, ultrasonography, radiographs, and serology may help distinguish pneumonia caused by \textit{R. equi} from that caused by other pathogens. In one study, white cell counts >20,000 cells/\textmu l, fibrinogen concentrations >700 mg/dl, and evidence of thoracic abscession were more likely to be found in foals with pneumonia caused by \textit{R. equi} than in foals with pneumonia caused by other bacterial pathogens.\textsuperscript{36} However, bacteriologic culture and/or polymerase chain reaction (PCR) amplification of the \textit{vapA} gene and cyto
tological examination of a tracheobronchial aspirate (TBA) are necessary to make a definitive diagnosis of pneumonia caused by \textit{R. equi}.

PCR amplification of the \textit{vapA} gene is more sensitive than bacterial culture.\textsuperscript{37–39} However, culture offers the advantage of detecting other bacterial pathogens present and permits in vitro susceptibility testing of the recovered pathogens. As a result, PCR amplification of the \textit{vapA} gene may be done in association with, but should not replace, bacterial culture.

On endemic farms, many foals without clinical disease have \textit{R. equi} in their trachea as a result of inhalation of contaminated dust or as a result of a subclinical infection.\textsuperscript{40} For this reason, culture or PCR amplification of \textit{R. equi} from a TBA should be interpreted in the context of cytological evaluation and clinical examination. A light growth of \textit{R. equi} from a foal with no clinical signs of respiratory disease, no cytological evidence of airway inflammation, and no ultrasonographic or radiographic evidence of pulmonary lesions is likely an incidental finding.

Several independent studies have recently evaluated the performance of available serological tests for diagnosis of infection caused by \textit{R. equi} on endemic farms.\textsuperscript{41–44} The serological tests evaluated were found to have low sensitivity, low specificity, or both. Improving either sensitivity or specificity of enzyme-linked immunosorbent assay (ELISA) assays by changing the cut-off value of the tests could only be done at the detriment of the other. The performance of each assay was not improved by sequential sampling of each foal at 2-wk intervals over time.\textsuperscript{44} Reliance on serology as the sole diagnostic test for \textit{R. equi} infections results in overdiagnosis of the disease and in missing infections in the early stages.

References and Footnotes