ACVIM Consensus Statement
J Vet Intern Med 2011;25:1209–1220

Consensus Statements of the American College of Veterinary Internal Medicine (ACVIM) provide the veterinary community with up-to-date information on the pathophysiology, diagnosis, and treatment of clinically important animal diseases. The ACVIM Board of Regents oversees selection of relevant topics, identification of panel members with the expertise to draft the statements, and other aspects of assuring the integrity of the process. The statements are derived from evidence-based medicine whenever possible and the panel offers interpretive comments when such evidence is inadequate or contradictory. A draft is prepared by the panel, followed by solicitation of input by the ACVIM membership which may be incorporated into the statement. It is then submitted to the Journal of Veterinary Internal Medicine, where it is edited prior to publication. The authors are solely responsible for the content of the statements.

Diagnosis, Treatment, Control, and Prevention of Infections Caused by *Rhodococcus equi* in Foals

S. Giguère, N.D. Cohen, M. Keith Chaffin, N.M. Slovis, M.K. Hondalus, S.A. Hines, and J.F. Prescott

Abstract: *Rhodococcus equi*, a Gram-positive facultative intracellular pathogen, is one of the most common causes of pneumonia in foals. Although *R. equi* can be cultured from the environment of virtually all horse farms, the clinical disease in foals is endemic at some farms, sporadic at others, and unrecognized at many. On farms where the disease is endemic, costs associated with morbidity and mortality attributable to *R. equi* may be very high. The purpose of this consensus statement is to provide recommendations regarding the diagnosis, treatment, control, and prevention of infections caused by *R. equi* in foals.

Key words: Antimicrobials; Bacterial species; Infectious diseases; Microbiology; Parenchymal disease; Pneumonia; Respiratory tract.

Pneumonia is a major cause of disease and death in foals. *Rhodococcus equi*, a Gram-positive facultative intracellular pathogen, is one of the most common causes of pneumonia in foals. Although *R. equi* can be cultured from the environment of virtually all horse farms, the clinical disease in foals is endemic at some farms, sporadic at others, and unrecognized at many. On farms where the disease is endemic, costs associated with morbidity and mortality may be very high. The purpose of this consensus statement is to provide recommendations regarding the diagnosis, treatment, control, and prevention of infections caused by *R. equi* in foals. The level of evidence for a given recommendation was subjectively characterized as strong, moderate, or weak. Strong evidence indicates that a given recommendation is supported by controlled prospective studies, or extensive experimental and observational studies yielding consistent, coherent results. Moderate evidence indicates that a recommendation is based on clinicopathologic data and retrospective case series, or that controlled prospective studies have given conflicting results. Weak evidence indicates that the recommendation is based on uncontrolled studies. Background information about the clinical manifestations, pathogenesis, virulence, and immunity to *R. equi* is provided in a companion review article.

Abbreviations:

HIP	hyperimmune plasma
MIC	minimum inhibitory concentration
PCR	polymerase chain reaction
PELF	pulmonary epithelial lining fluid
TBA	tracheobronchial aspirate

From the Departments of Large Animal Medicine (Giguère), University of Georgia, Athens, GA; Department of Large Animal Clinical Sciences, College of Veterinary Medicine, Texas A&M University, College Station, TX (Cohen, Chaffin); Hagyard Equine Medical Institute, Lexington, KY (Slovis); The Department of Infectious Diseases (Hondalus), University of Georgia, Athens, GA; Department of Veterinary Microbiology and Pathology, Washington State University, Pullman, WA (Hines); and Department of Pathobiology, University of Guelph, Guelph, Ontario, Canada (Prescott)

Corresponding author: S. Giguère, DVM, PhD, DACVIM, Department of Large Animal Medicine, College of Veterinary Medicine, University of Georgia, 501 D.W. Brooks Drive, Athens, GA 30602; e-mail: gigueres@uga.edu

Submitted September 22, 2011; Revised September 22, 2011; Accepted October 4, 2011.

10.1111/j.1939-1676.2011.00835.x

Diagnosis

Recommendation

Pneumonia. The definitive diagnosis of bronchopneumonia caused by *R. equi* should be based on bacterio-

logic culture or amplification of the *vapA* gene (hereafter *vapA*) using polymerase chain reaction (PCR) from a tracheobronchial aspirate (TBA) obtained from a foal with one or more of the following: (1) clinical signs of lower respiratory tract disease; (2) cytological evidence of septic airway inflammation; or (3) radiographic or ultrasonographic evidence of bronchopneumonia. Amplification of *vapA* using PCR may be done in conjunction with, but should not replace, bacterial culture, because it does not permit identification of other bacterial pathogens and in vitro antimicrobial susceptibility testing of *R. equi* isolates. The level of evidence for this recommendation is strong.

Extrapulmonary Disorders. The definitive diagnosis of extrapulmonary infections (eg, abdominal abscess, osteomyelitis) caused by *R. equi* must rely on bacteriologic culture or PCR amplification of *vapA* from samples from the site of infection. The diagnosis of extrapulmonary disorders from sites at which *R. equi* cannot be detected (eg, uveitis or polysynovitis) should be based on isolation of *R. equi* from a TBA or other primary sites of infection. The diagnosis of enterocolitis caused by *R. equi* is problematic because isolation of *R. equi* from feces cannot be taken as evidence of enterocolitis caused by *R. equi*. The level of evidence for these recommendations is strong.

Rationale/Justification

Differentiating between lower respiratory tract infections caused by *R. equi* and those caused by other pathogens is problematic, especially at farms without previous history of *R. equi* infections. Diagnostic tests, including white blood cell concentration (WBC), measurement of fibrinogen concentrations, ultrasonography, and radiography, may help raise the degree of suspicion that pneumonia in a given foal may be caused by *R. equi* rather than by another microorganism. In 1 study, WBC >20,000 cells/μL, fibrinogen concentration >700 mg/dL, and evidence of pulmonary abscessation were more likely to be found in foals with pneumonia caused by *R. equi* than in foals with pneumonia caused by other bacteria.[1] However, there is a considerable overlap in distributions, which precludes the use of fibrinogen concentrations and WBC for diagnosis or prognosis for an individual foal.[1,2]

Thoracic radiography and ultrasonography are useful in evaluating the severity of pneumonia and response to treatment. Ultrasonographic or radiographic detection of lung abscesses raises the degree of suspicion that pneumonia in a given foal is caused by *R. equi*. However, detection of pulmonary abscesses, although commonly used as a screening test (see below), is not a definitive diagnostic test.

Independent studies evaluating the performance of serological tests available for diagnosis of infection caused by *R. equi* at endemic farms have demonstrated these tests either have low sensitivity, low specificity, or both.[3–6] Improving either sensitivity or specificity of ELISA assays by changing the cut-off value of the tests could only be done to the detriment of the other. The presence of antibodies indicates exposure, subclinical infection, or maternal transfer of antibodies, but it does not necessarily indicate infection leading to clinical disease. The current state of knowledge precludes serology to be used as a diagnostic test for *R. equi* pneumonia.

Bacteriologic culture or PCR amplification of *vapA* from a tracheobronchial aspirate (TBA) is therefore the only acceptable ways of establishing a diagnosis of *R. equi* pneumonia. In 1 study, only 7 (64%) of the 11 foals with positive *R. equi* culture at necropsy, and 57 (64%) of the 89 foals with radiographic evidence of lung abscessation, yielded *R. equi* from culture of a TBA.[7] However, in 2 other studies, all 17 foals in which *R. equi* was isolated from lung parenchyma at necropsy yielded the organism from culture of TBAs obtained antemortem, suggesting that culture of a TBA is a valid method for diagnosing *R. equi* pneumonia.[2,8] Larger case series are required to more accurately estimate the sensitivity of TBA culture for diagnosis of *R. equi* pneumonia in foals. Specificity of the test must also be considered. Multiple other pathogens are often isolated concurrently with *R. equi*. Foals without clinical disease may have *R. equi* in their trachea as a result of subclinical disease or from inhaling *R. equi* in contaminated environments. At a farm with endemic *R. equi* pneumonia, 77 of 216 (35%) foals sampled had positive TBA cultures, but remained free of clinical signs of respiratory disease throughout the season.[9] For this reason, culture or PCR amplification of *R. equi* from a TBA should be interpreted in the context of cytological and clinical findings. Detection of *R. equi* from a foal without clinical signs of respiratory disease, cytological evidence of septic airway inflammation, or ultrasonographic or radiographic evidence of pulmonary lesions is probably an incidental finding. Amplification of *vapA* using PCR has been shown to be more sensitive than bacterial culture in most, but not all studies.[10–12] However, increased sensitivity may also result in a higher incidence of false-positive results because of the detection of very small numbers of *R. equi* present as environmental contaminants. Moreover, culture offers the advantage of detecting other bacterial pathogens present, and permits in vitro susceptibility testing of recovered pathogens. As a result, PCR amplification of *vapA* may be done in conjunction with, but should not replace, bacterial culture. The use of PCR assays based on genes other than *vapA* is not recommended, as these assays would also detect environmental isolates lacking the virulence plasmid which are not known to cause disease in foals.

Isolation of *R. equi* from nasal or fecal swabs cannot be taken as evidence of disease because of infection with *R. equi*. *Rhodococcus equi* can be cultured from the feces of healthy horses even if they live at farms without history of *R. equi* pneumonia.[13–15] Quantitative culture of the feces of foals at weekly intervals has been advocated to aid in early diagnosis of *R. equi* infections, because the fecal concentration of *R. equi* increased at the same time as clinical signs of respiratory disease appeared in a few foals.[14] However, a single fecal sample from a foal has no diagnos-

tic value because of individual and farm-to-farm variation in the number of *R. equi* in feces.[13–15] Furthermore, a negative fecal culture may not be helpful in excluding *R. equi* infection, because only 5 (17%) of 30 foals with confirmed *R. equi* pneumonia had positive fecal cultures.[9] Similarly, bacterial culture and PCR amplification of nasal swabs are insensitive for diagnosis of *R. equi* pneumonia.[10,11,16]

Treatment

Recommendation

The combination of a macrolide (erythromycin, azithromycin, or clarithromycin) with rifampin is the recommended treatment for infection caused by *R. equi*, based on in vitro activity data, pharmacokinetic studies, and retrospective studies. The level of evidence for this recommendation is moderate, with no randomized controlled studies available to substantiate it.

Although evidence exists that foals infected with macrolide- and rifampin-resistant isolates of *R. equi* might have a worse prognosis than foals infected with susceptible isolates,[17] there is currently inadequate evidence to recommend a specific drug for the treatment of foals infected with resistant isolates.

Rationale/Justification

A wide variety of antimicrobial agents are active against *R. equi* in vitro. However, many of these drugs are reported to be ineffective in vivo, probably because of poor cellular uptake and resulting low intracellular concentrations. For example, in a retrospective 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.[18] The combination of rifampin and erythromycin became the treatment of choice in the 1980s and has apparently reduced foal mortality relative to historical data.[7,18] However, there are no controlled studies directly comparing the efficacy of erythromycin and rifampin to that of other antimicrobial agents. In recent years, clarithromycin or azithromycin, both newer-generation macrolides, often replace erythromycin in the combination with rifampin.[19] Macrolides and rifampin are highly active against *R. equi* in vitro, but only exert bacteriostatic activity.[20] As a result, macrolides exert time-dependent activity against *R. equi* in vitro. The minimum concentration at which 90% of *R. equi* isolates are inhibited (MIC_{90}) is 0.12, 0.25, and 1.0 μg/mL, for clarithromycin, erythromycin, and azithromycin, respectively.[21] The combination of a macrolide and rifampin is synergistic both in vitro and in vivo, and the use of the 2 classes of drugs in combination reduces the likelihood of *R. equi* resistance to either drug.[20,22]

The recommended dosage for rifampin is 5 mg/kg q12 h, orally.[23] The recommended dosages for macrolides are listed in Table 1. Several formulations of erythromycin are commercially available. Although each formulation shows slight differences in bioavailability and elimination, they all result in therapeutic concentrations at recommended dosages. However, the bioavailability of erythromycin in foals is lower when foals are not fasted (mean ± SD: 26 ± 15% when fasted and 8 ± 7% when fed).[24] Advantages of azithromycin and clarithromycin over erythromycin in foals include considerably enhanced oral bioavailabilities especially in the absence of fasting, prolonged half-lives, and much higher concentrations in pulmonary epithelial lining fluid (PELF) and bronchoalveolar cells (Table 1).[25–28] These properties of the newer-generation macrolides contribute to their lower dosages and longer dosing intervals.

In general, plasma concentrations of macrolides in humans and cattle are considerably lower than the MIC of the pathogens against which these drugs have been proven to be effective. Thus, plasma concentrations of macrolides are considered poor predictors of in vivo efficacy against respiratory pathogens, whereas drug concentrations at the site of infection provide more clinically relevant information.[29] Measurement of drug concentration in PELF and cells collected by bronchoalveolar lavage is a widely used method to estimate antimicrobial concentrations at the site of infection for antimicrobials intended to treat lower respiratory tract infections in humans.[29,30] Concentrations of clarithromycin in PELF and bronchoalveolar cells of foals are considerably higher than concentrations reported after administration of azithromycin or erythromycin to foals (Table 1).[26–28] However, clarithromycin concentrations at these sites decrease rapidly, whereas the release of azithromycin from cells is much slower, resulting in sustained concentrations of azithromycin in tissues for days after discontinuation of treatment.[25–28] There are no data from randomized prospective studies comparing the relative efficacy of erythromycin, clarithromycin, and azithromycin in pneumonic foals. In a retrospective study, the combination clarithromycin-rifampin was significantly more effective than erythromycin-rifampin or azithromycin-rifampin, especially in foals with severe radiographic lesions.[19] However, these results must be interpreted with caution, because foals were not randomly distributed to treatment groups and because of biases from retrospective data. Nevertheless, these data are the best available evidence to guide macrolide selection in foals with *R. equi* pneumonia. Recent studies demonstrate that concurrent treatment with rifampin considerably decreases plasma, PELF, and bronchoalveolar cell concentrations of clarithromycin and potentially other macrolides.[31,32] However, there are no studies in foals comparing the clinical efficacy of the combination of a macrolide with rifampin to a macrolide alone. Until it is documented that a macrolide alone is as effective as the combination with rifampin, the combination of a macrolide (erythromycin, azithromycin, or clarithromycin) with rifampin remains the recommended treatment.

Resolution of clinical signs, normalization of plasma fibrinogen concentrations, and radiographic or ultrasonographic resolution of lung lesions are commonly

Table 1. Recommended dosages, minimum inhibitory concentrations, as well as plasma and pulmonary concentrations (mean ± SD) of macrolides commonly used for the treatment of *Rhodococcus equi* infections in foals.

	Erythromycin	Clarithromycin	Azithromycin
Recommended oral dosage (mg/kg)	25q 6–8 h	7.5 q12 h	10 q24–48[a]
MIC_{90} of *R. equi* isolates (μg/mL)[b]	0.25	0.12	1.00
Oral bioavailability (%)	25 ± 15[c]–8 ± 7[d]	57 ± 12[d]	54 ± 23[d]
Single oral dose (10 mg/kg; fasted)			
C_{max} plasma (μg/mL)	0.80 ± 0.74	0.94 ± 0.31	0.83 ± 0.19
C_{max} PELF (μg/mL)	ND	48.96 ± 13.26	10.00 ± 7.46
C_{max} BAL cells (μg/mL)	1.02 ± 1.11	74.20 ± 45.80	49.92 ± 26.94
$t_{1/2}$ plasma (h)	2.2 ± 2.6	4.0 ± 2.1	25.7 ± 15.4
$t_{1/2}$ PELF (h)	ND	8.6 ± 4.2	34.8 ± 30.9
$t_{1/2}$ BAL cells (h)	ND	10.7 ± 7.1	54.4 ± 17.5
Steady state at recommended dosages (fed)			
C_{max} plasma (μg/mL)	0.38 ± 0.32	0.88 ± 0.19	0.63 ± 0.10
C_{max} PELF (μg/mL)	NA	76.23 ± 59.43	11.51 ± 12.67
C_{max} BAL cells (μg/mL)	NA	269.00 ± 232.24	89.68 ± 44.25

PELF, pulmonary epithelial lining fluid; BAL, bronchoalveolar lavage. ND, not determined because quantifiable erythromycin activity was not detected in a sufficient number of animals or time points. NA, data not available. Adapted from:[21,24,26,28,90]

[a]Administration q24 h for 5 days and q48 h thereafter.

[b]MIC_{90}: Minimum inhibitory concentration that inhibit growth of at least 90% of isolates.

[c]Mean (±SD) oral bioavailability after fasting.

[d]Mean (±SD) bioavailability with ad libitum access to milk, water, and hay.

used to guide the duration of treatment that generally ranges between 3 and 12 weeks, depending on the severity of the initial lesions and response to treatment. Foals treated based on subclinical lesions identified during ultrasonographic screening typically do not require as long a treatment period as foals in respiratory distress with severe pulmonary lesions. As addressed below, many foals with subclinical ultrasonographic lesions clear the infection without treatment.[33] Currently, there are no validated criteria to differentiate foals with subclinical ultrasonographic lesions that will clear the infection from those that will progress to clinical disease.

Although well tolerated by most foals, the combination of erythromycin, clarithromycin, or azithromycin with rifampin commonly causes diarrhea.[19] Often, the diarrhea is self-limiting and does not necessitate cessation of treatment;[19] however, affected foals should be monitored carefully, because some may develop severe diarrhea, leading to dehydration and electrolyte loss that necessitate intensive fluid therapy and cessation of oral macrolides. The incidence of diarrhea in foals treated with erythromycin-rifampin has ranged between 17 and 36%.[19,34] During periods of very hot or humid weather, an idiosyncratic reaction characterized by severe hyperthermia and tachypnea has been described in foals treated with erythromycin.[34] Anecdotal reports suggest that these reactions may also occasionally occur with newer macrolides. Severe enterocolitis has also been reported in mares whose foals are treated with erythromycin, presumably by disrupting the mare's normal colonic microflora after ingestion of small amounts of active drug during coprophagia or by contamination of feeders or water buckets with drug present on the foal's muzzle. This complication seems to be rare. Enterocolitis in mares has been reproduced experimentally by administration of subtherapeutic doses of erythromycin.[35] In some cases, the severe enterocolitis in the mares of treated foals is associated with *Clostridium difficile*.[36]

Availability of a long-acting macrolide antimicrobial agent providing sustained therapeutic concentrations at the site of infection would result in less frequent administration, which in turn might improve compliance. Tulathromycin, a semisynthetic macrolide approved for use in swine and cattle, has been shown to concentrate in the bronchoalveolar cells of foals after intramuscular administration.[37] Tulathromycin was recently compared with azithromycin-rifampin for the treatment of foals with subclinical pneumonia, as identified using ultrasonographic screening on a farm with a high cumulative incidence of *R. equi* infections. Although differences in survival were not statistically significant, pulmonary abscesses, 1 week after initiation of treatment with tulathromycin, were significantly larger, and duration of treatment was significantly longer, indicating that tulathromycin is not as effective as standard treatment with azithromycin–rifampin.[38] These results might be explained by the fact that tulathromycin is poorly active against *R. equi* in vitro with a $MIC_{90} > 64$ μg/mL.[39] This concentration is more than 100-fold higher than achievable tulathromycin concentrations in bronchoalveolar cells after intramuscular administration at the recommended dose of 2.5 mg/kg.[37] Tilmicosin, another long-acting macrolide approved for use in swine and cattle, is also poorly active against *R. equi* (MIC_{90} of 32 μg/mL), and administration to foals sometimes results in swelling at the site of injection.[27] As a result, tulathromycin and tilmicosin are not recommended for use in foals with pneumonia caused by *R. equi*. In contrast, gamithromycin, a long-acting macrolide approved for

the treatment and prevention of respiratory disease in nonlactating cattle, is active against *R. equi* in vitro (MIC_{90} = 1.0 μg/mL).[40] Intramuscular administration of gamithromycin at a dosage of 6 mg/kg maintains bronchoalveolar cell concentrations above the MIC_{90} for *R. equi* for approximately 7 days.[40] However, treatment with gamithromycin is not recommended until the clinical efficacy and the safety of the drug have been established.

Treatment of foals infected with macrolide- and rifampin-resistant isolates or treatment of foals with adverse reactions to the combination macrolide–rifampin. Although the vast majority of *R. equi* isolates from foals are highly susceptible to macrolides and rifampin, strains resistant to either drug class have been encountered. Rifampin should not be used alone, because this increases the chance of resistance developing,[20,41] as a result of mutations in the RNA polymerase β subunit encoded by the *rpoB* gene.[42,43] Progressive development of resistance to both erythromycin and rifampin during treatment is extremely rare, but has been reported.[44] In a recent study, the overall prevalence of resistant isolates in Texas and Florida over a 10-year-period was 4%.[17]In the same study, the odds of death were 7 times higher in foals infected with resistant isolates.[17] In addition, the study demonstrated that isolates of *R. equi* susceptible to macrolides were sometimes misclassified as resistant; therefore, it is reasonable to request retesting/validation of resistance by the testing laboratory. The molecular mechanisms of macrolide resistance of *R. equi* isolates have not been determined, but *R. equi* isolates resistant to erythromycin, clarithromycin, or azithromycin are almost invariably resistant to the other 2 macrolides.

Treatment of foals developing severe diarrhea during macrolide treatment, or treatment of foals infected with resistant isolates, is problematic because of the limited range of effective alternatives. Macrolide- and rifampin-resistant isolates of *R. equi* are susceptible in vitro to fluoroquinolones, aminoglycosides, oxazolidinones, and glycopeptide antimicrobials.[17,39] In 1 study, 18 of 24 isolates were also susceptible to chloramphenicol, tetracycline, and trimethoprim-sulfamethoxazole.[17] Currently, there are no data to indicate the preferred antimicrobial agent(s) for the treatment of foals infected with isolates resistant to macrolides and rifampin.

Although objective data regarding efficacy are lacking, several other classes of antimicrobial agents have been used to treat *R. equi* in foals. Oral doxycycline in combination with rifampin has been used with anecdotal success to treat foals with pneumonia caused by *R. equi*. The recommended dosage for doxycycline in foals is 10 mg/kg q12 h orally.[45] This dosage results in serum, pulmonary epithelial lining fluid, and bronchoalveolar cell concentrations above the MIC_{90} of *R. equi* isolates (1.0 μg/mL) for the entire dosing interval. Chloramphenicol can be administered orally and achieves high concentrations within phagocytic cells in other species. The recommended dosage regimen is 50 mg/kg q6 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. High doses of a trimethoprim-sulfonamide combination (30 mg/kg of combination q8 or 12 h, orally) have been used alone or in combination with rifampin in foals with mild or early *R. equi* pneumonia, or for continued treatment in foals responding well to other antimicrobials.[18] Currently, there are insufficient data to recommend the use of these antimicrobial agents for treating infections caused by *R. equi*.

Ancillary Therapies. Nursing care, provision of adequate nutrition and hydration, and maintaining the foal in a cool and well-ventilated environment are important. Humidified oxygen delivered by pharyngeal insufflation in moderately hypoxemic foals, or by percutaneous transtracheal oxygenation in severely hypoxemic patients, is indicated.[46] Judicious use of nonsteroidal anti-inflammatory drugs might reduce fever and improve attitude and appetite in febrile, lethargic, anorectic foals. Nebulization with saline, antimicrobial agents, or bronchodilators has been advocated but there are no data to either support or refute these therapeutic practices. Immune-mediated extrapulmonary disorders such as polysynovitis generally resolve with successful treatment of the accompanying pneumonia. Exercise should be limited but not eliminated in foals with polysynovitis. In addition to appropriate systemic antimicrobial treatment, foals with *R. equi* septic arthritis or osteomyelitis often require aggressive local treatment such as joint lavage, surgical debridement, and IV or intraosseous regional limb perfusion with antimicrobial agents. The prognosis for foals with abdominal abscesses is poor, although rare cases will respond to long-term antimicrobial treatment.[47,48] Surgical removal or marsupialization has been attempted in some foals, but abdominal adhesions usually result in inability to resect the abscess.

Prognosis. Before the introduction of the combination of erythromycin and rifampin as the treatment of choice in the early 1980s, the prognosis of *R. equi* infected foals was poor with survival rates as low as 20%.[49] Using erythromycin and rifampin, Hillidge reported a successful outcome (as assessed by survival) in 50 (88%) of 57 foals with confirmed *R. equi* pneumonia.[7] Studies from referral centers, where severely affected cases are probably more prevalent, have revealed survival proportions ranging between 59 and 72%.[19,50,51] In contrast, the survival rate at farms using a screening program to identify and treat foals with subclinical lesions has resulted in survival proportions of nearly 100%.[52,53] It is likely, however, that many of these foals would have recovered without treatment.[33]

The impact of *R. equi* infections on future athletic performance has been examined. No significant differences in total earnings, average earning index, and age at the 1st race were observed when comparing 30 horses that previously had *R. equi* pneumonia with

either their dams' other progeny or the North American averages.[54] More recently, 54% of 83 foals (N = 45) that survived *R. equi* pneumonia had at least 1 racing start when compared with 65% of their birth cohort, suggesting that horses contracting *R. equi* pneumonia as foals may be somewhat less likely to race as adults. However, consistent with a previous report,[54] the racing performance of those foals that raced was not different from that of the US racing population.[50] In summary, prognosis for performance after successful treatment of uncomplicated *R. equi* pneumonia should be regarded as excellent.

Control and Prevention

Methods for control and prevention include using screening tests for early detection of *R. equi* pneumonia, environmental management, chemoprophylaxis, and prevention using either passive or active immunization.

Screening

Recommendation. Implementing some form of screening for early identification is recommended for controlling *R. equi* pneumonia at farms with recurrent history of foals affected by this disease. The level of evidence for this recommendation is relatively weak, with no controlled studies available to substantiate it.

Rationale/Justification. Pneumonia caused by *R. equi* is an insidious disease in which clinical signs may not be apparent until pathologic changes are well progressed.[55,56] Consequently, the rationale for screening is the supposition that detecting foals in the early stages of disease will improve therapeutic outcomes, because evidence exists that advanced progression of disease is associated with poorer prognosis. Controlled studies documenting reduction in mortality attributable to *R. equi* pneumonia after screening, are lacking. In the authors' experience, the apparent incidence (morbidity) will generally be increased by screening testing whenever positive screening test results are considered presumptively diagnostic. The reason that positive screening results are often interpreted as presumptively diagnostic is because the predictive value of positive results of screening tests at endemic farms is considered to be high. Moreover, substantiating the cause of pneumonia by isolating virulent *R. equi* from fluid obtained by tracheobronchial aspiration is eschewed by many practicing veterinarians at breeding farms because of risks of the procedure posed to foals, costs for the procedure, the aforementioned presumed high positive predictive value of test results, and because false-negative test results may be more likely in early stages of disease. Subclinical disease has been described after both experimental and natural infection.[9,57] In general, the cumulative incidence of clinical signs of pneumonia attributed to *R. equi* at affected farms in the United States has been approximately 10–20% from birth to weaning (although many farms may experience greater cumulative incidences).[58,59] In contrast, the cumulative incidence at *R. equi*-endemic farms of foals with sonographic evidence of pulmonary abscessation or consolidation is generally between 30 and 60%, in the authors' anecdotal experience. The finding that cumulative incidences of clinical disease tend to be lower than those described anecdotally for sonographically detectable lesions indicates that many pre/subclinically affected foals may resolve without intervention. Because it remains unknown what proportion or which specific foals might recover spontaneously from subclinical disease, and because *R. equi* infections can cause severe disease, many breeding farms elect to treat all foals with positive results of screening tests. Uncontrolled studies suggest that screening may reduce the mortality attributable to *R. equi* pneumonia.[53,60] Thus, in the absence of a vaccine or other effective methods for preventing this devastating disease, screening is recommended to control *R. equi* at endemic farms. Implementing screening testing at farms that are only sporadically affected by *R. equi* pneumonia may not be warranted.

A variety of screening techniques performed serially have been described, including visual inspection of foals, monitoring rectal temperatures, clinical signs of pneumonia or extrapulmonary disorders, hematologic parameters, serology, and thoracic imaging using either radiography or ultrasonography, with the empiric recommendation that screening begin around 3 weeks of age.[61] To the authors' knowledge, systematic comparisons among these tests have not been performed. Thus, a specific recommendation for any particular screening test cannot be made, and it is likely that the optimal approach for screening may vary among farms on the basis of cumulative incidence of disease, resources available for control and prevention, and preferences of the attending veterinarian(s) and farm management.

Some tests appear to be ineffective for screening. Current knowledge indicates that serum concentrations of either antibodies against *R. equi*, serum amyloid A, or plasma fibrinogen do not appear to be useful screening tests[4,6,62]; further evaluation of serum amyloid A with newer methods of detection and more frequent sampling may be warranted.

White blood cell concentrations performed at monthly intervals appeared to have clinically acceptable sensitivity and specificity for early detection of *R. equi* pneumonia at 1 farm (viz, sensitivity of approximately 79% and specificity of 91% at a cut-off value of 15,000 cells/μL).[3] The principal limitations of using WBC for screening are the time and effort required to collect blood samples from foals, the lag from time of submission, and the potential for false-positive results attributable to other infectious or inflammatory conditions that may be common among foals or stress-associated leukocytosis.

To the authors' knowledge, use of ultrasonography for screening to detect *R. equi* pneumonia has not been systematically evaluated. As a result of the various advantages and limitations associated with this

approach to screening, it has both proponents and opponents. Advantages of thoracic ultrasonography include (1) the procedure can be performed relatively quickly for an individual foal; (2) competence with the procedure can be rapidly developed; (3) results are immediately available; (4) the procedure may be more sensitive than radiography for detecting lesions in their early stages of development or in certain regions where soft tissue structures are superimposed over regions of the lung;[63] (5) results are specific for the presence of pulmonary pathology (as contrasted with, for example, results of WBC); and (6) evidence from uncontrolled studies that mortality attributable to *R. equi* was eradicated using ultrasonographic screening.[53] Disadvantages of ultrasonographic screening include the costs to the farm for repetitive sonographic examinations, the increased labor needed to handle foals repetitively, and an increased number of foals treated for presumptive *R. equi* pneumonia as a result of the aforementioned apparent increase in cumulative incidence. A consequence of any screening program that results in a greater numbers of foals being treated for presumptive *R. equi* pneumonia is that it will lead to higher costs to the farm/foal-owners, increased risk of adverse events associated with treatment,[34] and further development of resistance to macrolides.[17] Despite the limitations of screening methods, it is the authors' belief that, in the absence of a highly effective method for prevention such as a vaccine, diligent application of screening tests in foals for earlier detection of disease is an important tool for controlling *R. equi* pneumonia at farms where it is endemic.

It remains unclear whether treatment of all subclinically affected foals is appropriate. A recent controlled clinical trial at an endemic farm demonstrated that many foals with subclinical pulmonary lesions recover without treatment, and that treatment of foals having subclinical pulmonary lesions with azithromycin-rifampin did not accelerate recovery compared with a placebo.[33] These results indicate that mass antimicrobial treatment of all foals with small subclinical pulmonary abscesses was unnecessary at that farm. The extent to which these results may be extrapolated to other farms remains unknown because the proportion of foals that recover without treatment may vary by farm, geographic region, and age at which foals lesions are detected. Additional studies are needed to determine the risks and benefits of treating subclinically affected foals.

Environmental Management

Recommendation. Although *R. equi* pneumonia has been positively associated with the density of mares and foals at farms and airborne concentrations of virulent *R. equi*, and negatively associated with foaling at pasture, there is inadequate evidence to recommend environmental interventions to control or prevent *R. equi* pneumonia.

Rationale/Justification. Some farms do not have affected foals, some farms have foals affected sporadically, and some farms experience the disease on a recurrent basis, suggesting that environmental conditions influence the occurrence of this disease. The odds of developing *R. equi* pneumonia appear to be related to the density of mares and foals per acre at horse breeding farms.[58,59,64,65]A reduction in the number of mares at a farm reportedly reduced the cumulative incidence of *R. equi* relative to preceding years,[8] and the authors are aware of similar anecdotes from farms. However, such data must be interpreted with caution in light of the considerable year-to-year variation in incidence that can occur within farms.[66] The impact of reducing density of mares and foals for decreasing the incidence of *R. equi* pneumonia will remain unresolved until observational or experimental studies can be conducted to address this question. It seems reasonable that reducing density be considered as an approach for decreasing the incidence of *R. equi* pneumonia.

Rhodococcus equi is a soil saprophyte that is also found commonly in herbivore feces. Geochemical properties of soil have been associated with odds of disease caused by *Mycobacterium avium* ssp. *paratuberculosis*,[67] another facultative intracellular pathogen phylogenetically related to *R. equi*. In Texas, however, soil geochemistry did not appear to be associated with occurrence of *R. equi* pneumonia at farms;[68] similar studies from other geographic regions have not been reported, to the authors' knowledge, although the odds of recovering virulent *R. equi* from soil samples tended to be greater from soils with pH < 6 than from samples that had pH ≥ 6, in Australia.[69] Neither the presence nor the concentration of virulent *R. equi* in soil is positively associated with either increased odds or increased cumulative incidence of *R. equi* pneumonia at breeding farms in North America or Australia.[65,69,70] Removing feces from paddocks or avoiding spreading manure on pastures (whether manure was composted or not) did not decrease the odds of farms being affected by *R. equi* pneumonia;[64] however, these management practices are probably important for controlling parasitic and other infectious diseases.

Airborne concentrations of *R. equi* were correlated with disease incidence at Thoroughbred breeding farms in Australia.[69] Factors associated with increased air concentration of *R. equi* at these farms included site (holding pens/lanes relative to paddocks), warmer ambient temperature, less soil moisture, reduced grass height, and later date during the foaling season.[69] In Kentucky, concentrations of airborne *R. equi* were lower between midnight and 6 AM than later times, and the presence of horses at the sampling site appeared to increase the airborne concentrations of virulent *R. equi*;[71] these findings suggest that airborne concentrations may be increased by greater activity and density of horses at sites where foals are housed. Irrigation of holding pens at a farm with foals affected by *R. equi* was demonstrated to reduce the frequency of recovery of virulent *R. equi* in air samples from these pens.[72] These results should be interpreted with caution, however, because samples were collected in sequential years (rather than with contemporaneous controls); moreover, there was no

significant reduction in cumulative incidence of *R. equi* pneumonia at the farm.[72] It remains to be determined whether the positive association between airborne concentrations of virulent *R. equi* with cumulative incidence of *R. equi* pneumonia is a cause or effect. Thus, it is uncertain whether methods for reducing airborne concentrations would be effective for controlling or preventing *R. equi* pneumonia.

Evidence exists that foaling at pasture may reduce the occurrence of *R. equi* pneumonia. In a survey, foaling at pasture was significantly associated with decreased odds of *R. equi* pneumonia;[59] however, this observation could have been confounded by other management practices or other unaccounted factors. The airborne concentrations of virulent *R. equi* in barns were significantly lower than in paddock samples from 3 farms in Ireland.[73] Being born and maintained in pasture significantly reduced the cumulative incidence of *R. equi* pneumonia at a breeding farm in Brazil,[74] but these findings must be interpreted with considerable caution pending replication because they were based on historical controls. Moreover, there may be practical and veterinary medical considerations that prevent this strategy from being adapted by some farms, even if it were effective.

A practical management question often posed to the authors is whether foals with *R. equi* pneumonia should be isolated from other foals. Relative to adult horses, foals typically shed higher concentrations of virulent *R. equi* in their feces,[75] and 2 pneumonic foals shed higher concentrations of *R. equi* in their feces than did unaffected foals from the same environment.[14] Thus, foals represent a source of environmental contamination, and pneumonic foals appear to pose a greater risk. Although it might seem prudent to restrict diseased foals from being housed in paddocks that will be used to hold other mares and foals, there is no evidence to indicate that shedding by affected foals into the environment causes pneumonia in other foals. Air samples from the breathing zone of pneumonic foals had higher concentrations of virulent *R. equi* than environmental air samples collected from lanes and pens at farms at their farms.[76] However, concentrations of virulent *R. equi* from air samples collected from the breathing zones were not significantly different between pneumonic foals and healthy controls, indicating that affected foals do not represent a greater risk than other foals for aerosol transmission. There is no compelling evidence that *R. equi* infection is contagious among foals, and exposure to virulent *R. equi* appears to be widespread in the environment of foals.[65,69,70] Thus, currently there is no evidence to indicate that affected foals should be isolated from other foals.

In summary, no environmental management practice has sufficient evidence on which to base recommendations for controlling and preventing *R. equi* pneumonia. Further evaluation of certain practices, such as foaling at pasture and reducing either airborne concentrations of virulent *R. equi* or density of mares and foals, is merited.

Chemoprophylaxis

Recommendation. Chemoprophylaxis to prevent *R. equi* pneumonia with macrolides or other classes of antimicrobial drugs is not recommended because of conflicting evidence of efficacy and concerns for promoting resistance of *R. equi* and other bacteria to these drugs. The level of evidence for this recommendation is moderate.

Rationale/Justification. Because an effective vaccine is lacking, use of antimicrobial agents to prevent *R. equi* pneumonia has been examined. Two studies from different laboratories using different study designs have evaluated the use of azithromycin for chemoprophylaxis. In a randomized, controlled trial conducted in the United States among 338 foals at 10 farms, the cumulative incidence of *R. equi* pneumonia was reduced from 21% among untreated foals to 5% among foals that received azithromycin (10 mg/kg; PO; q48 h for the first 14 days of life beginning on the first day of life).[77] In contrast, in a study conducted among 70 foals at a large breeding farm in Germany, the incidence of abscessing pneumonia was not significantly different between foals that received azithromycin (10 mg/kg; PO; q24 h for the first 28 days of life) for prevention of abscessing pneumonia (cumulative incidence = 60%) and foals that did not receive azithromycin for chemoprophylaxis (cumulative incidence = 69%); however, the age at onset of abscessing pneumonia was delayed in treated foals.[52] Neither study was fully masked, nor were placebos used. The reason for the discrepancy between studies remains unknown, but it does not appear to relate to either the dosage or duration of azithromycin treatment. Other possible explanations include differences between studies in case definitions, the extent of selection bias, methods of randomization, incidence of disease, risk factors for disease, and possibly drug formulation. Nevertheless, use of azithromycin is not considered an acceptable approach for chemoprophylaxis because widespread use of this drug would create greater pressure for emergence of macrolide resistance among bacteria. Evidence exists that prognosis is worse for foals with *R. equi* pneumonia from which macrolide-resistant isolates have been recovered.[17]

Gallium maltolate is a metal-based compound with antimicrobial properties that has been demonstrated to reduce replication of *R. equi* in pure culture,[78] and within macrophages[79] to reduce tissue concentrations of *R. equi* in mice after experimental infection,[78] and to be bioavailable[80] and safe in foals.[81] Chemoprophylaxis with gallium maltolate (30 mg/kg; PO; q24 h for the first 14 days of life) failed to reduce the incidence of *R. equi* pneumonia in a placebo-controlled trial of 438 foals at 12 farms in the United States.[82]

Passive Immunization

Recommendation. Administration of commercially available and licensed plasma containing antibody against *R. equi* is recommended as an aid for prevent-

Table 2. Effectiveness of transfusion of hyperimmune plasma to prevent *Rhodococcus equi* foal pneumonia: published reports of studies with contemporaneous controls.

Incidence[a] with Plasma (Proportion)	Incidence[a] without Plasma (Proportion)	Relative Risk Reduction[b] (%)	$P < .05$	Number of Administrations (Age at Administration [days])	Reference
3% (3/101)	43% (6/14)	93	Yes	1 (1–60)	91
5.7% (14/246)	53.4% (7/13)	89	Yes	1 (1–40)	8
6.3% (1/16)	27.7% (23/83)	77	No	1 (10–39)	92
19.1% (13/68)	30.0% (24/80)	37	No	2 (1–10 and 30–50)	93
29.0 (103/355)	45.3% (58/128)	36	Yes	2[c] (1–3 and 14–30)	82
6.8% (2/29)	4.6% (5/107)	−48	No	Not provided	66
29.4% (10/34)	21.1% (12/57)	−39	No	1 (≤ 2)	94

[a]Incidence = cumulative incidence of *R. equi* pneumonia among foals transfused with hyperimmune plasma (1st column) or foals from the same farm that did not receive plasma.

[b]Relative risk reduction = (incidence without plasma – incidence with plasma) × 100% (incidence without plasma).

[c]75% of foals received 2 doses. The other 25% received only 1 dose at 1–3 days of age.

ing pneumonia caused by *R. equi* at endemic farms. The level of evidence for this recommendation is moderate. However, the ideal age and minimal effective dose of hyperimmune plasma (HIP) require further research. Transfusion of HIP is not completely effective and therefore does not eliminate the need for screening or careful monitoring of foals at risk.

Rationale/Justification. Intravenous administration of HIP obtained from horses vaccinated against *R. equi* using various antigens has generally proved effective in significantly reducing the severity of *R. equi* pneumonia in foals after experimental challenge.[57,83,84] However, studies evaluating the efficacy of various HIP preparations under field conditions have given equivocal results. Although the data are conflicting and not all trials have shown a statistically significant reduction in the cumulative incidence of *R. equi* pneumonia, 5 of 7 studies have demonstrated reduction of relative risk, suggesting some benefit of HIP transfusion (Table 2). The studies summarized in Table 2 used different immunization protocols to hyperimmunize the donors. As a result, it is not possible to make recommendations regarding the most appropriate immunization protocols for plasma donors. Intravenous administration of purified immunoglobulins obtained from horses immunized with recombinant VapA and VapC to foals reduced the severity of pneumonia after high-dose experimental challenge with *R. equi* to a degree similar to that provided by commercially available HIP.[85] This demonstrates that immunoglobulins, predominantly against VapA and VapC, are responsible for the protection conferred by HIP. Therefore, administration of plasma obtained from horses that are not hyperimmunized against *R. equi* is not recommended. As endemicity of *R. equi* at farms does not appear to be explained by farm-specific isolates, and because foals may be infected with multiples strains,[86] there is no need to administer plasma that is produced from immunizing horses with isolates of *R. equi* obtained from a given farm; transfusion of plasma from horses that have been immunized against any virulent (ie VapA-positive) strain of *R. equi* should suffice. Use of HIP licensed as an aid in the control of *R. equi* pneumonia is recommended (rather than plasma simply obtained from horses hyperimmunized against *R. equi*), because licensure ensures standard of potency, purity, and safety. Currently, there is insufficient information to recommend one brand of licensed antibody product over another.

The optimal amount of plasma to be transfused and the optimal age at which transfusion should occur remain to be determined. Administration of HIP 9 days after aerosol infection of foals with *R. equi* did not confer protection,[87] suggesting that administration of HIP before infection is important. As a result of evidence that many foals become infected early in life,[88] it is commonly recommended that foals receive transfusion of at least 1 L of HIP no later than the 2nd day of life. Because early administration may result in the decline of passively transferred antibody to a nonprotective level at a time when foals are still susceptible to *R. equi* and when environmental challenge is high, it is a common practice to administer a 2nd dose of HIP at 2–4 weeks of age.

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

Active Immunization

Recommendation. There is inadequate evidence to recommend active immunization of mares or foals to control or prevent *R. equi* pneumonia.

Rationale/Justification. The evidence from mouse models, immune adult horses, and the study of other similar intracellular pathogens strongly suggests that an active immunization strategy to prevent *R. equi* disease will need to induce antigen-specific Type 1 cell-mediated responses. Antibody responses alone are unlikely to be protective. At a minimum, protective responses produced by an efficacious vaccine are likely

to include CD4+ Th1 lymphocytes that secrete interferon-gamma in response to *R. equi* antigens and (possibly) CD8+ T lymphocytes that recognize and kill *R. equi* infected cells (see companion article in the same issue). There also may be other as yet undefined requirements. As foals are exposed and possibly infected in the first days or weeks of life, an effective immunization strategy will likely need to be initiated shortly after birth.

The relative immunologic hyporesponsiveness that characterizes early life probably represents the primary obstacle to an active immunization strategy. However, there is evidence that vaccination is an achievable goal. For example, immunologic studies have demonstrated adult-like IFN-gamma responses of foals to *R. equi*.[89] Likewise, even at endemic farms where all foals are presumably exposed to high doses of virulent *R. equi*, a majority of foals effectively resist disease and go on to develop protective immune responses that prevent rhodococcal pneumonia throughout adult life. A novel strategy will probably be required to induce or at least prime these responses in the first weeks of a foal's life. In a promising study, intragastric inoculation of neonatal foals with virulent *R. equi* protected foals from a subsequent respiratory challenge that produced severe disease in controls.[84] Inoculation of virulent bacteria is not an acceptable real-world strategy. However, it unequivocally demonstrates that young foals can mount protective immune responses. An effective vaccine to prevent rhodococcal pneumonia in foals is unlikely to come easily. A long-term view encompassing basic science research and empirical vaccine trials will be required.

References

1. Leclere M, Magdesian KG, Kass PH, et al. Comparison of the clinical, microbiological, radiological and haematological features of foals with pneumonia caused by *Rhodococcus equi* and other bacteria. Vet J 2011;187:109–112.

2. Lavoie JP, Fiset L, Laverty S. Review of 40 cases of lung abscesses in foals and adult horses. Equine Vet J 1994;26:348–352.

3. Giguère S, Hernandez J, Gaskin JM, et al. Evaluation of WBC concentration, plasma fibrinogen concentration, and an agar gel immunodiffusion test for early identification of foals with *Rhodococcus equi* pneumonia. J Am Vet Med Assoc 2003;222:775–781.

4. Giguère S, Hernandez J, Gaskin J, et al. Performance of five serological assays for diagnosis of *Rhodococcus equi* pneumonia in foals. Clin Diagn Lab Immunol 2003;10:241–245.

5. Phumoonna T, Muscatello G, Chicken C, et al. Clinical evaluation of a peptide-ELISA based upon N-terminal B-cell epitope of the VapA protein for diagnosis of *Rhodococcus equi* pneumonia in foals. J Vet Med B Infect Dis Vet Public Health 2006;53:126–132.

6. Martens RJ, Cohen ND, Chaffin MK, et al. Evaluation of 5 serologic assays to detect *Rhodococcus equi* pneumonia in foals. J Am Vet Med Assoc 2002;221:825–833.

7. Hillidge CJ. Use of erythromycin-rifampin combination in treatment of *Rhodococcus equi* pneumonia. Vet Microbiol 1987;14:337–342.

8. Muller NS, Madigan JE. Methods of implementation of an immunoprophylaxis program for the prevention of *Rhodococcus equi* pneumonia: results of a 5-year field study. Proc Am Assoc Equine Pract 1992;38:193–201.

9. Ardans AA, Hietala SK, Spensley MS, et al. Studies of naturally occuring and experimental *Rhodococcus equi*. Proc Am Assoc Equine Pract 1986;32:129–144.

10. Pusterla N, Wilson WD, Mapes S, et al. Diagnostic evaluation of real-time PCR in the detection of *Rhodococcus equi* in faeces and nasopharyngeal swabs from foals with pneumonia. Vet Rec 2007;161:272–275.

11. Sellon DC, Besser TE, Vivrette SL, et al. Comparison of nucleic acid amplification, serology, and microbiologic culture for diagnosis of *Rhodococcus equi* pneumonia in foals. J Clin Microbiol 2001;39:1289–1293.

12. Rodriguez-Lazaro D, Lewis DA, Ocampo-Sosa AA, et al. Internally controlled real-time PCR method for quantitative species-specific detection and *vapA* genotyping of *Rhodococcus equi*. Appl Environ Microbiol 2006;72:4256–4263.

13. Nakazawa M, Sugimoto C, Isayama Y. Quantitative culture of *Rhodococcus equi* from the feces of horse. Natl Inst Anim Health Q (Tokyo) 1983;23:67–68.

14. Takai S, Iimori S, Tsubaki S. Quantitative fecal culture for early diagnosis of *Corynebacterium* (*Rhodococcus*) *equi* enteritis in foals. Can J Vet Res 1986;50:479–484.

15. Takai S, Ohkura H, Watanabe Y, et al. Quantitative aspects of fecal *Rhodococcus* (*Corynebacterium*) *equi* in foals. J Clin Microbiol 1986;23:794–796.

16. Hashikura S, Higuchi T, Taharaguchi S, et al. Evaluation of nasotracheal aspiration as a diagnostic tool for *Rhodococcus equi* pneumonia in foals. Equine Vet J 2000;32:560–564.

17. Giguère S, Lee E, Williams E, et al. Determination of the prevalence of antimicrobial resistance to macrolide antimicrobials or rifampin in *Rhodococcus equi* isolates and treatment outcome in foals infected with antimicrobial-resistant isolates of *R. equi*. J Am Vet Med Assoc 2010;237:74–81.

18. Sweeney CR, Sweeney RW, Divers TJ. *Rhodococcus equi* pneumonia in 48 foals: Response to antimicrobial therapy. Vet Microbiol 1987;14:329–336.

19. Giguère S, Jacks S, Roberts GD, et al. Retrospective comparison of azithromycin, clarithromycin, and erythromycin for the treatment of foals with *Rhodococcus equi* pneumonia. J Vet Intern Med 2004;18:568–573.

20. Nordmann P, Ronco E. *In-vitro* antimicrobial susceptibility of *Rhodococcus equi*. J Antimicrob Chemother 1992;29:383–393.

21. Jacks S, Giguère S, Nguyen A. In vitro susceptibilities of *Rhodococcus equi* and other common equine pathogens to azithromycin, clarithromycin and 20 other antimicrobials. Antimicrob Agents Chemother 2003;47:1742–1745.

22. Prescott JF, Nicholson VM. The effects of combinations of selected antibiotics on the growth of *Corynebacterium equi*. J Vet Pharmacol Ther 1984;7:61–64.

23. Castro LA, Brown MP, Gronwall R, et al. Pharmacokinetics of rifampin given as a single oral dose in foals. Am J Vet Res 1986;47:2584–2586.

24. Lakritz J, Wilson WD, Marsh AE, et al. Effects of prior feeding on pharmacokinetics and estimated bioavailability after oral administration of a single dose of microencapsulated erythromycin base in healthy foals. Am J Vet Res 2000;61:1011–1015.

25. Davis JL, Gardner SY, Jones SL, et al. Pharmacokinetics of azithromycin in foals after i.v. and oral dose and disposition into phagocytes. J Vet Pharmacol Ther 2002;25:99–104.

26. Jacks S, Giguère S, Gronwall PR, et al. Pharmacokinetics of azithromycin and concentration in body fluids and bronchoalveolar cells in foals. Am J Vet Res 2001;62:1870–1875.

27. Womble A, Giguère S, Murthy YV, et al. Pulmonary disposition of tilmicosin in foals and in vitro activity against *Rhodococcus equi* and other common equine bacterial pathogens. J Vet Pharmacol Ther 2006;29:561–568.

28. Suarez-Mier G, Giguère S, Lee EA. Pulmonary disposition of erythromycin, azithromycin, and clarithromycin in foals. J Vet Pharmacol Ther 2007;30:109–115.

29. Drusano GL. Infection site concentrations: Their therapeutic importance and the macrolide and macrolide-like class of antibiotics. Pharmacotherapy 2005;25:150S–158S.

30. Kiem S, Schentag JJ. Interpretation of antibiotic concentration ratios measured in epithelial lining fluid. Antimicrob Agents Chemother 2008;52:24–36.

31. Peters J, Block W, Oswald S, et al. Oral absorption of clarithromycin is nearly abolished by chronic comedication of rifampicin in foals. Drug Metab Dispos 2011;39:1543–1649.

32. Venner M, Peters J, Hohensteiger N, et al. Concentration of the macrolide antibiotic tulathromycin in broncho-alveolar cells is influenced by comedication of rifampicin in foals. Naunyn Schmiedebergs Arch Pharmacol 2010;381:161–169.

33. Venner M, Rodiger A, Laemmer M, et al. Failure of antimicrobial therapy to accelerate spontaneous healing of subclinical pulmonary abscesses on a farm with endemic infections caused by *Rhodococcus equi*. Vet J 2011; Sep 14. doi.org/10.1016/j.tvjl.2011.07.004 [Epub ahead of print].

34. Stratton-Phelps M, Wilson WD, Gardner IA. Risk of adverse effects in pneumonic foals treated with erythromycin versus other antibiotics: 143 cases (1986–1996). J Am Vet Med Assoc 2000;217:68–73.

35. Gustafsson A, Baverud V, Gunnarsson A, et al. The association of erythromycin ethylsuccinate with acute colitis in horses in Sweden. Equine Vet J 1997;29:314–318.

36. Baverud V, Franklin A, Gunnarsson A, et al. *Clostridium difficile* associated with acute colitis in mares when their foals are treated with erythromycin and rifampicin for *Rhodococcus equi* pneumonia. Equine Vet J 1998;30:482–488.

37. Scheuch E, Spieker J, Venner M, et al. Quantitative determination of the macrolide antibiotic tulathromycin in plasma and broncho-alveolar cells of foals using tandem mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci 2007;850:464–470.

38. Venner M, Kerth R, Klug E. Evaluation of tulathromycin in the treatment of pulmonary abscesses in foals. Vet J 2007;174:418–421.

39. Carlson K, Kuskie K, Chaffin K, et al. Antimicrobial activity of tulathromycin and 14 other antimicrobials against virulent *Rhodococcus equi* in vitro. Vet Ther 2010;11:E1–E9.

40. Berghaus LJ, Giguère S, Sturgill TL, et al. Plasma pharmacokinetics, pulmonary distribution, and in vitro activity of gamithromycin in foals. J Vet Pharmacol Ther 2011; Mar 28. doi: 10.1111/j.1365-2885.2011.01292.x. [Epub ahead of print]

41. Takai S, Takeda K, Nakano Y, et al. Emergence of rifampin-resistant *Rhodococcus equi* in an infected foal. J Clin Microbiol 1997;35:1904–1908.

42. Fines M, Pronost S, Maillard K, et al. Characterization of mutations in the *rpoB* gene associated with rifampin resistance in *Rhodococcus equi* isolated from foals. J Clin Microbiol 2001;39:2784–2787.

43. Asoh N, Watanabe H, Fines-Guyon M, et al. Emergence of rifampin-resistant *Rhodococcus equi* with several types of mutations in the rpoB gene among AIDS patients in northern Thailand. J Clin Microbiol 2003;41:2337–2340.

44. Kenney DG, Robbins SC, Prescott JF, et al. Development of reactive arthritis and resistance to erythromycin and rifampin in a foal during treatment for *Rhodococcus equi* pneumonia. Equine Vet J 1994;26:246–248.

45. Womble A, Giguère S, Lee EA. Pharmacokinetics of oral doxycycline and concentrations in body fluids and bronchoalveolar cells of foals. J Vet Pharmacol Ther 2007;30:187–193.

46. Hoffman AM, Viel L. A percutaneous transtracheal catheter system for improved oxygenation in foals with respiratory distress. Equine Vet J 1992;24:239–241.

47. Valdes A, Johnson JR. Septic pleuritis and abdominal abscess formation caused by *Rhodococcus equi* in a foal. J Am Vet Med Assoc 2005;227:960–963.

48. Reuss SM, Chaffin MK, Cohen ND. Extrapulmonary disorders associated with *Rhodococcus equi* infection in foals: 150 cases (1987–2007). J Am Vet Med Assoc 2009;235:855–863.

49. Elissalde GS, Renshaw HW, Walberg JA. *Corynebacterium equi*: An interhost review with emphasis on the foal. Comp Immunol Microbiol Infect Dis 1980;3:433–445.

50. Ainsworth DM, Eicker SW, Yeagar AE, et al. Associations between physical examination, laboratory, and radiographic findings and outcome and subsequent racing performance of foals with *Rhodococcus equi* infection: 115 cases (1984–1992). J Am Vet Med Assoc 1998;213:510–515.

51. Chaffin MK, Martens RJ. Extrapulmonary disorders associated with *Rhodococcus equi* pneumonia in foals: Retrospective study of 61 cases (1988–1996). Proc Am Assoc Equine Pract 1997;43:79–80.

52. Venner M, Reinhold B, Beyerbach M, et al. Efficacy of azithromycin in preventing pulmonary abscesses in foals. Vet J 2009;179:301–303.

53. Slovis NM, McCracken JL, Mundy G. How to use thoracic ultrasound to screen foals for *Rhodococcus equi* at affected farms. Proc Am Assoc Equine Pract 2005;51:274–278.

54. Bernard B, Dugan J, Pierce S, et al. The influence of foal pneumonia on future racing performance. Proc Am Assoc Equine Pract 1991;37:17–18.

55. Giguère S, Prescott JF. Clinical manifestations, diagnosis, treatment, and prevention of *Rhodococcus equi* infections in foals. Vet Microbiol 1997;56:313–334.

56. Prescott JF. *Rhodococcus equi*: An animal and human pathogen. Clin Microbiol Rev 1991;4:20–34.

57. Martens RJ, Martens JG, Fiske RA, et al. *Rhodococcus equi* foal pneumonia: Protective effects of immune plasma in experimentally infected foals. Equine Vet J 1989;21:249–255.

58. Chaffin MK, Cohen ND, Martens RJ. Evaluation of equine breeding farm characteristics as risk factors for development of *Rhodococcus equi* pneumonia in foals. J Am Vet Med Assoc 2003;222:467–475.

59. Cohen ND, O'Conor MS, Chaffin MK, et al. Farm characteristics and management practices associated with development of *Rhodococcus equi* pneumonia in foals. J Am Vet Med Assoc 2005;226:404–413.

60. Prescott JF, Machang'u R, Kwiecien J, et al. Prevention of foal mortality due to *Rhodococcus equi* pneumonia on an endemically affected farm. Can Vet J 1989;30:871–875.

61. Cohen ND, Chaffin MK, Martens RJ. Control and prevention of *Rhodococcus equi* pneumonia in foals. Compend Contin Educ Pract Vet 2000;22:1062–1070.

62. Cohen ND, Chaffin MK, Vandenplas ML, et al. Study of serum amyloid A concentrations as a means of achieving early diagnosis of *Rhodococcus equi* pneumonia. Equine Vet J 2005;37:212–216.

63. Ramirez S, Lester GD, Roberts GR. Diagnostic contribution of thoracic ultrasonography in 17 foals with *Rhodococcus equi* pneumonia. Vet Radiol Ultrasound 2004;45:172–176.

64. Chaffin MK, Cohen ND, Martens RJ. Evaluation of equine breeding farm management and preventative health practices as risk factors for development of *Rhodococcus equi*

pneumonia in foals. J Am Vet Med Assoc 2003;222:476–485.

65. Cohen ND, Carter CN, Scott HM, et al. Association of soil concentrations of *Rhodococcus equi* and incidence of pneumonia attributable to *Rhodococcus equi* in foals on farms in central Kentucky. Am J Vet Res 2008;69:385–395.

66. Chaffin MK, Cohen ND, Martens RJ, et al. Foal-related risk factors associated with development of *Rhodococcus equi* pneumonia on farms with endemic infection. J Am Vet Med Assoc 2003;223:1791–1799.

67. Ward MP, Perez AM. Association between soil type and paratuberculosis in cattle herds. Am J Vet Res 2004;65:10–14.

68. Martens RJ, Cohen ND, Chaffin MK, et al. Association of pneumonia in foals caused by *Rhodococcus equi* with farm soil geochemistry. Am J Vet Res 2002;63:95–98.

69. Muscatello G, Anderson GA, Gilkerson JR, et al. Associations between the ecology of virulent *Rhodococcus equi* and the epidemiology of *R. equi* pneumonia on Australian thoroughbred farms. Appl Environ Microbiol 2006;72:6152–6160.

70. Martens RJ, Takai S, Cohen ND, et al. Association of disease with isolation and virulence of *Rhodococcus equi* from farm soil and foals with pneumonia. J Am Vet Med Assoc 2000;217:220–225.

71. Kuskie KR, Smith JL, Wang N, et al. Effects of location for collection of air samples on a farm and time of day of sample collection on airborne concentrations of virulent *Rhodococcus equi* at two horse breeding farms. Am J Vet Res 2011;72:73–79.

72. Muscatello G, Gilkerson JR, Browning GF. Effects of selective intensive irrigation on the incidence and prevalence of *Rhodococcus equi* pneumonia on an endemically affected farm. Aust Equine Vet 2006;25:79–80.

73. Muscatello G, Gerbaud S, Kennedy C, et al. Comparison of concentrations of *Rhodococcus equi* and virulent *R. equi* in air of stables and paddocks on horse breeding farms in a temperate climate. Equine Vet J 2006;38:263–265.

74. Malschitzky E, Neves AP, Gregory RM, et al. Reduzir o uso da cocheira a incidencia de infeccoes por *Rhodococcus equi* em potros. A Hora Veterinaria 2005;24:27–30.

75. Takai S, Ohbushi S, Koike K, et al. Prevalence of virulent *Rhodococcus equi* in isolates from soil and feces of horses from horse-breeding farms with and without endemic infections. J Clin Microbiol 1991;29:2887–2889.

76. Muscatello G, Gilkerson JR, Browning GF. Detection of virulent *Rhodococcus equi* in exhaled air samples from naturally infected foals. J Clin Microbiol 2009;47:734–737.

77. Chaffin MK, Cohen ND, Martens RJ. Chemoprophylactic effects of azithromycin against *Rhodococcus equi*-induced pneumonia among foals at equine breeding farms with endemic infections. J Am Vet Med Assoc 2008;232:1035–1047.

78. Harrington JR, Martens RJ, Cohen ND, et al. Antimicrobial activity of gallium against virulent *Rhodococcus equi* in vitro and in vivo. J Vet Pharmacol Ther 2006;29:121–127.

79. Martens RJ, Miller NA, Cohen ND, et al. Chemoprophylactic antimicrobial activity of gallium maltolate against intracellular *Rhodococcus equi*. J Equine Vet Sci 2007;27:341–345.

80. Martens RJ, Mealey K, Cohen ND, et al. Pharmacokinetics of gallium maltolate after intragastric administration in neonatal foals. Am J Vet Res 2007;68:1041–1044.

81. Martens RJ, Harrington JR, Cohen ND, et al. Gallium therapy: A novel metal-based antimicrobial strategy for potential control of *Rhodococcus equi* foal pneumonia. Proc Am Assoc Equine Pract 2006;52:219–221.

82. Chaffin MK, Cohen ND, Martens RJ, et al. Evaluation of the efficacy of gallium maltolate for chemoprophylaxis against pneumonia caused by *Rhodococcus equi* infection in foals. Am J Vet Res 2011;72:945–957.

83. Prescott JF, Nicholson VM, Patterson MC, et al. Use of *Rhodococcus equi* virulence-associated protein for immunization of foals against *R. equi* pneumonia. Am J Vet Res 1997;58:356–359.

84. Hooper-McGrevy KE, Wilkie BN, Prescott JF. Virulence-associated protein-specific serum immunoglobulin G-isotype expression in young foals protected against *Rhodococcus equi* pneumonia by oral immunization with virulent *R. equi*. Vaccine 2005;23:5760–5767.

85. Hooper-McGrevy KE, Giguère S, Wilkie BN, et al. Evaluation of equine immunoglobulin specific for *Rhodococcus equi* virulence-associated proteins A and C for use in protecting foals against *Rhodococcus equi*-induced pneumonia. Am J Vet Res 2001;62:1307–1313.

86. Cohen ND, Smith KE, Ficht TA, et al. Epidemiologic study of results of pulse-field gel electrophoresis of isolates of *Rhodococcus equi* obtained from horses and horse farms. Am J Vet Res 2003;64:153–161.

87. Chaffin MK, Martins RJ, Martens JG. Therapeutic effects of immune plasma in foals with *Rhodococcus equi* pneumonia. Equine Vet J 1991;12(suppl):23–29.

88. Horowitz ML, Cohen ND, Takai S, et al. Application of Sartwell's model (lognormal distribution of incubation periods) to age at onset and age at death of foals with *Rhodococcus equi* pneumonia as evidence of perinatal infection. J Vet Intern Med 2001;15:171–175.

89. Jacks S, Giguère S, Crawford PC, et al. Experimental infection of neonatal foals with *Rhodococcus equi* triggers adult-like gamma interferon induction. Clin Vaccine Immunol 2007;14:669–677.

90. Womble AY, Giguère S, Lee EA, et al. Pharmacokinetics of clarithromycin and concentrations in body fluids and bronchoalveolar cells of foals. Am J Vet Res 2006;67:1681–1686.

91. Madigan JE, Hietala S, Muller N. Protection against naturally acquired *Rhodococcus equi* pneumonia in foals by administration of hyperimmune plasma. J Reprod Fertil Suppl 1991;44:571–578.

92. Higuchi T, Arakawa T, Hashikura S, et al. Effect of prophylactic administration of hyperimmune plasma to prevent *Rhodococcus equi* infection on foals from endemically affected farms. Zentralbl Veterinarmed [B] 1999;46:641–648.

93. Giguère S, Gaskin JM, Miller C, et al. Evaluation of a commercially available hyperimmune plasma product for prevention of naturally acquired pneumonia caused by *Rhodococcus equi* in foals. J Am Vet Med Assoc 2002;220:59–63.

94. Hurley JR, Begg AP. Failure of hyperimmune plasma to prevent pneumonia caused by *Rhodococcus equi* in foals. Aust Vet J 1995;72:418–420.