Rhodococcus equi Pneumonia

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Rhodococcus equi is the most devastating cause of pneumonia in foals between 3 weeks and 5 months of age. Much progress has been made in recent years in understanding the epizootiology and pathogenesis of R. equi infections. This article reviews recent advances with emphasis on diagnosis, treatment, and prevention of R. equi infections. Author's address: Department of Large Animal Clinical Sciences, College of Veterinary Medicine, University of Florida, Gainesville, FL 32610. © 2001 AAEP.

1. Introduction

Rhodococcus equi, a Gram-positive facultative intracellular pathogen, is the most devastating cause of pneumonia in foals between 3 weeks and 5 months of age. R. equi has also emerged as a significant opportunistic pathogen in immunosuppressed people, especially those infected with the human immunodeficiency virus.1,2 Although all horse farms are likely to be infected to various degree with R. equi and antibody is widespread in horse populations, the clinical disease is enzootic and devastating on some farms, sporadic on others, and unrecognized on most. On farms where the disease is enzootic, costs associated with veterinary care, early diagnosis, long-term therapy, and mortality of foals may be very high. In addition to significant immediate costs, R. equi pneumonia has a long-term detrimental effect on the equine industry because foals that recover from the disease are less likely to race as adults.3 Much progress has been made in recent years in understanding the epizootiology and pathogenesis of R. equi infections. These advances have been valuable in the development of rapid diagnostic techniques and more effective strategies for the control of the disease. This article reviews recent advances with emphasis on diagnosis, treatment, and prevention of R. equi infections.

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, make early clinical diagnosis difficult. Early clinical signs often only include 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 pneumonia progresses, clinical signs may include decreased appetite, lethargy, fever, tachypnea, and increased effort of breathing characterized by nostril flaring and increased abdominal effort. Cough and bilateral nasal discharge are inconsistent findings. A smaller percentage of affected foals may present with a more devastating subacute form. These foals may be found dead or, more commonly, present in acute respiratory distress with a high fever and no previous history of clinical respiratory disease. Foals with the subacute form of the disease have a poor prognosis despite appropriate therapy.

NOTES
Extrapulmonary manifestations of rhodococcal infections may also occur. Intestinal lesions are present in approximately 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. In the same study, only 4% of the foals had intestinal lesions without pneumonia. The intestinal form of *R. equi* infection is characterized by a multifocal ulcerative enterocolitis and typhlitis over the area of the Peyer’s patches with granulomatosus or suppurrative inflammation of the mesenteric and/or colonic lymph nodes. Occasionally, a single large abdominal abscess (usually in a mesenteric lymph node), often causing adhesion to the large or small bowel, is the only finding. Clinical signs associated with the abdominal form of the disease may include fever, depression, anorexia, weight loss, colic, and diarrhea. Marked gastrointestinal lymphatic obstruction associated with increased protein concentration in the peritoneal fluid and hypoproteinemia may lead to ascites giving foals a pot-bellied appearance. Such foals have a poor prognosis because of the extensive granulomatous inflammation of the colonic mucosa and submucosa and mesenteric lymph nodes.

Immune-complex deposition within synovial structures leads to polysynovitis in approximately one-third of cases with *R. equi* pneumonia. The tibiotalars and stifle joints are most commonly affected. Occasionally all joints are affected. The degree of joint effusion is variable and, in most cases, lameness is not apparent or limited to a stiff gait. Cytological examination of the synovial fluid usually reveals a non-septic mononuclear pleocytosis and bacteriologic culture of the synovial fluid is negative. Histologic examination of the synovial membrane reveals lymphoplasmacytic synovitis. Fluorescein-labeled anti-equine IgG staining of the synovial membrane of three affected foals revealed evidence of immunoglobulin within the synovial membrane. Rheumatoid factors (i.e., antibodies directed against autologous or heterologous Fc portion of immunoglobulin) were also identified in the synovial fluid of a foal with non-septic joint effusion and *R. equi* pneumonia. Local therapy of the affected joints is not indicated as the effusion usually resolves without any apparent consequences as the primary infection resolves with antimicrobial therapy. The presence of a non-septic polysynovitis in a foal between 3 weeks and 6 months of age is highly suggestive of *R. equi* infection and deserves further investigation (see section 7. Diagnosis). Immune complex deposition may also contribute to the development of uveitis, anemia and thrombocytopenia in some foals.

Bacteremic spread of the organism from the lungs or gastrointestinal tract may occasionally result in septic arthritis or osteomyelitis. However, foals can occasionally develop *R. equi* septic arthritis or osteomyelitis without apparent lung or other source of infection. The degree of lameness of foals with septic arthritis distinguishes them from foals with immune-mediated polysynovitis. In equivocal cases, bacterial culture and cytological examination of the synovial fluid should be performed. In addition to appropriate antimicrobial therapy (see treatment), foals with *R. equi* septic arthritis and osteomyelitis often require aggressive local therapy. *R. equi* vetebral 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 and renal abscessation. Ulcerative lymphangitis, cellulitis, and subcutaneous abscesses have also been reported. Disease due to *R. equi* is rare in adult horses. There are a few reports of sporadically occurring illness similar to that observed in foals involving primarily lungs or colon and related lymph nodes, or rarely as a wound infection. In one adult horse, acquired combined immunodeficiency of unknown origin led to *R. equi* septicemia and lung abscessation.

Rarely, the organism has also been isolated from infertile mares and aborted fetuses.

### 3. Pathogenesis

*R. equi* is a facultative intracellular pathogen and its infectivity in vitro is limited to cells of the monocyte-macrophage lineage. The ability of *R. equi* to persist in, and eventually destroy, alveolar macrophages seems to be the basis of its pathogenicity. Intracellular persistence correlates with the absence of phagosome-lysosome fusion and phagocytosis of *R. equi* by equine macrophages is not associated with a functional respiratory burst. As opposed to resident macrophages, interferon (IFN)-γ-activated mouse macrophages produce both reactive oxygen (ROI) and reactive nitrogen (RNI) intermediates. These two radicals combine to form peroxynitrite, which efficiently kills *R. equi*. Neither ROI nor RNI alone is sufficient to mediate killing of *R. equi*. Optimal binding of *R. equi* to mouse macrophages in vitro requires complement and is mediated leukocyte complement receptors type 3 (CR3, CD11b/CD18). Entry of several microorganisms into macrophages after adherence to complement receptors has been shown to allow them to avoid the toxic consequences of the oxidative burst. Opsonization of *R. equi* with specific antibody is associated with increased phagosome-lysosome fusion and significantly enhances killing of *R. equi* by equine macrophages suggesting that the mechanism of cellular entry can mediate the fate of the bacteria. As opposed to macrophages, neutrophils from foals and adult horses are fully bactericidal and killing of *R. equi* is considerably enhanced by specific opsonizing antibody.

The ability of *R. equi* to induce disease in foals likely depends on both host and microbial factors. Knowledge of the virulence mechanisms of *R. equi* were largely speculative until the discovery of the
virulence plasmid. Unlike most environmental R. equi, isolates from pneumonic foals typically contain 80–90 kb plasmids encoding a family of seven closely related virulence-associated proteins designated VapA and VapC to VapH. 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 coordinately regulated by temperature with VapA. Plasmid-cured derivatives of virulent R. equi strains lose their ability to replicate and survive in macrophages (Fig. 1). Plasmid-cured derivatives also fail to induce pneumonia and are completely cleared from the lungs of foals two weeks following heavy intrabronchial challenge, confirming the absolute necessity of the large plasmid for the virulence of R. equi (Fig. 2). 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. Simultaneous expression of all the vap-like genes may be necessary for virulence. The precise role of each of these genes in the pathophysiology of R. equi infections remains to be determined.

Cell wall mycolic acid-containing glycolipids may also contribute to virulence of R. equi. Strains with a longer carbon chain mycolic are more virulent as determined by lethality and granuloma formation in mice than those with shorter chains. Other unexplored candidates as virulence factors include capsular polysaccharides as well as cholesterol oxidase, choline phosphohydrolase, and phospholipase C exoenzymes (“equi factors”). However, both capsule and exoenzymes are produced by virulent as well as by avirulent strains suggesting that their contribution to virulence, if any, is insignificant in comparison to plasmid-mediated functions.

4. Epidemiology

R. equi is a soil organism with simple growth requirements. Intestinal carriage in adult herbivores is passive and only represents acquisition from contaminated grass, but the organism multiplies in the intestine of the foal up to about three months of age, reaching numbers up to 10^9 colony forming units per gram of feces. Foals develop primary pneumonia or secondary bacteremia, with mortality rates ranging from 8% to 50% depending on the severity of the infection and the availability of appropriate treatment. Nonsuppurative lesions may persist for several years despite antibiotic therapy, leading to chronic respiratory disease and a decrease in performance. The organism is usually isolated from the lungs and nasal passages of affected foals, and it can be cultured from the feces of recovered foals.

Fig. 1. Role of the large plasmid in survival and replication of R. equi in macrophages. Mouse peritoneal macrophages were infected in vitro with either the plasmid-containing and VapA-expressing strain 103^+ or with its plasmid-cured derivative (103^-) using an infectivity ratio of 5 bacteria/macrophage. At 48 h post infection, 103^+ infected monolayers were heavily infected (A, arrows) whereas monolayers infected with 103^- had only a few remaining intracellular bacteria (B).

Fig. 2. Pathological findings in foals infected intrabronchially with R. equi strain 103^+ or 103^- (A) Cross section of a cranioventral lung in a foal infected with R. equi 103^- showing no lesions on day 14 postinfection. (B) Cross section of a cranioventral lung lobe in a foal infected with R. equi 103^+ showing multiple well-defined nodular areas of pulmonary consolidation on day 14 postinfection.
units (CFU)/g of feces.\textsuperscript{37} Since pneumonic foals swallow infected sputum, the virulent bacteria so swallowed can multiply in their intestine. Therefore, the manure of \textit{R. equi} affected foals is likely a major source of progressive contamination of the environment with virulent organisms. Under suitable conditions of high summer temperatures, \textit{R. equi} can multiply in the environment by 10,000-fold in only 2 weeks.\textsuperscript{38} A single gram of soil contaminated with foal manure may therefore, under favorable conditions, contain millions of virulent \textit{R. equi}. Inhalation of dust particles laden with virulent \textit{R. equi} is the major route of pneumonic infection. 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{39} Although all horse farms are likely infected with \textit{R. equi}, the incidence of clinical disease varies from farm to farm. This probably reflects differences in environmental and management conditions, as well as differences in virulence of isolate. Although the total numbers of \textit{R. equi} in the environment may be similar in farms with and without a history of \textit{R. equi} infections, farms with enzootic disease tend to be more heavily infected with virulent \textit{R. equi}.\textsuperscript{27} In a survey of the prevalence of virulent \textit{R. equi} at horse breeding farms in Japan, the organism was isolated from almost all soil samples, at numbers of $10^2$–$10^3$ CFU per gram of soil. The vast majority of these isolates did not contain plasmids and were avirulent. Virulent \textit{R. equi} isolates containing 80–90 kb plasmids and expressing VapA were cultured from 24 of the 31 farms examined. On those farms, virulent \textit{R. equi} represented 1.7 to 23.3% of all isolates.\textsuperscript{40}

5. \textit{R. equi} Isolates in Immunosuppressed Humans

Analysis of 39 isolates from immunocompromised human patients with and without AIDS revealed that only eight contained an 80–90 kb plasmid, expressed VapA, and were virulent for mice.\textsuperscript{41} These results suggested that opportunistic infections in immunocompromised patients can be caused by both mouse-virulent and avirulent \textit{R. equi} strains. Therefore, the pathogenesis of \textit{R. equi} infection in immunocompromised human patients appears to be different from the pathogenesis in foals, in which the virulence plasmid is always found. In a recent study characterizing the plasmid content, expression of the virulence associated proteins, and mouse virulence of 19 \textit{R. equi} isolates from human patients with or without AIDS, a new category of \textit{R. equi} was defined. Isolates from patients with AIDS tended either to be virulent ($10^6$ bacteria needed for lethality in mice) and possess a 80–90 kb plasmid encoding VapA and, or to have “intermediate virulence” ($10^5$ bacteria needed for lethality) with one of four distinct large plasmids encoding a 20-kDa antigen (VapB) related to, but distinct from, VapA. Most of the non-AIDS isolates are avirulent ($>10^8$ bacteria needed for lethality) and do not express these antigens.\textsuperscript{42} In another study, \textit{R. equi} was isolated from 73.9% of soil samples collected from 115 parks and 49 yards in Japan. The number of \textit{R. equi} in those samples ranged from $10^1$–$10^5$ CFU per gram of soil. None of the 1294 isolates from those samples expressed VapA or VapB, suggesting that the human environment is not a significant route of exposure to virulent or intermediately virulent \textit{R. equi}.\textsuperscript{43} In contrast, 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{44} To the author’s knowledge, intermediately virulent isolates (expressing VapB) have never been isolated from foals with naturally acquired \textit{R. equi} infections. Experimentally, heavy intrabronchial challenge with intermediately virulent \textit{R. equi} results in pneumonia but at a dose much higher than that required for induction of pneumonia with VapA expressing strains.\textsuperscript{45}

6. Immunity

\textbf{Antibody-Mediated Immunity}

Immunity to \textit{R. equi} pneumonia in foals likely depends on both the antibody and cell-mediated components of the immune system but its exact basis remains to be determined. The age of development of \textit{R. equi} pneumonia coincides with and may in part be related to the decline of maternally derived antibodies.\textsuperscript{46} However, the strongest evidence for a role of antibody in protection against \textit{R. equi} is the partially protective effect of passively transferred anti–\textit{R. equi} hyperimmune (HI) equine plasma (see section 10). The mechanisms by which HI plasma confers protection are not completely understood. The list of possible effector molecules includes antibody and non-specific factors such as fibroactin, complement components, collectins, cytokines, and acute phase proteins. Opsonization of \textit{R. equi} with specific antibody has been shown to promote phagocytosis and killing of \textit{R. equi} by alveolar macrophages, identifying antibody as a critical component of HI plasma.\textsuperscript{17} In all the studies evaluating the protective effect of HI plasma, plasma donors were immunized with whole cell vaccines or a mixture of several soluble antigens, making it impossible to determine the role of antibody against defined antigens of \textit{R. equi}.

Recent studies have focused more specifically against the role of antibody against plasmid-encoded virulence-associated proteins (Vap). First, a monoclonal antibody to VapA and serum from horses immunized with partially purified VapA have opsonizing activity.\textsuperscript{47} Moreover, purified immunoglobulins obtained from horses vaccinated with partially purified VapA protected mice against intraperitoneal challenge with virulent \textit{R. equi} compared with mice administered immunoglobulins from non-immunized horses.\textsuperscript{48} More recently, in-
travenous administration of purified immunoglobulins obtained from horses immunized with recombinant VapA and VapC to foals was found to reduce the severity of pneumonia following heavy experimental challenge with R. equi. In the same study, the degree of protection conferred by purified anti-VapA and -VapC immunoglobulins was similar to that provided by commercially available HI plasma.

Cell-Mediated Immunity

Because of the facultative intracellular nature of R. equi, cell-mediated immune mechanisms are thought to be of major importance in resistance. Almost all knowledge of cell-mediated immunity to R. equi infections comes from infection of mice. Deficiencies in the complement component C5, phagocytic cells, and NK cells in mice do not impair the pulmonary clearance of virulent R. equi. In contrast, functional T lymphocytes are absolutely required for the clearance of virulent (plasmid and VapA positive) R. equi in mice. However, athymic nude mice (lacking functional T lymphocytes) clear plasmid-cured derivatives from their lungs within one week of infection suggesting that, as opposed to virulent organisms, clearance of avirulent plasmid-negative strains in mice does not require functional lymphocytes and depends mainly on innate defense mechanisms.

The two major mechanisms by which T lymphocytes mediate clearance of intracellular pathogens are secretion of cytokines and direct cytotoxicity. Although both CD4+ (helper) and CD8+ (cytotoxic) T cells contribute to host defense against R. equi in mice, CD4+ T lymphocytes play the major role and are absolutely required for complete pulmonary clearance. The mouse CD4+ Th cells can be divided in 2 subsets based on the cytokines they produce. The Th1 subset produces mainly IFN-γ and IL-2 and is mainly responsible for macrophage activation and cell-mediated immunity. The Th2 subset produces mainly IL-4, IL-5, and IL-10, which mainly promote humoral immunity. Studies in mice have clearly shown that a Th1 response is sufficient to effect pulmonary clearance of R. equi while a Th2 response is detrimental. How these findings in mice relate to the foal remains to be determined. Analogy to human immunodeficiency virus–related R. equi pneumonia suggest either that foals are immunocompromised in some way or that infection with virulent R. equi alters immune response in foals. The cytokine response of foals infected with virulent and avirulent R. equi has recently been investigated. Foals infected intrabronchially with a virulence plasmid-containing strain of R. equi showed marked reduction in IFN-γ mRNA expression by bronchial lymph node CD4+ T lymphocytes compared to CD4+ T cells similarly isolated from foals infected with an avirulent plasmid-cured derivative of the same strain. In addition, IL-10, a cytokine known to downregulate a Th1 response in other species, was only expressed in the lungs of foals infected with the virulent strain. These findings suggest that virulent R. equi have an immunomodulating effect important in the pathogenesis of infection.

7. Diagnosis

The distinction between lower respiratory tract infections caused by R. equi and that caused by other pathogens is problematic especially on farms with no previous history of R. equi infections. Many diagnostic tests including a complete blood count (CBC), measurement fibrinogen concentrations, radiographs, and serology may help distinguish R. equi pneumonia from that caused by other pathogens. However, bacteriologic culture or polymerase chain reaction (PCR) amplification combined with cytological examination of tracheobronchial aspirate (TBA) are necessary to make a definitive diagnosis of R. equi pneumonia.

Clinical Laboratory Tests

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 nonsurvivors than in survivors whereas another study showed no difference between the 2 groups. In a review of 40 cases of lung abscesses there was a trend toward higher fibrinogen concentrations and WBC counts for the foals from which R. equi was isolated from a TBA as opposed to foals from which another pathogen was isolated. However, in all these studies there was a considerable overlap in ranges, which preclude the use of fibrinogen concentrations and WBC as diagnostic tests or prognostic indicators for an individual animal.

Imaging Techniques

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 (Fig. 3). The consolidated lesions are often seen as more discrete nodular and cavitary lesions compatible with pulmonary abscessation (Fig. 3). Although in one study nonsurvivors tended to have more severe radiographic lesions than survivors, many survivors have very severe radiographic lesions and radiographs should not be used as the sole criterion for prognosis and euthanasia. In foals less than 4 months of age radiographic evidence of nodular lung lesions and lymphadenopathy are highly suggestive of R. equi infections. However, in foals of 4 months and older S. zooepidemicus is another common cause of lung abscesses. Ultrasonography is a helpful
diagnostic tool when lung involvement includes peripheral areas but may not be as useful as radiography to evaluate the 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 (Fig. 4). To the author’s knowledge, comparison between ultrasonography and radiography has not been critically evaluated in foals with *R. equi* pneumonia. Nevertheless, 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. A sector scanner equipped with a 7.5 or 5.0 MHz transducer is preferred but the linear scanner commonly used in equine reproduction can also be used.

**Serology**

A number of workers have investigated serological approaches to diagnose *R. equi* infections in foals. The two most commonly used types of assays are agar gel immunodiffusion (AGID), and enzyme-linked immunosorbent assay (ELISA). Serologic diagnosis of *R. equi* infections is problematic because the widespread exposure of foals to this organism at a young age leads to antibody production.
without necessarily producing clinical disease. In addition, maternally derived antibody causes positive reactions with sensitive ELISA assays which further confound the interpretation of the test. We recently evaluated the accuracy of AGID and 3 previously described ELISA assays by comparing serological results to culture of a tracheobronchial aspirate in 100 foals with pneumonia. The serological tests evaluated were found to either have low sensitivity, low specificity, or both. Improving either sensitivity or specificity of ELISA assays by changing the cut-off value of the tests could only be done at the detriment of the other. Simple reliance on serology as a diagnostic test for respiratory diseases is likely an incidental finding. In a recent study all 17 foals with confirmed R. equi pneumonia appeared. However, in 2 other studies, all 17 foals in which R. equi was isolated from the lung parenchyma at necropsy, were previously found to yield the organism on culture of a TBA suggesting that culture of a TBA is a consistent and reliable method of diagnosing R. equi pneumonia. Larger case series are required to determine the exact sensitivity of TBA for the diagnosis of R. equi pneumonia in foals. Multiple other pathogens are often isolated along with R. equi. Foals without clinical disease exposed to contaminated environments may have R. equi in their trachea as a result of inhalation of contaminated dust. In one study conducted on a farm with enzootic R. equi pneumonia 77 (35%) of 216 foals sampled had positive TBA cultures but no signs of respiratory disease. For this reason, bacteriologic culture of a TBA should be interpreted in the context of cytological evaluation, physical examination, and laboratory results. A light growth of R. equi from a foal with no clinical signs of respiratory disease, normal fibrinogen concentrations and WBC count, and no cytological evidence of airway inflammation is likely an incidental finding. In a recent study, the use of PCR amplification based on the VapA gene sequence was found to be a more sensitive mean of identifying R. equi in TBA samples than bacterial culture, especially if the foal sampled is concurrently being treated with antimicrobial agents. However, increased sensitivity may also result in a higher incidence of false positive results due to the detection of very small numbers of R. equi present as environmental contaminants. PCR amplification may be done in association with, but should not replace, bacterial culture because it does not permit identification of concurrent bacterial pathogens and in vitro antimicrobial susceptibility testing.

Positive R. equi culture from nasal or fecal swabs cannot be taken as evidence of infection. R. equi can be cultured from the feces of normal horses even if they live in farms with no history of R. equi pneumonia. The quantitative culture of the feces of foals at weekly intervals has been advocated as an aid in early diagnosis of R. equi infections in foals because the bacterial count per gram of feces increased at the same time as clinical signs appeared. However, a single fecal sample from a foal has no diagnostic value because of individual as well as farm to farm variation in the number of R. equi in the feces. Furthermore, a negative fecal culture may not be helpful in ruling out R. equi infection since, in one study, only 5 (17%) of 30 foals with confirmed R. equi pneumonia had positive fecal cultures. Similarly, bacterial culture and PCR amplification of nasal swabs are poorly sensitive for the diagnosis of R. equi pneumonia.

8. Treatment

A wide variety of antimicrobial agents are effective against R. equi in vitro. However, because R. equi is an intracellular pathogen surviving and replicating in macrophages and it 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. The combination of erythromycin and rifampin has become the treatment of choice for R. equi infections in foals and has dramatically reduced foal mortality since its introduction. The two drugs are bacteriostatic against R. equi but highly effective in vitro. Their combination is synergistic both in vitro and in vivo and use in combination reduces the likelihood of resistance to either drug. Rifampin and, to a lesser extent, erythromycin are lipid soluble, allowing them to penetrate caseous material. Rifampin is concentrated approximately 2-fold in phagocytes and is effective against many intracellular bacteria. Erythromycin is concentrated 10- to 20-fold in granulocytes and alveolar macrophages by an active mechanism but, despite high intracellular concentrations, its intracellular antibacterial activity at best equals that against extracellular bacteria. The concentration of erythromycin within lysosomes may explain the poor efficacy of the drug against intracellular pathogens since many of them, like R. equi, can prevent phagolysosome fusion. Alternatively, acidity within the phagolysosome may reduce the efficacy of erythromycin. The recommended dosage regimen for rifampin is 5 mg/kg q 12 h or 10 mg/kg q 24 h orally.
ommended dosage of the esters or salts of erythromycin is 25 mg/kg q 6–8 h, orally. A third antimicrobial agent may be necessary if another pathogen resistant to erythromycin and rifampin is isolated in significant numbers along with R. equi. The combination of gentamicin or amikacin with erythromycin or rifampin in vitro give significant antagonistic activity against R. equi compared with either drug alone. Therefore, administration of an aminoglycoside with either erythromycin or rifampin is not recommended for the treatment of R. equi infections. Resolution of clinical signs, normalization of plasma fibrinogen and radiographic or ultrasonographic resolution of lung lesions are commonly used to guide the duration of therapy which generally ranges between 4 and 9 weeks.

Although well tolerated by most foals, erythromycin commonly causes fecal consistency to soften. Most of the time this effect is self-limiting and does not necessitate cessation of therapy but these foals should be monitored carefully because some may develop severe diarrhea. During surges of very hot weather an idiosyncratic reaction characterized by severe hyperthermia and tachypnea has been described in foals treated with erythromycin. Administration of antipyretic drugs and placing the foal in a cold environment will treat this problem. Clostridium difficile enterocolitis has also been observed occasionally in the dams of nursing foals while the foals are being treated with oral erythromycin presumably because coprophagic behavior leads to ingestion of sufficient active erythromycin to perturb the intestinal flora of the mare. Even though the vast majority of R. equi isolates from foals are sensitive to erythromycin and rifampin resistant strains to either drugs have been encountered. Therapy of foals infected with resistant isolates is problematic because of the limited range of alternative effective drugs.

Alternative Antimicrobial Agents

High doses of the trimethoprim-sulfonamide (TMS) combination (30 mg/kg of combination q 8–12 h, orally) may be effective in foals with mild or early R. equi pneumonia without marked evidence of pulmonary abscessation or for continued therapy in foals responding well to other antimicrobials. However, TMS is unlikely to be as effective as erythromycin and rifampin for the treatment of severe R. equi pneumonia with extensive abscessation because of its poor activity in caseous material and against most intracellular pathogens. Chloramphenicol can also be administered orally and achieves high concentrations within phagocytic cells. The recommended dosage regimen is 50 mg/kg q 6 h orally. However, the fact that only 70% of R. equi isolates are susceptible to this drug and the potential human health risk make this drug a less attractive alternative.

Azithromycin and clarithromycin are two newer generation macrolides commonly used in human medicine. Compared to erythromycin, they are more chemically stable, have a greater bioavailability, and achieve higher concentrations in phagocytic cells and tissues. In people, the incidence and the severity of side effects for these two new macrolides are also considerably decreased when compared to erythromycin. The need for other effective and safer antimicrobial agents for the treatment of R. equi infections in foals makes azithromycin and clarithromycin attractive alternatives. We recently investigated the pharmacokinetics of these two antimicrobial agents in 2- to 4-month-old foals and determined their in vitro minimal inhibitory concentrations (MIC) for common equine pathogens. Based on the pharmacokinetic parameters, the MIC of Rhodococcus equi isolates, and pharmacodynamic parameters correlated with a favorable outcome of infection in other species, azithromycin, at a dose of 10 mg/kg q 24 h orally, or clarithromycin, at a dose of 7.5 mg/kg q 12 h orally would likely be appropriate for the treatment of R. equi infections in foals. Additional studies are required to confirm the efficacy and safety of these dosages in a clinical setting. Until otherwise proven, the combination of erythromycin and rifampin should remain the first line of therapy for R. equi infections in foals.

9. 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, Hillidge reported a successful outcome (as assessed by survival) in 50 (88%) of 57 foals with confirmed R. equi pneumonia. However, until recently there was no information on the impact of R. equi infections on future athletic performance. Recently, the records of 115 foals diagnosed with R. equi pneumonia and treated with erythromycin and rifampin were reviewed. The survival rate was 72%. Death was more likely in foals presented with respiratory distress and the non-survivors had more severe radiographic abnormalities on admission than survivors. Of the survivors, 54% 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, racing performance of foals that raced as adults was not significantly different from that of the US racing population.

10. Control of R. equi Infections on Farms where the Disease is Enzootic

Approaches for the successful control of R. equi infections on enzootic farms depend on 1) a decrease in the size of infective challenge, 2) earlier recognition and therapy of the disease, and 3) passive immunization. These approaches have recently been reviewed in detail.
Decreasing the Size of Infective Challenge

The management and environmental factors associated with development of *R. equi* pneumonia have never been critically investigated. Nevertheless, there seems to be a progressive build up of infection on horse farms with prolonged use. Thus, enzootic farms are likely to be those used for breeding horses for many years, those with heavy concentrations of mares and foals, and those located where summer temperatures are high, where the soil type is sandy, and where dust is extensive. Large numbers of foals kept on bare, dusty, manure-containing paddocks will result in heavy challenge, with clinical disease maintaining virulent bacteria. It is important to house foals in well-ventilated, dust-free areas, and to avoid dirt paddocks and crowding. Pneumonic foals must be isolated, because they are the major source of contamination of the environment with virulent organisms, and their manure composted. Pasture must be rotated to decrease dust formation and consequent inhalation of *R. equi*. Any sandy or dirt areas should ideally be planted with grass and made “off limits” to foals or, alternatively, irrigation may be useful in decreasing dust formation and encouraging grass. Manure should be regularly removed from paddocks and composted. Because mares and foals tend to congregate around water sources and under shade in hot summers, reduction in the size of mare–foal bands may reduce the destruction of grass and exposure to barren soil. Dispersal of foals onto grass paddocks will reduce dust formation and, therefore, the number of inhaled bacteria. Ingestion of low number of organisms via grazing on contaminated grass may actually have the beneficial effect of immunizing foals orally.82

Earlier Recognition of the Disease

*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. Early recognition of the disease with isolation and appropriate treatment of infected foals will reduce losses, limit the spread of virulent organisms in the environment, and decrease the cost of therapy. In one study on a farm with enzootic *R. equi* problems, twice weekly complete physical examination with auscultation of the lungs was successful in promoting early diagnosis and preventing mortality.83 Other suggested approaches for early identification of *R. equi* infected foals on enzootic farms include periodic measurement of fibrinogen concentrations, serological surveillance using AGID, and lung ultrasonography. However, to the author’s knowledge, the usefulness of these approaches has never been critically investigated. We recently assessed the value of monthly measurement of WBC counts and fibrinogen concentrations compared to bimonthly serological surveillance using the AGID test for early identification of *R. equi* infected foals on a large breeding farm with enzootic *R. equi* infections. We monitored 165 foals during the entire breeding season. Diagnosis of *R. equi* pneumonia was confirmed in pneumonic foals by culture of a tracheobronchial aspirate. WBC count was found to be a more sensitive and specific indicator of early *R. equi* pneumonia than measurement of fibrinogen concentrations. Foals with leukocyte counts >14,000 cells/µl should either be subjected to more diagnostic tests (e.g., tracheobronchial aspirate) or treated. Foals with leukocyte counts >13,000 but <14,000 cells/µl should be retreated weekly until resolution of leukocytosis. The sensitivity and specificity of the AGID assay were respectively 76.2% and 50.5%, limiting the usefulness of this test for early identification of *R. equi* infected foals on enzootic farms.

Passive Immunization

Intravenous administration of HI plasma obtained from horses vaccinated against *R. equi* using various antigens has proved effective in significantly reducing the incidence of *R. equi* pneumonia in foals following both experimental and natural challenge in some studies.84,85 However, other studies have failed to identify significant differences in the incidence of naturally acquired *R. equi* pneumonia between HI plasma recipients and control foals,86,87 suggesting that the efficacy of a particular HI plasma product cannot be extrapolated to another product prepared in a similar but not identical fashion. In North America, most horse farms relying on HI plasma for the prevention of *R. equi* pneumonia use commercially available HI plasma products. Hyperimmune plasma obtained from horses vaccinated with *R. equi* antigens is commercially available from two companies, both currently holding a conditional USDA license for these products.8a,b However, to the author’s knowledge, the efficacy of these commercially available plasma products in protecting against *R. equi* pneumonia has, until recently, never been critically investigated.

To determine the efficacy of the Lake Immunogenics Inc. HI plasma,8a 165 Thoroughbred foals on a farm with enzootic *R. equi* infections were randomly assigned to two treatment groups (HI plasma or non-treated controls). Transfused foals were given two 950-ml doses of HI plasma by rapid intravenous infusion. The first dose was given between 1 and 10 days of age, and the second dose was given between 30 and 50 days of age. A tracheobronchial aspirate for bacterial culture was performed on every foal with clinical signs of lower respiratory tract disease. The incidence of *R. equi* pneumonia in foals without failure of passive transfer of immunity was lower in foals transfused with HI plasma (19.1%) than in non-treated controls (30%) (p = 0.09). All transfused foals that developed *R. equi* pneumonia were diagnosed with the disease prior to administration of the second dose of HI plasma. The median age at diagnosis of *R. equi* pneumonia on this particular farm was 36 days. Therefore,
administration of the second dose of HI plasma around 25 days of age may have been more beneficial.

A different approach was used to assess the efficacy to the Veterinary Dynamics Inc. HI plasma. Briefly, seven healthy 3-week-old pony foals received 950-ml of HI plasma 24 h prior to heavy intrabronchial challenge with virulent R. equi. Eleven foals not receiving HI plasma were also infected using an identical protocol. All foals were euthanized 2 weeks post-challenge, their lung lesions were assessed, and the numbers of R. equi in their lungs were enumerated. Compared to the untreated group, lung lesions were less severe and bacterial numbers were significantly lower in transfused foals (p < 0.01). Collectively, the results of these independent studies show that the two commercially available anti–R. equi HI plasma products evaluated were found to have high anti-VapA antibody titers. These antibodies may be responsible for the protective effect observed (see section 6), although the possibility that antibodies against other antigens or molecules other than antibody contribute to protection cannot be excluded. These studies also emphasize that protection conferred by HI plasma is not complete. For optimal control of the disease on enzootically infected farms, administration of hyperimmune plasma should be combined with other control strategies, such as attempts at decreasing the size of infective challenge and early identification and treatment of infected foals.

The ideal time for administration of HI plasma and determination of the minimal effective dose require further research. Administration of HI plasma prior to infection with R. equi is important. However, early administration may result in the decline of passively transferred antibody to a non-protective level at a time when foals are still highly susceptible to R. equi and environmental challenge is high. Therefore, intravenous administration of 1 of HI plasma within the first week of life followed by a second administration at approximately 25 days of age, although expensive, may be the best approach on farms with high morbidity rates. A single administration at the beginning of the warm season may be advisable for foals born early during the year in geographic areas where cold winter temperatures reduce environmental challenge. These recommendations are only guidelines and the best time for administration of HI plasma may in fact vary depending on the geographic location of the farm, the size of infective challenge, and the age at which most foals on the farm develop clinical signs.

References and Footnotes


Lake Immunogenics, Inc., Ontario, NY 14519.

Veterinary Dynamics, Inc., Templeton, CA 93465.