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Rhodococcus equi Infections (28 Apr 2000)

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Introduction

Rhodococcus equi is one of the most important causes of disease in foals between 1 and 6 months of age, with most foals showing clinical signs between 4 and 12 weeks of age. Infection is most commonly characterized by a subacute to chronic bronchopneumonia sometimes accompanied by ulcerative typhlocolitis and mesenteric lymphadenopathy. *R. equi* may also be isolated from the cervical lymph nodes of pigs whereas in goats the organism may occasionally cause granulomatous lesions in the liver resulting in wasting and death of young animals [1, 2]. Infection in other species is rare and usually associated with immunosuppression. For example, *R. equi* has emerged as a significant opportunistic pathogen in immunosuppressed people, especially those infected with HIV [3, 4]. This article is a summary of recent advances in understanding pathogenesis of *R. equi* infections in foals. Current concepts in diagnosis, treatment and control are also discussed.

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.

Extrapulmonary manifestations of rhodococcal infections may also occur. Intestinal manifestations are present in approximately 50 % of pneumonic foals presented for necropsy [5]. However, the majority of foals with *R. equi* pneumonia do not show clinical signs of intestinal disease. 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 granulomatous or suppurative inflammation of the mesenteric and/or colonic lymph nodes [5].

Immune-complex deposition within synovial structures leads to polysynovitis in approximately one-third of cases with *R. equi* pneumonia [6, 7, 8]. The tibiotarsal and stifle joints are most commonly 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. Local therapy of the affected joints is not indicated as the effusion usually resolves without any apparent consequences as the pneumonia resolves. Immune complex deposition may also contribute to the development of uveitis, anemia and thrombocytopenia in some foals [9]. Bacteremic spread of the organism from the lungs or gastrointestinal tract may occasionally result in septic arthritis and/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* vertebral osteomyelitis resulting in spinal cord compression has also been reported [10, 11, 12]. Other rare extrapulmonary manifestations of *R. equi* infections in foals include panophthalmitis, guttural pouch empyema, sinusitis, pericarditis, nephritis, and hepatic and renal abscessation [9]. Ulcerative lymphangitis, cellulitis, and subcutaneous abscesses have also been reported. Disease due to *R. equi* is rare in adult horses.

Pathogenesis

R. equi is a facultative intracellular pathogen and its infectivity in vitro is limited to cells of the monocyte-macrophage lineage [13]. 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 [14, 15]. Phagocytosis of *R. equi* by equine macrophages is not associated with a functional respiratory burst [16] and, at least in humans, the L-arginine-NO pathway is not required for intracellular killing of this organism [17]. Optimal binding of *R. equi* to mouse macrophages in vitro requires complement and is mediated by Mac-1, a leukocyte complement receptor type 3 (CR3, CD11b/CD18) [13]. 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 [18]. Opsonisation 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 [14]. 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 [19-22].

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 recent discovery of the virulence plasmid [23, 24]. Unlike most environmental *R. equi*, isolates from pneumonic foals typically contain 85-90 kb plasmids encoding a highly immunogenic, lipid-modified virulence-associated protein (VapA) [25-28]. VapA is expressed on the bacterial surface and its expression is temperature regulated, occurring between 34°C and 41°C [29]. Plasmid-cured derivatives of virulent *R. equi* strains lose their ability to replicate and survive in macrophages [30]. 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* [30, 31]. 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 [30].

However, these results do not totally rule out a role for VapA in virulence. Recent sequencing adjacent to vapA in the 85-kb plasmid of *R. equi* has revealed six other genes with approximately 40% overall amino acid identity with vapA. At least three of those genes are transcribed when *R. equi* is cultured in vitro and at least one of those gene products is recognized by serum from a naturally infected foal. Identification of multiple genes with considerable homology indicates that these virulence-associated genes constitute a gene family in *R. equi* [32]. 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 [33]. 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.

Immunity

Immunity to *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 *R. equi* pneumonia coincides with and may in part be related to the decline of maternally-derived antibodies [34].

However, the strongest evidence for a role of antibody in protection against *R. equi* is the protective effect of passively transferred anti-*R. equi* hyperimmune equine plasma (See section on prevention).

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

do not impair the pulmonary clearance of virulent *R. equi* [35]. In contrast, functional T lymphocytes are absolutely required for the clearance of virulent (plasmid and VapA positive) *R. equi* in mice [36-38]. 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 [37].

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 [38-40]. The mouse CD4+ Th cells can be divided in 2 subsets based on the cytokines they produce. The Th1 subset produces mainly IFN-g 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 [36, 41]. 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-g 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 [42]. 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 [42]. These findings suggest that virulent *R. equi* have an immunomodulating effect important in the pathogenesis of infection.

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 10⁵/g of feces [43]. Inhalation of dust particles laden with virulent *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 haematogenously acquired pneumonia unless the foal has multiple exposures to large numbers of bacteria [44]. Although all horse farms are likely infected with *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 *R. equi* in the environment may be similar in farms with and without a history of *R. equi* infections, farms with enzootic disease tend to be more heavily infected with virulent *R. equi* [45]. In a survey of the prevalence of virulent *R. equi* at horse breeding farms in Japan, the organism was isolated from almost all soil samples, at numbers of 10²-10⁵ colony forming units per gram of soil. The vast majority of these isolates did not contain plasmids and were avirulent. Virulent *R. equi* isolates containing 85 to 90 kb plasmids and expressing VapA were cultured from 24 of the 31 farms examined. On those farms, virulent *R. equi* represented 1.7 to 23.3% of all isolates [46]. *R. equi* can also be isolated from areas never inhabited by horses. In one study, *R. equi* was isolated from 73.9% of soil samples collected from 115 parks and 49 yards in Japan [47].

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), fibrinogen level, radiographs, and serology may help distinguish *R. equi* pneumonia from that caused by other pathogens. However, bacteriologic culture combined with cytological examination of tracheobronchial exudate are still the "gold standards" used to arrive at a definitive diagnosis. 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 [8]. 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 or abscessation is the most common radiographic abnormality. 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.

Although a number of serological tests have been described, 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 may cause positive reactions in some serological assays which further confound the interpretation of the test. There is also a lack of sensitivity and specificity data on which to assess the significance of most commercially available serological assays and simple reliance on serology as a diagnostic test for *R. equi* infections may result in overdiagnosis of this disease and in missing infections in the early stages [48]. Serologic tests may be more useful at the farm level to detect overall exposure (see section on prevention) than at the individual level to diagnose clinical infection with *R. equi*.

Bacteriologic culture combined with cytological examination of a tracheobronchial aspirate is therefore the only acceptable way to arrive at a definitive diagnosis of *R. equi* pneumonia. Multiple other pathogens may be isolated along with *R. equi*. Foals without clinical disease exposed to contaminated environments may have *R. equi* in their tracheas as a result of inhalation of contaminated dust [49]. For this reason, bacteriologic culture of a TBA should be interpreted in the context of cytological evaluation, physical examination and laboratory results. To date culture results have been as sensitive as PCR based assays and offer the advantage of allowing in vitro antimicrobial susceptibility testing [50, 51]. On endemically infected farms, it is important not to assume that every coughing foal with an elevated temperature is suffering from *R. equi* infection. Streptococcus zooepidemicus infection may cause bronchopneumonia with or without abscess formation in young foals and is far more readily treated than *R. equi* infection.

Treatment

The combination of erythromycin and rifampin has become the treatment of choice for *R. equi* infections in foals. The two drugs are bacteriostatic against *R. equi* [52] but their combination is synergistic both in vitro and in vivo [52-54]. Furthermore, their use in combination reduces the likelihood of resistance to either drug [52]. The recommended dosage regimen for rifampin is 5 mg/kg every 12 hours or 10 mg/kg every 24 hours orally [55-57]. Recommended dosage of the acid soluble estolate or ethylsuccinate esters of erythromycin is 25 mg/kg every 8 or 12 hours [57, 58]. A third antimicrobial agent may be necessary if another pathogen resistant to erythromycin or 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 [52, 54]. 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 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 [59]. 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 [60]. 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 [48].

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 % [61]. Using erythromycin and rifampin, Hillidge [57] 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 [62]. The survival rate was 72 %. Death was more likely in foals presented with respiratory distress and the non-survivors had a 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 [62].

Control

There is a progressive build up of infection on horse farms with prolonged use so that 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 the soil type is sandy and 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. Pasture must be rotated to decrease dust formation and by 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. Early recognition of *R. equi* cases with isolation and treatment of infected foals will reduce losses, prevent the spread of virulent organism and limit the cost of therapy. Careful daily observation of foals, daily recording of foals' temperatures, measurement of plasma fibrinogen every two weeks, periodic ultrasonographic examination of the lungs and serological surveillance have all been used to successfully promote early diagnosis on enzootic farms [63].

The use of one liter of hyperimmune (HI) plasma obtained from donors vaccinated with *R. equi* antigens has become the mainstay of prevention of this disease in foals on enzootically affected farms, since it has been shown to be highly effective in reducing illness and death in most studies [64-69]. Further studies are necessary to confirm definitively the role of anti-*R. equi* antibody as the factor present in plasma that mediates protection, and if so, against which antigen(s) of *R. equi* these antibodies are directed. The ideal time for administration of HI plasma and determination of the minimal effective dose also require further research. Anti-*R. equi* antibody following administration of HI plasma are significantly increased and maintained at high levels for approximately 30 days in most foals [69]. Therefore, administration of 1 L of HI plasma within the first week of life followed by a second administration at approximately 25-30 later, although expensive, may be the best approach on farms with high morbidity rates. In farm with lower morbidity rates a single administration between 10 to 21 days of age may be advisable. These recommendations are only guidelines and it is important to realize that the best time for administration of HI plasma may 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.

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