Review of the Epidemiology and Ecology of *Rhodococcus equi*

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The concentration of airborne virulent *R. equi* is closely correlated with the prevalence of *R. equi* disease on endemically affected farms. Virulent *R. equi* is certainly aerosolised in association with dust, but foals are also an important source of airborne virulent *R. equi* as they breathe off high concentrations in exhaled air. Both clinically ill and asymptomatic foals have high concentrations of virulent *R. equi* in their breath and thus may be an important source of bacteria for susceptible foals. Authors’ address: Equine Infectious Disease Laboratory, The University of Melbourne, Victoria 3010, Australia; e-mail: jrgilk@unimelb.edu.au. © 2007 AAEP. *Presenting author.

*Rhodococcus equi* is a Gram-positive, facultative intracellular coccobacillus in the order Actinomycetales. *R. equi* is largely a soil inhabitant that survives and multiplies in the gastrointestinal tract, and it is found in the fecal matter of grazing animals.¹ Thus, *R. equi* is often described as a coprophilic soil-associated actinomycete.² Growth of *R. equi* is optimal in soil enriched with horse feces that is at neutral to alkaline soil pH and an ambient temperature of ~30°C; the organism thrives on the volatile fatty acids found in horse fecal material.³ The organism has been shown to be acid tolerant,⁴ and expression of virulence genes are regulated by pH and nutrient stresses.⁵ Soil pH may play a role in virulent *R. equi* survival and replication outside the host. A positive relationship between acidic soil pH and an increase in the proportion of *R. equi* in the soil that were virulent organisms has been recently shown.⁶ The capacity to reduce the virulent *R. equi* burden in the soil by modifying the soil environment and soil pH is currently being explored (unpublished results). Virulent strains of *R. equi* possess an extrachromosomal DNA element known as the virulence-associated plasmid. The presence of the plasmid was associated with virulence.⁷–⁹ Each equine virulence plasmid contains a family of eight virulence-associated protein (vap) genes (vapA, vapC-vapI and pseudo vapE) situated on a pathogenicity island that occupies approximately one-third of the plasmid.¹⁰ The expression of several members of the vap gene family are thermally and pH regulated. Optimal expression occurs under mildly acidic (pH ~ 5–6.5) conditions of 37–38°C.⁵,¹¹,¹² Importantly, expression of these genes are upregulated when *R. equi* are grown within the macrophage.⁵ These findings underpin the importance of the vap gene family in *R. equi* pathogenesis, because they facilitate intracellular survival and replicate in the alveolar macrophages of the host. The importance of vapA in the
pathogenesis of *R. equi* infection and disease was highlighted in a recent study where a deletion mutant strain of *R. equi* that lacked a 7.9-kb DNA region of the plasmid spanning five *vap* genes (*vapA, C, D, E, and F*) was constructed. This mutant was attenuated in the mouse model, was unable to replicate in the lungs, and was rapidly cleared from the murine respiratory tract. The reinserterion of *vapA* alone into the locus was sufficient to restore virulence to the strain in the mouse model, whereas significant attenuation was maintained in strains where the other *vap* genes (*vapC, D, E, or F*) were reintroduced. These findings confirmed *vapA* as an essential virulence factor.

Fecal excretion of both virulent and avirulent *R. equi* is known to play an important role in contamination of the soil environment on equine farms. The concentration of *R. equi* in the fecal matter of horses varies. Most adult horses shed 100–1000 colony-forming units (cfu) of *R. equi* per gram of feces with virulent strains compromising <10% of the fecal *R. equi* population. In contrast, foals shed much higher concentrations of *R. equi*, between 1000–10,000 *R. equi* cfu/g of feces, and virulent strains compromise 10–40% of the fecal *R. equi* population. Unpublished data. Between the ages of 3 and 12 wk, fecal excretion of *R. equi* in the healthy foal reaches its peak (unpublished data). Between the ages of 3 and 12 wk, fecal excretion of *R. equi* in the healthy foal reaches its peak (unpublished data).

In foals with *R. equi* pneumonia, the fecal concentrations can be as high as 10^9 *R. equi* cfu/g of feces, or more than the fecal *R. equi* population being virulent has been observed (unpublished data). These observations formed the basis of the hypothesis that the feces of foals, especially foals with *R. equi* pneumonia, are the most important source of virulent *R. equi* contamination on farms. Therefore, many authors have suggested that fecal removal is a useful tool in reducing environmental burdens.

*R. equi* pneumonia occurs endemically on some farms and sporadically or not at all on many farms. The prevalence and severity also varies on endemic farms from season to season.

A recent study in Australia estimated the prevalence of *R. equi* pneumonia in foals on Thoroughbred studs to be 12.9%, and the case fatality rate was 6.3% (unpublished results). Farm characteristics such as high foal density, large population, large farm size, and large proportion of transient mares during the breeding season have been associated with increases in disease incidence and prevalence.

A commonly held view among veterinarians and Rhodococcus research scientists is that the most significant factor associated with increased risk of *R. equi* pneumonia is the level of environmental *R. equi*, specifically the concentration in soil and aerosolized dust. Studies comparing the soil around the *R. equi* population on farms with varying disease prevalence have indicated that the level of virulent *R. equi* in the soil of a farm was not an accurate reflection of disease prevalence or disease risk. The fecal-oral cycle of *R. equi* transmission in the horse means that contamination of the soil on a farm is progressive and insidious, although probably irrelevant as long as the virulent organism remains soil bound and unable to be inhaled.

Aerosol infection through dust has been thought to be the major route of pulmonary infection in foals. Recent epidemiological studies based on culture of airborne bacteria have highlighted the significance of airborne virulent *R. equi* burden in determining the prevalence and risk of *R. equi* pneumonia. A survey involving 22 Australian Thoroughbred breeding farms showed that the prevalence and incidence of *R. equi* pneumonia was associated with the airborne virulent *R. equi* burden and the age of the foal when sampled. Farms with high airborne virulent *R. equi* burdens experienced high disease prevalence. Environmental factors such as low soil moisture, poor grass cover, and high ambient temperature aided aerosolization of the organism from soil and were associated with high concentrations of airborne virulent *R. equi*. There was also an association between the age of the foal and the occurrence of disease. The majority of *R. equi* pneumonia cases (175 of 269 cases) were observed in foals (1–2 mo old). The incidence of *R. equi* pneumonia peaked during periods when the level of airborne virulent *R. equi* burdens were high, and this also corresponded with periods when the number of 1- to 3-mo-old foals in residence on the farms was greatest. Specific sites on farms have been suggested to be primary sources of *R. equi* respiratory infection. Early studies showed that the *R. equi* environmental concentration in the stable area where the foals were kept on a farm with endemic *R. equi* pneumonia differed significantly from the *R. equi* concentration on other farms where disease was not endemic. This difference was not obvious elsewhere on these farms. Other work has shown an association between dusty stables and increased risk of *R. equi* pneumonia. In a recent Australian study, specific sites that were commonly dry, bare, and dusty, particularly the laneways along which horses are moved between pens, paddocks, and holding pens (where horses are confined before procedures such as breeding, veterinary treatment, and farriery), had approximately double the airborne virulent *R. equi* concentration detected on grass-covered paddocks. Studies investigating the airborne *R. equi* population in Ireland where climatic conditions are different from Australian conditions (pasture cover and soil moisture are both higher) indicated that the indoor stable environment was the site where significant aerosol virulent *R. equi* challenge was likely to occur. The frequency of detecting airborne virulent *R. equi* in the stables was significantly greater than in the paddock, and the odds of a foal encountering an airborne virulent *R. equi* in the stable environment was 17.3 times greater than in the paddock on endemically affected farms. These findings support the anecdotal and experimental evidence that inhalation of virulent *R.
equi is the main route of pulmonary infection of the foal. This also indicates that the size of the aerosol virulent R. equi challenge influences the likelihood of disease.

Recently, it has been suggested that R. equi can be contagious as well as infectious, and an aerosol route of transmission of virulent R. equi has been proposed after detection of virulent R. equi in the exhaled breath of foals. Detection of virulent R. equi, an intracellular pathogen of the lower respiratory tract, in the exhaled breath of foals with overt clinical signs of R. equi bronchopneumonia and from other foals that were asymptomatic, suggested a possible contagious epidemiology. Air samples were collected in yards before muster, during the mustering of mares and foals, and after the dust had settled in the yards. The concentrations of virulent R. equi and the proportion of R. equi that were virulent in the air samples collected from the respiratory zone of foals after the dust had settled were significantly higher than levels of virulent R. equi detected during or before mustering. The odds of detecting virulent R. equi in the respiratory zone were 9.6 times greater than the odds of detecting airborne virulent R. equi in the dust from pens into which the foals were mustered. Thus, foals were more likely to encounter aerosolized virulent R. equi through contact with other foals than from dust. These findings show that aerosol transmission of virulent R. equi can occur and may be a means of continued disease spread despite management strategies designed to reduce aerosol burdens derived from inhalation of contaminated dust.

Management strategies that aim to reduce the likelihood of susceptible foals encountering high levels of aerosolized virulent R. equi are the key to reducing the risk of disease on farms. In Australia, the use of irrigation of yards and lanes before mustering significantly reduced the generation of virulent R. equi aerosols, effectively halving the risk of R. equi pneumonia in foals. Management should also aim to avoid confining susceptible foals in areas with soil of low water-holding capacity (i.e., sandy areas) and also try to maintain good pasture cover in paddocks and yards used to house foals to decrease the risk of aerosolization of the organism. Where foals are routinely stabled, strategies that either reduce the time spent in the stable or methods to improve air hygiene in stables need to be explored. The confinement of large mobs of foals together in yards or pens will likely result in highly trafficked, dusty environments with higher concentrations of fecal soil-derived virulent R. equi in the air foals breath. In addition, increased foal density increases the potential for foal-to-foal respiratory contact, which will maximize the likelihood of a significant pulmonary insult in susceptible foals. Recent results from other groups support this hypothesis, and findings suggest a relationship between foal group size, foal density, and risk of R. equi pneumonia. Given the potential for direct foal-to-foal aerosol transmission, management strategies to limit the time that foals spend congregate in crowded yards and to reduce mob sizes may need to be included in addition to measures to minimize the risk of exposure through aerosol dust. These approaches are likely to considerably reduce the prevalence of R. equi pneumonia on farms.

References


