Rhodococcus equi infections in foals

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Introduction

Rhodococcus equi, a gram-positive facultative intracellular pathogen replicating in macrophages, is one of the most important causes of disease in foals between three weeks and six months of age, with most foals showing clinical signs before the age of four months. *R. equi* has also been increasingly recognized as an important cause of pneumonia in immunosuppressed people, especially those infected with human immunodeficiency virus. *R. equi is* considered to be a saprophytic inhabitant of the soil. Although all horse farms are likely to be infected to various degrees with *R. equi* and antibody is widespread in the horse population, the clinical disease is enzootic and devastating on some farms, is sporadic on others, and is unrecognized on most. This probably reflects differences in environmental (temperature, dust, soil pH, soil type) and management conditions as well as differences in virulence of isolates. On enzootic farms the disease leads to significant financial loss because of the cost of therapy and occasional death of foals.

Although the virulence of *R. equi* likely depends on many determinants, isolates from pneumonic foals characteristically contain a large plasmid which encodes a gene responsible for the expression of a virulence associated protein (VapA) on the surface of the bacteria at temperatures > 34°C. Foals experimentally infected with virulent (plasmid containing) *R. equi* develop severe pneumonia whereas plasmid cured derivatives are rapidly cleared and fail to induce lesions.[1] Inhalation of dust particles laden with virulent *R. equi* is the most important route of pneumonic infection in foals. Ingestion of the organism is a significant route of exposure but does not lead to hematogenously acquired pneumonia unless the foal has multiple exposure to very large numbers of bacteria

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, make 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, cough, fever, tachypnea, and labored breathing. Nasal discharge is an inconsistent finding.

Because ultrasonographic screening for early detection has become routine practice at many farms endemic for pneumonia caused by *R. equi*, the most frequently recognized form of *R. equi* infection on those farms is a subclinical form in which foals develop ultrasonographic evidence of peripheral pulmonary consolidation or abscessation without manifesting clinical signs.[2,3] On those farms, the cumulative frequency of sonographically visible areas of focal pulmonary consolidation or abscessation considerably exceeds the historical frequency of clinical pneumonia attributed to *R. equi* indicating that many subclinically affected foals might spontaneously recover without therapy. In two independent studies at endemic farms, 80% to 90% of foals with ultrasonographic lesion recovered without antimicrobial therapy.[4,5] The proportion of such subclinically affected foals that progress to clinically-apparent disease might vary by farm, geographical region, and age at which foals are examined.

Extrapulmonary manifestations of rhodococcal infections are common. In a retrospective study of 150 foals with *R. equi* infections, 111 (74%) had at least one of 39 extrapulmonary disorders.[6] 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.[6] Intestinal lesions are present in approximately 50% of foals with *R. equi* pneumonia presented for necropsy.[7] However, the majority of foals with *R. equi* pneumonia do not show clinical signs of intestinal disease. Abdominal lesions may include ulcerative enterocolitis and typhlitis 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.[7] Polysynovitis is present in approximately 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 reveals a non-septic mononuclear pleocytosis and bacteriologic culture of the synovial fluid is negative.[8] 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 diskospondylitis 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.[6]

Diagnosis

The distinction between lower respiratory tract infections caused by *R. equi* and that caused by other pathogens is problematic especially at farms without previous history of *R. equi* infections. Diagnostic tests, including white blood cell concentration, measurement of fibrinogen concentrations, ultrasonography, and radiography, may help raise the degree of suspicion that pneumonia in a given foal may be caused by *R. equi* rather than by another microorganism. However, the definitive diagnosis of bronchopneumonia caused by *R. equi* should be based on bacteriologic culture or amplification of the *vapA* gene by polymerase chain reaction (PCR) from a tracheobronchial aspirate (TBA) obtained from a foal with: 1) clinical signs of lower respiratory tract disease, 2) cytological evidence of septic airway inflammation, and/or 3) radiographic or ultrasonographic evidence of bronchopneumonia. Amplification of *vapA* by PCR may be done in conjunction with, but should not replace bacterial culture because it does not permit identification of other bacterial pathogens and *in vitro* antimicrobial susceptibility testing of *R. equi* isolates.[9] The definitive diagnosis of extrapulmonary infections (e.g. abdominal abscess, osteomyelitis) caused by *R. equi* must rely on bacteriologic culture or PCR amplification of *vapA* from samples from the site of infection. The diagnosis of extrapulmonary disorders from sites at which *R. equi* cannot be detected (e.g. uveitis or polysynovitis) should be based on isolation of *R. equi* from a TBA or other primary sites of infection. The diagnosis of enterocolitis caused by *R. equi* is problematic because isolation of *R. equi* from feces cannot be taken as evidence of enterocolitis caused by *R. equi*.[9]

Hyperfibrinogenemia is the most consistent laboratory finding in foals with *R. equi* pneumonia, although rare cases may have normal fibrinogen concentrations. Neutrophilic leukocytosis with or without monocytosis is also common. One study showed significantly higher fibrinogen concentrations and white blood cell counts (WBC) in non-survivors than in survivors whereas other studies showed no difference between the 2 groups. In one study, WBC >20,000 cells/µL, fibrinogen concentration >700 mg/dL, and evidence of pulmonary abscessation were more likely to be found in foals with pneumonia caused by *R. equi* than in foals with pneumonia caused by other bacteria.[10] However, there is a considerable overlap in distributions, which precludes the use of fibrinogen concentrations and WBC for diagnosis or prognosis for an individual foal.

Thoracic radiography is useful in evaluating the severity of pneumonia and in assessing response to therapy. A prominent alveolar pattern characterized by ill-defined regional consolidation is the most common radiographic abnormality. The consolidated lesions are often seen as more discrete nodular and cavitary lesions consistent with pulmonary abscessation. Although non-survivors tend to have more severe radiographic lesions than survivors, many survivors have very severe radiographic lesions thus radiographs should not be used as the sole criterion for prognostication and euthanasia.[11] Ultrasonography is a helpful diagnostic tool when lung involvement includes peripheral areas but may not be as useful as radiography to evaluate the full extent of lung lesions since abscesses with overlying aerated lung will not be detected. However, in most horses and foals with pulmonary abscessation the periphery of the lung is affected, enabling the ultrasonographer to successfully image some of the abscesses. Early ultrasonographic lesions are non-specific and may only include irregularities of the pleural surface. These lesions may progress to form focal areas of consolidation of various sizes. In more chronic cases, well circumscribed, encapsulated abscesses can be detected. Ultrasonography is very useful in evaluating the severity of pneumonia and in assessing response to therapy especially for equine practitioners who do not have access to thoracic radiography. Ultrasonography is also a useful tool for detection of some abdominal abscesses and for screening for *R. equi*-infected foals on farms where the disease is endemic (see section on control). Ultrasonographic or radiographic detection of lung abscesses raises the degree of suspicion that pneumonia in a given foal is caused by *R. equi*. However, detection of pulmonary abscesses, while commonly used as a screening test (see below), is not a definitive diagnostic test.

Independent studies evaluating the performance of serological tests available for diagnosis of infection caused by *R. equi* at endemic farms have demonstrated these tests have either low sensitivity, low specificity, or both.[12-15] Improving either sensitivity or specificity of ELISA assays by changing the cut-off value of the tests could only be done to the detriment of the other. The presence of antibodies indicates exposure, subclinical infection, or maternal transfer of antibodies but it does not necessarily indicate infection leading to clinical disease. The current state of knowledge precludes serology being used as a diagnostic test for *R. equi* pneumonia.

Treatment

A wide variety of antimicrobial agents are active against *R. equi* in vitro. However, because *R. equi* is a facultative intracellular pathogen surviving and replicating in macrophages and therefore causes granulomatous lesions with thick caseous material, many of these drugs are ineffective in vivo. For example, in one study all 17 foals with *R. equi* pneumonia treated with the combination of penicillin and gentamicin died despite the fact that all isolates were sensitive to gentamicin.[8] The combination of rifampin and erythromycin became the treatment of choice in the 1980s and has dramatically reduced foal mortality since its introduction. In recent years, clarithromycin or azithromycin, two newer generation macrolides, often replace erythromycin in the combination with rifampin. Macrolides and rifampin are highly active against *R. equi* in vitro but only exert bacteriostatic activity. Of the three macrolides listed above clarithromycin is the most active against *R. equi* in vitro. The combination of a macrolide and rifampin is synergistic both in vitro and in vivo and the use of the two classes of drugs in combination reduces the likelihood of *R. equi* resistance to either drug. Rifampin and macrolides are lipid soluble, allowing them to penetrate cell membranes and caseous material.

Advantages of azithromycin and clarithromycin over erythromycin in foals include enhanced oral bioavailability, prolonged half-lives, and much higher concentrations in bronchoalveolar cells and pulmonary epithelial lining fluid.[16] These properties of the newer generation macrolides contribute to their lower dosages and longer dosing intervals. Concentrations of clarithromycin in pulmonary epithelial lining fluid and bronchoalveolar cells of foals at steady state are considerably higher than concentrations reported following daily administration of azithromycin to foals. However, clarithromycin concentrations at these sites decrease rapidly, whereas the release of azithromycin from cells is much slower, resulting in sustained concentrations of azithromycin in tissues for days following discontinuation of therapy. In a retrospective study, the combination clarithromycin-rifampin was significantly more effective than erythromycin-rifampin or azithromycin-rifampin, especially in foals with severe radiographic lesions.[17]

Although well-tolerated by most foals, macrolides commonly cause diarrhea. Most of the time the diarrhea is self-limiting and does not necessitate cessation of therapy but affected foals should be monitored carefully because some may develop severe diarrhea, leading to dehydration and electrolyte loss that necessitate intensive fluid therapy and cessation of oral macrolides. The incidence of diarrhea in foals treated with erythromycin-rifampin has ranged between 17 and 36%. In most cases, diarrhea was mild and self-limiting. In the same study, the incidence of severe diarrhea necessitating administration of IV fluids was not significantly different between groups of foals treated with azithromycin-rifampin, clarithromycin-rifampin or erythromycin-rifampin. During surges of very hot weather an idiosyncratic reaction characterized by severe hyperthermia and tachypnea has been described in foals treated with erythromycin. Anecdotal reports suggest that these reactions may occasionally occur with newer macrolides as well. Administration of antipyretic drugs and placing the foal in a cool environment will treat this problem. Severe enterocolitis has also been reported in mares whose foals are being treated with erythromycin, presumably due to disruption of the mare's normal colonic microflora following ingestion of small amounts of active drug during coprophagia or from contamination of feeders or water buckets with drug present on the foal's muzzle.

Prognosis

Prior to the introduction of the combination erythromycin-rifampin as the recommended treatment, the prognosis of *R. equi* pneumonia was poor with reported mortality rate as high as 80%. Using erythromycin and rifampin a successful outcome (as assessed by survival) in 50 (88%) of 57 foals with confirmed *R. equi* pneumonia has been reported. However, until recently there was no information on the impact of *R. equi* infections on future athletic performance. Recently, a large collaborative study involving several major veterinary hospitals was conducted in order to definitively assess the influence of prior *R. equi* pneumonia on racing performance. The records of 115 foals (49 Thoroughbreds [TB] and 66 Standardbreds [SB]) that had chest radiographs and were diagnosed with *R. equi* pneumonia based on culture of a TBA were reviewed.[18] All cases were treated with erythromycin and rifampin between 1984 and 1994. The survival rate was significantly higher in SB (80%) than in TB (61%). Death was more likely in foals presented with respiratory distress and the non-survivors had a higher radiographic score on admission than survivors. Of the survivors 54% (for both SB and TB) had at least one racing start as opposed to 65% for the control population suggesting that horses contracting *R. equi* pneumonia as foals are slightly less likely to race. However, those foals that raced performed as well as expected.

Prevention

Screening

R. equi pneumonia is often not recognized until it is well advanced and, therefore, difficult to treat. Even severely affected foals may appear to suckle and behave normally to a casual observer. The rationale for screening is the assumption that detecting foals in the early stages of disease along with appropriate treatment of affected foals will improve outcome. It is important to emphasize that screening methods are not diagnostic tests. A useful screening test is one in which the probability of disease is high with a positive test result (high positive predictive value) and very low with a negative test result (high negative predictive value). The higher the prevalence of disease at a given farm, the higher the positive predictive value of a given test will be. Therefore, depending on the prevalence of *R. equi* infections on a given farm, a positive result of a screening test could be a basis to perform a diagnostic test (low to moderate prevalence) or to initiate therapy (high prevalence).

A variety of screening techniques performed serially have been described, including visual inspection of foals for clinical signs of pneumonia, monitoring rectal temperatures, hematological parameters, serology, and thoracic imaging using either radiography or ultrasonography, with empiric recommendation that screening begin around three weeks of age. Systematic comparisons of these tests have not been performed. Thus, a specific recommendation for any particular screening test cannot be made, and it is likely that the optimal approach for screening may vary among farms on the basis of cumulative incidence of disease, resources available for control and prevention, and preferences of the attending veterinarian(s) and farm management.

Over the past decade, control of *R. equi* infections at many farms where the disease is endemic has relied on early detection of subclinical pulmonary disease using thoracic ultrasonography and initiation of treatment with antimicrobial agents prior to development of clinical signs.[2,3,19] Ultrasonography of the chest offers several advantages over other screening tests: 1) results are specific for the presence of pulmonary pathology; 2) the procedure can be performed relatively quickly for an individual foal; 3) results are available immediately; and 4) the procedure may be more sensitive than radiography for detecting lesions in their early stages of development or in certain regions where soft tissue structures are superimposed over regions of the lung. Although controlled studies are lacking periodic ultrasonography of the chest appears to have decreased mortality due to *R. equi* pneumonia at some farms. [2,3,19]

However, recent double blinded randomized placebo controlled studies documented that approximately 88% of foals with small pulmonary lesions (sum of lesion diameters [or abscess score] of 1-10 cm) recover without antimicrobial therapy.[4,20] In addition, antimicrobial treatment of foals with small ultrasonographic lesions did not significantly hasten lesion resolution compared to administration of a placebo.[4,20] The use of ultrasonography for screening to detect *R. equi* pneumonia was recently evaluated at an endemic farm with personnel and veterinarians blinded to screening results.[5] Of 270 foals enrolled in the study, 216 (80%) developed sonographically visible pulmonary consolidation whereas only 17% of foals developed clinically apparent *R. equi* pneumonia.[5] Pulmonary lesions resolved without clinically apparent illness or antimicrobial therapy in 79% of foals with ultrasonographic lesions.[5] In the aforementioned study, the cumulative sensitivity of ultrasonography was very good (89%) but cumulative specificity was low (62%).[5]

Because it is impossible to know which specific foals might recover spontaneously from subclinical disease, and because *R. equi* infections can cause severe disease, many breeding farms elect to treat all foals with ultrasonographic lesions. This approach has resulted in an increased number of foals treated for presumptive *R. equi* pneumonia. The temporal association between this widespread use of macrolides and rifampin as a result of ultrasonographic screening and a perceived increase in the frequency of detection of resistant isolates in the last decade[21] suggest that this practice may not be innocuous. Emergence of widespread macrolide- and rifampin-resistance at a farm after widespread use of these drugs was instituted as part of an ultrasonographic screening program has been documented.[22] On that particular farm, 20%-40% of *R. equi* isolates from pneumonic foals were resistant to macrolides and rifampin.[22] Currently, it is unknown if macrolide- and rifampin- resistance is widespread on many horse farms relying on mass antimicrobial therapy of foals with ultrasonographic lesions or if it is an isolated problem. In addition to the cost and risk for selection for resistant bacteria, unnecessary mass antimicrobial therapy of foals with pulmonary lesions might lead to development of life threatening adverse reactions (e.g. diarrhea or hyperthermia) in some foals.

The spontaneous resolution of ultrasonographic lesions in a large proportion of foals with ultrasonographic lesions,[4,5,20] combined with the apparent increase in macrolide- and rifampin-resistance at some farms[22] support the need to stop the practice of mass macrolide treatment of all subclinically affected foals with ultrasonographic lesions. The goal should be to more accurately identify, of the many subclinically infected foals, which few are likely to go on to develop disease and hence require treatment. The degree of severity and the number of ultrasonographic lesions that warrant therapy are unknown and may vary by farm, geographical region, and age at which lesions are detected. Additional studies are needed to establish better criteria to determine the need for therapy in subclinically affected foals and to better quantify the risks versus benefits of treating foals with subclinical ultrasonographic lesions.

Passive immunization

Intravenous administration of hyperimmune plasma (HIP) obtained from horses vaccinated against *R. equi* using various antigens has generally proved effective in significantly reducing the severity of *R. equi* pneumonia in foals following experimental challenge. However, studies evaluating the efficacy of various HIP preparations under field conditions have given equivocal results. Although the data are conflicting and not all trials have shown a statistically significant reduction in the cumulative incidence of *R. equi* pneumonia, five of seven studies have demonstrated reduction of relative risk, suggesting some benefit of HIP.[23] Use of HIP licensed as an aid in the control of *R. equi* pneumonia is recommended (rather than plasma simply obtained from horses hyperimmunized against *R. equi*) because licensure ensures standard of potency, purity, and safety. Currently, there is insufficient information to recommend one brand of licensed antibody product over another.

The optimal amount of plasma to be transfused and the optimal age at which transfusion should occur remain to be determined. Administration of HIP nine days after aerosol infection of foals with *R. equi* did not confer protection,[24] suggesting that administration of HIP prior to infection is important.

Because of evidence that many foals become infected early in life, it is commonly recommended that foals receive transfusion of at leastone liter of HIP no later than the second day of life. Because early administration may result in the decline of passively transferred antibody to a non-protective level at a time when foals are still susceptible to *R. equi* and when environmental challenge is high, it is common practice to administer a second dose of HIP at two to four weeks of age.

Transfusion of HIP is not completely effective and therefore does not eliminate the need for careful monitoring of foals at risk. In addition to being incompletely effective, transfusion of HIP carries some risk to foals, both in terms of trauma that may occur during handling and adverse reactions to transfusions. The process is also time- and labor-intensive, and expensive. The cost effectiveness of transfusion depends on the value of the foals and the prevalence of disease at a given farm.

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