Rhodococcus equi–What is New This Decade?

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KEYWORDS

• *Rhodococcus equi* • Pneumonia • Hyperimmune plasma • Antimicrobial resistance • Foal

KEY POINTS

- Foals are infected with *Rhodococcus equi* shortly after birth.
- Many infected foals develop self-resolving subclinical disease.
- Treatment of subclinical disease has led to the development of antimicrobial-resistant strains.
- Administration of hyperimmune plasma minimizes the severity of pneumonia but does not prevent infection.
- Better biomarkers are needed to aid targeted treatment and minimize the development of resistance to antimicrobials.
- Questions remain regarding prophylactic strategies against this pathogen.

Our understanding of *R equi* pathogenesis has changed over the last decade, and this knowledge has translated to a new set of recommendations regarding prophylaxis, treatment, and diagnosis. Before thoracic ultrasonography became widely available, it was proposed that foals became infected with *R equi* around 3 to 6 month of age, the period of time when the clinical signs were observed. With the introduction of thoracic ultrasonography as a screening tool, it became apparent that foals were infected early in life and disease progressed slowly until the development of clinical signs months after the initial infection. Unfortunately, early diagnosis of infection was also associated with an increased rate of treatment of subclinical foals and rapid selection of antimicrobial resistant (AMR) strains of *R equi*. The goal of this article is to provide the equine practitioner with a useful summary of the current recommendations that are supported by new literature and to highlight the areas that require further investigation.

RHODOCOCCUS EQUI IN FOALS

Rhodococcus equi (*R equi*) remains the most common cause of subacute or chronic granulomatous bronchopneumonia in foals less than 5 months of age.[1,2] This disease

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continues to have a major financial impact on the horse industry due to the cost and labor associated with treatment and prevention strategies and the lack of a commercial vaccine.

R equi is a gram-positive intracellular bacterial pathogen that is normally present in the environment and in the manure of healthy herbivores.[3] Under breeding farm situations, the environmental concentration of *R equi* increases because the organism replicates in horse manure and infected foals shed larger amounts of virulent *R equi* in their feces.[4] Conditions that favor aerosolization of *R equi* such as high foal density and hot dry weather have been recognized as risk factors for *R equi* infection.[5] The role that exhaled *R equi* has in the epidemiology of *R equi* infection remains to be determined.[6] Virulent and avirulent strains of *R equi* exist and both are commonly isolated from horse manure, air, and soil in equine farms.[7] Virulent strains carry a virulence plasmid and express a highly immunogenic surface protein called virulence-associated protein A (VapA). This protein is required for intracellular survival of the bacterium inside macrophages. Strains lacking the *vapA* gene cannot successfully replicate inside the macrophage and therefore are considered avirulent to foals.[8] Most clinically affected foals carry vapA-positive strains but clinical samples should be tested for the presence of the *vapA* gene to confirm virulence.

PATHOGENESIS OF *RHODOCOCCUS EQUI*

R equi does not affect adult horses unless these are immunocompromised. To date, which specific age-related factors are responsible for this susceptibility remains to be determined. Immunosuppressed people, cats, camelids, and dogs are also susceptible to infection.[9–12]

Early on, it was assumed that foals were infected closer to the development of clinical signs (3–6 months of age), time that coincided with the natural decrease of maternal antibodies. The insidious character of the disease along with the ability of foals to compensate for the progressive loss of functional lung,[13] made it hard to identify early age as a risk factor until thoracic ultrasonography made it possible to observe pulmonary lesions as early as 1 month of age in subclinical animals.[14] It is now well established that foals are infected shortly after birth and they become less susceptible as they age.[15–17] Exposure of foals to airborne virulent *R equi* during the first 2 weeks of life is associated with the development of disease[17]; the role oral exposure plays in disease development is unclear. Once inhaled, virulent *R equi* infects alveolar macrophages where the organism replicates until macrophage necrosis occurs. Continuous macrophage death and reinfection of new macrophages leads to the characteristic abscess formation.[13] Similar to *Mycobacterium tuberculosis*, *R equi* is slow growing and clinical signs do not develop for months.[18] This understanding of the time of infection has changed prophylactic and treatment recommendations and has been key for the improvement of research models to study this disease.

Unfortunately, there are no small animal models to replicate this condition. Mice are not susceptible to *R equi* infection unless they are immunocompromised and develop systemic instead of respiratory disease.[19] Guinea pigs seem to be resistant to pulmonary infection with virulent *R equi.*[20] Although some of these nonequine models are useful to perform initial in vitro and in vivo testing,[21] foal models are needed for the final evaluation of prophylactic methods, vaccines, and for the understanding of *R equi* pathogenesis in equids. This situation poses challenges for *R equi* research. Housing of mares and foals is expensive, mare's gestation is long, and foals are not genetically identical, which results in variable results and the need for studies with larger sample sizes.

CLINICAL SIGNS

The clinical signs of *R equi* infection vary with the location of the infection. The pulmonary form, which is the most common presentation, results in clinical signs of pneumonia of variable severity. Early on, foals may develop fever, anorexia, and lethargy, which are followed by tachypnea, increased respiratory effort (nostril flaring), cough, and nasal discharge as the disease progresses.[22] These signs are worse in hot, humid environments.[23] In farms where *R equi* infection is endemic, thoracic ultrasonography reveals rates of subclinical infection (pulmonary lesions consistent with abscessation or consolidation in foals without clinical signs) above 50% of the foal population. Without treatment, 70% to 85% of these foals will remain subclinical and will heal over time.[24] However, 20% to 25% of these foals will develop clinical signs of pneumonia that requires treatment.[7,24,25] Overall, uncomplicated pulmonary infection carries a high survival rate (>90%) but severe pneumonia may result in higher mortality rates (19%).[23]

Extrapulmonary lesions are not uncommon and can affect a wide variety of organ systems. Moreover, multiple systems may be affected at once. Thus, it is important to closely evaluate extrapulmonary sites in foals with suspected or confirmed *R equi* infection. *R equi* bacteremia may be responsible for these presentations but an association between a positive blood culture and a specific extrapulmonary presentation has not been found.[26] Abdominal manifestations, the most common extrapulmonary presentation, can cause severe disease and high mortality rate. Foals usually present with diarrhea, fever, anorexia, and lethargy and may have leukopenia and hypoproteinemia because of granulomatous enterocolitis or enterotyphlocolitis. Milder forms present with soft manure, poor growth rates, and rough haircoat. Abdominal lymphadenitis is typically detected postmortem but may occasionally be visible on abdominal ultrasonography. Abdominal abscessation can lead to septic peritonitis.[26] Subclinical pyogranulomatous hepatitis has also been identified during necropsy.[26] Both, immune-mediated and septic polysynovitis can occur because of *R equi* infection. Foals with immune-mediated polysynovitis have effusion in multiple joints but no signs of lameness and respond well to corticosteroid treatment.[27] In contrast, foals with septic joint/s or osteomyelitis are lame and require aggressive treatment. Unilateral or bilateral uveitis, defined as the presence of aqueous flare, fibrin hypopyon, and/or hyphema, has been described in infected foals and may result from septic or immune-mediated processes.[26–29] Although foals with severe uveitis were less likely to survive, more studies are needed before this clinical finding can be used as a definitive prognosticator.[28] Immune-mediated hemolytic anemia has also been reported.[29,30] Vertebral body osteomyelitis can present with a variety of neurologic signs depending on lesion location. Other presentations such as mediastinal lymphadenopathy, pericarditis, subclinical granulomatous meningitis are less common.[26]

DIAGNOSIS

Early diagnosis of *R equi* is key for successful treatment because this organism responds poorly to routinely used antimicrobials such as beta-lactam and aminoglycoside combinations, or potentiated sulfonamides.[31] Differentiation of *R equi* from other causes of bacterial pneumonia based solely on clinical signs is not possible because the clinical signs are nonspecific to *R equi*. Presumptive diagnosis can be made based on signalment, farm history of *R equi* infection, clinical signs, thoracic ultrasonography, or radiography as well as bloodwork changes.[23] However, a definitive diagnosis can only be achieved by culture and fluid analysis of a tracheobronchial aspirate (TBA)

in cases of pneumonia or by culture of a sample obtained from an extrapulmonary site. *R equi* pneumonia can be confirmed if the TBA sample is positive for virulent *R equi* and cytology is consistent with suppurative inflammation. A sterile TBA can be collected percutaneously or using a double-guarded aspiration catheter via endoscopy. Disadvantages of TBA fluid collection are the time required for bacterial culture (72 hours) and the procedural risks in cases of severe respiratory disease.[23] Bronchoalveolar lavage fluid (BALF) collection is easily performed in the field. Cytological evaluation of BALF from foals with *R equi* pneumonia had a higher neutrophil percentage than foals with other causes of bacterial pneumonia. However, the large overlap in the range of neutrophil percentage between both groups limits the diagnostic use of this test.[32]

Imaging of the lungs is useful to detect pulmonary pathologic condition and aids presumptive diagnosis. Thoracic radiographs of foals with *R equi* pneumonia show ill-defined soft tissue nodules with or without irregular areas of cavitation.[33] Superficial pulmonary abscessation and consolidation are also easily identifiable using thoracic ultrasonography.[33] Although these changes are common in foals with clinical and subclinical pneumonia, they are not pathognomonic for *R equi*. Radiographic evidence of thoracic abscessation in pneumonic foals showed a sensitivity of 71% and a specificity of 85% for the diagnosis of *R equi* pneumonia.[23] Bloodwork changes are nonpathognomonic for *R equi* pneumonia either. Leukocytosis characterized by mature neutrophilia and monocytosis, as well as high fibrinogen and globulin concentrations, are common in clinical and subclinical *R equi* infection.[23,34] Thus, bloodwork should not be used in isolation to decide the treatment of foals. These results are best used in combination with other parameters to promote targeted foal treatment.[35,36] Serum amyloid A (SAA) was not a reliable predictor of clinical *R equi* pneumonia in a study with a low number of foals but it was useful to evaluate disease progression and response to treatment.[37]

Other diagnostic tests, such as real-time quantitative polymerase chain reaction (qPCR) detection of virulent ($VapA^+$) *R equi* in feces, are being evaluated.[38] Fecal qPCR is a noninvasive, rapid diagnostic test but the presence of virulent *R equi* in the manure of normal and subclinical foals poses a challenge for its interpretation.[38] Serology should not be used as a screening tool in farms with endemic *R equi* because positive results will lead to unnecessary treatment of foals.[34] Serum IgG(T) was significantly higher in foals that developed *R equi* pneumonia after experimental and natural infection but more work under field conditions is needed before it can be recommended as a useful marker.[39]

Diagnosis of extrapulmonary lesions can be challenging depending on location and clinical signs. Extrapulmonary lesions should be suspected in foals from endemic farms that have bloodwork changes supportive of chronic infection (mature neutrophilia, monocytosis, thrombocytosis as well as increased SSA, fibrinogen, and globulins). Some lesions may only be recognized on postmortem evaluation.[26] Whenever accessible, samples should be collected and submitted for culture.

TREATMENT

Although there is no question that foals with clinical signs of *R equi* infection should be treated, the equine practitioner is faced with the challenge of deciding when to treat foals with subclinical disease (foals with ultrasonographic or bloodwork evidence of infection but without clinical signs of disease). Many farms with endemic *R equi* problems attempt to minimize the occurrence of rhodococcal pneumonia by early identification of infected foals using thoracic ultrasonography coupled with aggressive

antibiotic treatment.[14,40] Although this approach was thought to be beneficial in terms of reducing mortality, it is not without risks. The recommended macrolides used to treat *R equi* infections can cause mild, self-limiting diarrhea,[41] and hyperthermia in foals[42,43] as well as occasionally fatal colitis in mares.[44] Evidence indicates that the prevalence of rhodococcal infections on farms may be overestimated using routine ultrasound screening. Thus, the incidence of *R equi* pneumonia on farms that do not routinely use thoracic ultrasonography varies between 5% and 20%, whereas on farms that use ultrasound screening, the number of foals identified with lung lesions ranged from 29% to 64%.[14,40] These results, along with recent studies, suggest that many foals with small pulmonary lesions recover without antimicrobial therapy and that antimicrobial treatment of foals with small lesions (median abscess score $\leq$6–10 cm) does not significantly accelerate lesion resolution relative to administration of a placebo.[22,24,41]

More farms are now introducing treatment protocol changes with the goal of minimizing the number of foals that receive antimicrobials every year. Alteration of the treatment criteria to exclude foals with subclinical disease and small ultrasonographic lesions decreased the number of foals treated from 80% to 50% without increasing mortality in a farm.[36] In another farm, the addition of white blood cells and SSA to a thoracic ultrasonographic screening program reduced the number of foals treated without significantly increasing the risk of the development of clinical *R equi* pneumonia.[35] Establishing this type of program in a farm that uses a screen and treat program may be difficult for the practitioner due to the perceived risk of increased mortality because of the changes.[35] Moreover, there are no specific recommendations that can be applied to all farms at this time. Therefore, each veterinarian should develop an individualized screening program that gradually aims to minimize the number of foals treated on each farm.

A wide range of antimicrobials is active against *R equi* in vitro but only a few of these are effective in vivo likely due to the intracellular nature of this organism.[45] Thus, an appropriate antimicrobial treatment plan should be used for clinical cases with a presumptive diagnosis of *R equi* pneumonia, because this organism responds poorly to routine antimicrobials, such as beta-lactam and aminoglycoside combinations, or potentiated sulfonamides.[31] Foals 2 to 6 months of age with clinical signs of pneumonia and high white cell count (>20,000 cells/μL) and elevated fibrinogen concentration (>700 mg/dL) are likely to be infected with *R equi* (specificity 85%).[34] Mixed bacterial infections, most commonly with *Streptococcus* spp. and *Actinobacillus* spp., are not uncommon in foals with moderate-to-severe *R equi* pneumonia[23] but coinfection does not seem to negatively influence prognosis.[46] Mixed bacterial infections are rarer in mild-to-moderate *R equi* pneumonia cases.[32,46]

The recommended treatment of *R equi* infection is a combination of a macrolide and rifampin (5 mg/kg PO Q12 h or 10 mg/kg PO q24 h). Clarithromycin (7.5 mg/kg PO Q12 h) and azithromycin (10 mg/kg PO Q24 h for 5 days, Q48 h thereafter) are the most commonly used macrolides. Although administration of rifampin decreases oral absorption and plasma concentration of clarithromycin in foals,[47] the concentration of these drugs in pulmonary epithelial lining fluid and in pulmonary macrophages of foals remains above the minimum inhibitory concentration for *R equi* when this combination is used.[48,49] Moreover, the combination of drugs was found to be superior than monotherapy in mice.[50] Typically, antimicrobials are administered for 4 to 8 weeks[51] but duration of treatment varies with disease presentation and shorter courses may be beneficial.[52] Because the combination treatment can be expensive and labor intensive, drugs that can be used for monotherapy have been investigated. Tulathromycin, a macrolide that is given intramuscularly once a week (2.5 mg/kg) does

not seem to be effective when used alone[25,53] but was shown to have similar treatment efficacy as the azithromycin–rifampin combination in foals with mild to moderate to severe pneumonia when combined with oral rifampin (10 mg/kg Q24 h).[52] Intramuscular administration of gamithromycin (6 mg/kg, IM, once a week) was shown to be noninferior to azithromycin–rifampin. However, almost 60% of the foals developed significant side effects including colic that required treatment with analgesics and marked lameness. Local pain that lasted for 5 days was also observed when the drug was administered subcutaneously.[54,55] Intravenous gamithromycin (6 mg/kg, IV, once a week) through a catheter induces significantly fewer adverse effects but more research in needed to refine the dose and dosing interval in foals with *R equi.*[49,55] Additional research is needed before monotherapy can be recommended in moderate-to-severe *R equi* cases.

Aside from the described side effects related to local site of administration, macrolides cause diarrhea in a third of the treated foals. Although the diarrhea usually improves with treatment discontinuation, a subset of these foals may require supportive treatment; therefore, diarrheic foals should be closely monitored.[56] Foals should be kept in well-ventilated, cold areas while treated with macrolides. Macrolides cause a drug-induced anhidrosis, which in turn results in hyperthermia that may be fatal. Hyperthermic foals are usually tachypneic. Anhidrosis develops shortly after treatment is initiated and lasts for at least 3 weeks after discontinuation of treatment.[42,43] Increased liver enzymes may be observed in foals that receive rifampin.[24] An in-depth review of *R equi* treatment has been published recently.[51]

The efficacy of other drugs for the treatment of *R equi* has been investigated. When used as monotherapy, doxycycline failed to reduce the size of lung abscesses compared to other drugs and to placebo groups in experimental animal studies.[22] The combination of doxycycline and azithromycin had a similar therapeutic effect compared with the combination of rifampin and azithromycin in a randomized controlled clinical trial in foals with mild or subclinical pneumonia.[41] The study did not include an azithromycin group; thus, it is difficult to assess the real contribution of doxycycline to the combination. Gentamicin was shown to be among the most active drugs against *R equi* using an in vitro intracellular bactericidal assay[45] but failed to reach the mutant prevention concentrations in BAL cells and pulmonary fluid lining.[57] Intravenous liposomal gentamicin was effective for the treatment of *R equi* pneumonia after experimental infection but caused nephrotoxicity in 50% of the treated foals.[58] Alternative routes such as nebulization or different dosing intervals will be needed before this drug can be safely used in these foals.[58] The use of gallium maltolate is not recommended for treatment at this time because its efficacy in clinical cases has not been demonstrated.[59]

IMPACT OF SCREENING ON *RHODOCOCCUS EQUI* RESISTANCE

Prophylactic antimicrobial treatment of foals with subclinical lesions is not superior to the use of a placebo[22] and has led to a significant increase in AMR strains.[60–62] Farms that prophylactically treated foals based on thoracic ultrasonography screening programs had significantly higher concentrations of AMR strains in their soil than farms that did not routinely treat subclinical foals.[63] Resistance has also significantly increased in clinical samples from foals. Reports of resistance ranged from 0.7% to 3.7% before 2010 in studies from KY and Texas.[64,65] This number increased to 13% resistance for rifampin and 16% resistance to macrolides between 2007 and 2017 in KY.[65] Virulent *R equi* isolates resistant to rifampicin and macrolides were

more common in necropsied foals previously treated with mainstay dual therapy than in foals that did not receive any antimicrobial treatment.[66]

The development of AMR strains has multiple clinical implications. Macrolide resistance in macrolide-resistant isolates of *R equi* in the United States is caused by erm(46), an erythromycin-resistant methylase gene that has been identified only in *R equi* to date.[67,68] Strains that express the gene for resistance are resistant to all macrolides, lincosamides, and streptogramin B,[65] and the gene could transfer horizontally to other bacterium.[69] Foals that harbor resistant strains contaminate the environment via cough or manure. Treatment options are greatly minimized in phase of resistance. Alternative antimicrobials to treat AMR *R equi* infections such as imipenem are limited by their use in human medicine.[70] Moreover, this drug does not seem to achieve therapeutic concentrations in BAL cells or pulmonary lining fluid.[57] The limitations of other antimicrobials have been described in the treatment section. Survival of foals infected with resistant strains is significantly lower than survival of foals infected with antimicrobial susceptible strains.[65] When treated with a rifampin-macrolide combination, only 25% of the foals infected with a resistant strain survived to discharge in comparison with 69% survival rate for the foals infected with a nonresistant strain.[65] Epidemiological studies are needed to determine the duration of AMR persistence on farms and the possibility of resistance sharing between bacteria that might serve as a repository of antimicrobial-resistance genes that could threaten public health.[63]

PREVENTION

It is important to understand that absolute prevention of *R equi* infection is unlikely. Thus, even with a successful prophylactic program in place, foals are likely to develop pulmonary lesions identifiable using thoracic ultrasonography. The goal of a prophylactic program should be to decrease the incidence of clinical pneumonia and its severity because this will minimize the use of antimicrobials and the development of AMR strains.

To date, there is no commercially available vaccine to prevent infection against *R equi* or to minimize the frequency of clinical disease. Vaccination of neonatal foals is challenging because priming of the naïve neonatal immune system requires multiple vaccine doses.[71] As foals are infected shortly after birth,[7,16] complete vaccination will only be achieved after infection has occurred. Moreover, vaccine response may be affected by the presence of maternal antibodies.[72] Multiple vaccine candidates have been tested and failed to prevent infection in foals or have been only tested in nonequine models. A detailed summary of this early vaccine study has been published elsewhere.[72] A recent study evaluating a pilus (Rpl) vaccine administered during gestation to mares failed to decrease the severity of pneumonia in foals after experimental infection in spite of higher colostral antibodies against the pilus in vaccinated mares and higher serum and BALF antibody titers in foals born from vaccinated mares.[73] A vaccine based on a highly conserved bacterial polysaccharide (poly-N-acetylglucosamine or PNAG) was protective against intrabronchial challenge of 28-day-old foals[71] but failed to reduce the incidence of pneumonia in foals challenged shortly after birth.[74] As of now, the only vaccination method that protected foals against *R equi* after experimental infection was the administration of live, virulent *R equi* orally.[75] However, electron beam-inactivated *R equi*, which are structurally intact microorganisms, did not reduce the proportion of foals developing clinical pneumonia after experimental challenge.[76]

Because of the current vaccine situation, most farms with endemic *R equi* rely on *R equi*-specific hyperimmune plasma (Re-HIP) administration as a means for

prophylaxis. Early research reported mixed results with this practice[77–80] but newer data support its use based on clinical benefits. Intravenous administration of Re-Hip to neonates significantly reduced the severity of pneumonia after experimental challenge of 1-week-old foals.[81] Administration of a novel hyperimmune plasma, raised against β-1→6-poly-*N*-acetyl glucosamine (PNAG-HIP) shortly after birth was not superior to a commercially available Re-HIP product for protecting foals against natural development of *R equi* pneumonia.[82] Recently, the volume of Re-HIP administered has been evaluated. Foals that received 2 L of Re-HIP shortly after birth were 2.4 times less likely to develop clinical pneumonia than foals that received 1 L of Re-HIP at the same time. Transfusion of 2 L of Re-HIP also resulted in a lower proportion of foals (12% vs 32%) developing subclinical pneumonia identified by thoracic ultrasonography.[83] Moreover, the administration of 2 L of Re-HIP appeared safe.[83,84] More research evaluating the effect of the volume and time of Re-HIP administration is needed as the 2 studies previously described are limited by their retrospective nature. Foals that received Re-HIP shed less-virulent *R equi* in their manure when compared with foals that did not receive Re-HIP after experimental infection.[85] Additional research is needed to assess the potential benefit of Re-HIP administration in environmental contamination. Another area that requires further investigation is the mechanism of Re-HIP protection. It is tempting to speculate that *R equi*-specific antibodies are fully responsible for the acquired protection because the amount and activity of antibodies in Re-HIP are positively associated with the protection against *R equi*[86]; however, the role other proteins, such as complement, has been shown to be key for *R equi* opsonization.[71] Although recommended at this time, hyperimmune plasma administration is expensive, labor intensive, and mild side effects such as tachycardia, tachypnea, or hyperthermia, which may require the transfusion to be slowed down or discontinued, may occur.[87] Moreover, there is a large variation in the amount of antibodies present in Re-HIP between companies and among bags of the same lot number, which leads to variable amount of antibody levels after plasma administration.[88]

In summary, *R equi* infection is a common condition of foals typically characterized by self-resolving subclinical pneumonia. A subset (20%–30%) of infected foals will develop more severe disease and will require treatment. Extrapulmonary infection has a variable prognosis depending on the location of the infection. The challenge in the years to come is to develop better biomarkers of disease that may result in strategic antimicrobial use. Targeted treatment is imperative in face of the growing antimicrobial resistance seen in *R equi*. Prevention strategies such as the development of protective vaccines and the improvement of products used for passive immunization are also needed. Research in these areas is challenging because of our incomplete understanding of this disease and the need for foal-based research.

CLINICS CARE POINTS

- Pulmonary lesions seen on ultrasound without other clinical signs does not warrant antimicrobial treatment.
- Treat *R. equi* with a macrolide - rifampin combination unless resistance is documented.
- Intravenous administration of plasma shortly after birth appears to decrease disease severity.
- There is no information about which foals will develop clinical disease.

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