Survival of Foals with Experimentally Induced *Rhodococcus equi* Infection Given Either Hyperimmune Plasma Containing *R. equi* Antibody or Normal Equine Plasma*

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ABSTRACT

The purpose of this study was to determine if colostrum-deprived foals with experimentally induced *Rhodococcus equi* pneumonia have a decreased severity of the disease and decreased mortality rate when given hyperimmune (HI) *R. equi* antibody plasma (*R. equi* titer at least 100% and virulence-associated protein A [VapA] at least 10,000) prophylactically versus when given normal equine plasma (*R. equi* titer less than 20% and VapA less than 160). Sixteen colostrum-deprived foals (*R. equi* titer less than 5%) each received normal equine plasma in the first 24 hours of life (*R. equi* titer less than 20%). At 14 days of age, six foals were given normal equine plasma and 10 foals were given HI plasma. All foals were subsequently infected intrabronchially with a pathogenic strain of *R. equi* (2.5 × 10⁸ organisms) at 21 days of age. Repeated physical examinations, weight measurements, complete blood cell counts, fibrinogen measurements, and thoracic radiographs (ventrodorsal and lateral) were performed to help determine the severity of the disease. Foals given HI plasma had significantly higher *R. equi* ELISA titers (42.4%) than those given normal plasma (20.9%) on the day of experimental infection. Mortality rates and severity of disease were statistically similar (*P > .05) for the groups. Although none of the foals was treated with antibiotics, several with severe *R. equi* pneumonia recovered. Either HI or normal equine plasma administered to foals in the first few weeks of life...
caused no adverse effects and may be protective against *R. equi*, although the exact constituent responsible for protection is undetermined and requires further investigation.

## INTRODUCTION

*Rhodococcus equi* causes severe pneumonia along with various extrapulmonary disorders, such as enterocolitis, abdominal abscessation, immune-mediated synovitis, septic arthritis and physis, vertebral body osteomyelitis, mediastinal abscess, cellulitis, and subcutaneous abscess in foals 3 weeks to 5 months of age.1–10 High foal morbidity and mortality rates are accompanied by significant financial losses on breeding farms where the disease becomes endemic.11,12 Hyperimmune (HI) equine plasma containing *R. equi* antibodies given prophylactically to foals is protective against both naturally occurring *R. equi* pneumonia and experimentally induced disease,13–18 with few conflicting reports.19,20

The purpose of the present study was to determine if colostrum-deprived foals with experimentally induced rhodococcal pneumonia have a decreased severity of the disease and decreased incidence of mortality when HI plasma is given prophylactically compared with administration of normal equine plasma. This study was unique in that all foals were colostrum-deprived and received normal equine plasma with a low *R. equi* antibody on the first day of life, followed by either HI plasma or normal equine plasma at 14 days of age.

## MATERIALS AND METHODS

The experimental design is summarized in Figure 1.

### Animals

Sixteen newborn pony foals born between May 2 and July 1, 1999 were separated from their dams at birth and given normal equine plasma intravenously (Foal Immune, Lake Immunogenics, Inc., Ontario, NY) at 15 ml/kg. The plasma has an *R. equi* titer less than 20% and virulence-associated protein A [VapA] less than 16021,22 as a substitute for colostral antibodies. The normal equine plasma was collected from one horse that was not vaccinated for *R. equi* and had a low titer (26%). Normal equine plasma was administered to standardize *Rhodococcus*-specific IgG (albeit at a low titer) in all foals and to prevent septicemia from other causes. The foals were prevented from nursing the mare for 24 hours by a physical barrier and were tube fed 300 ml of milk replacer at 0, 6, 12, and 18 hours. The mares were not milked out during this time. At 24 hours of age, when the foal’s intestinal tract would no longer absorb immunoglobulins in significant amounts,23,24 the foal and mare were reunited and kept in a box stall at night and turned out on pasture during the day (weather permitting) for 2 weeks. The study was approved by the Institutional Animal Care and Use Committee.

Blood was collected from the foals on the day of birth before the normal plasma was administered and then again at 24 hours of age for determination of *R. equi* ELISA titers. Before the foals were experimentally infected, blood was drawn for an *R. equi* ELISA to determine the amount of circulating *R. equi* antibodies in each foal. IgG determinations at 24 and 48 hours of age were greater than 800 mg/dl for all foals, indicating adequate IgG levels for protection against septicemia and no significant absorption of colostral (and therefore *R. equi*) antibodies after 24 hours (Table 1).

None of the foals was treated for *R. equi* during the study after challenge. Two foals were treated for conjunctivitis with neomycin, bacitracin, and polymyxin ophthalmic ointment and the condition was resolved within 2 to 3 days. All foals were dewormed with ivermectin (200 µg/kg orally) at 5 weeks of age.
Normal and Hyperimmune Plasma Treatments

Foals were separated into two groups, using a table of random numbers. At 14 days of age, one group (n = 6) was assigned to receive normal equine plasma (15 ml/kg) from a horse not vaccinated with \( R. \) equi and having a low titer (\( R. \) equi titer 26%, VapA less than 160).\(^{21,22}\) This was the lowest titer that could be found among a group of nonvaccinated donor horses. The plasma was collected aseptically by machine plasmapheresis and frozen immediately. Samples were cultured for fungi and aerobic and anaerobic bacteria. All samples were stored at \(-28.9^\circ C\).

The second group of foals (n = 10) was treated with HI plasma (15 ml/kg). The HI plasma was produced from a single \( R. \) equi-immunized universal donor containing \( R. \) equi antibodies (Rhodococcus equi Antibody, Lake Immunogenics, Inc., Ontario, NY) (titer at least 100%, VapA at least 10,000).\(^{21,22}\) The donor was injected repeatedly subcutaneously with an acellular suspension of antigens derived from an isolate of \( R. \) equi collected from a foal with rhodococcal pneumonia. The HI plasma was collected and treated in the same manner as for the normal equine plasma.

Challenge

A suspension of pathogenic \( R. \) equi was prepared from ATCC 33701 capsular type 1 \( R. \) equi. The microorganisms were grown on trypticase soy agar for 2 days, then washed with phosphate buffered saline (PBS) to prepare individual aliquots for the inoculation of each
foal. These aliquots were stored frozen at –56.6˚C in a special freezing medium. Before inoculation of the foals, an aliquot was thawed and 50 µl of each of five dilutions (i.e., $10^{-2}$, $10^{-3}$, $10^{-4}$, $10^{-5}$, $10^{-6}$) were cultured on five blood agar plates and incubated at 370˚C for 48 hours. The colonies on each plate of each dilution were then counted and an average for each dilution was determined. The average number of colonies for each dilution was first multiplied by 20 to determine the number per ml and then was multiplied by the dilution factor to determine a number of viable bacteria/ml at that particular dilution. The bacterial counts for each dilution were averaged to determine the concentration of bacteria in the bronchiolar inoculum. The inoculum was diluted with saline to provide a 50-ml dose ($5.0 \times 10^6$ organisms/ml).

At 21 days of age, each foal was sedated with xylazine (2 mg/kg) and butorphanol (0.1 mg/kg). With the foal standing, an endoscope was passed nasally approximately 1.5 cm distal to the bifurcation of the trachea. A syringe containing the challenge inoculum was attached directly to the biopsy channel of the endoscope, and 25 ml of the *R. equi* suspension was injected. The channel was flushed with 25 ml of air and the remaining 25 ml of the inoculum was similarly injected into the other main bronchus. The endoscope was cleaned and disinfected between uses with 0.2% chlorhexidine solution.

**Evaluations**

Physical examinations and weight measurements were performed daily. Temperature, pulse, and respiratory rates were recorded twice daily, in the morning and afternoon. On Mondays, Wednesdays, and Fridays, blood was collected from each foal for a complete blood cell count, hematocrit, total protein, and fibrinogen levels. Because foals were born on different days, there was slight (1 to 3 days) variation in the schedule relative to the day of treatment and challenge.

Chest radiographs (lateral and ventrodorsal views) also were performed for each foal on Mondays, Wednesdays, and Fridays. To accomplish this radiographic procedure, xylazine (2 mg/kg) and butorphanol (0.10 mg/kg) were administered to each foal to establish recumbency. A board-certified radiologist who was blinded to treatment group assignments scored the radiographs. Lateral radiographs had two radiographic patterns: bronchointerstitial, where 0 = normal, 1 = 1% to 20%, 2 = 21% to 75%, and 3 = 76% to 100% of the lung affected and alveolar and/or abscession, where 0 = normal, 1 = 1% to 20%, 2 = 21% to 50%, and 3 = more than 50% of the lung affected.

**TABLE 1. *Rhodococcus equi* ELISA Titers in Colostrum-Deprived Foals Challenged with *R. equi* 1 Week After Treatment with Either Normal Equine Plasma or Hyperimmune *R. equi* Antibody Plasma at 2 Weeks of Age**

<table>
<thead>
<tr>
<th>Sampling Time</th>
<th>Median ELISA Value (%)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Normal Equine Plasma</td>
</tr>
<tr>
<td>1st day, before normal plasma</td>
<td>4.0</td>
</tr>
<tr>
<td>1st day, after normal plasma</td>
<td>19.9</td>
</tr>
<tr>
<td>21 days of age, when challenged</td>
<td>20.9*</td>
</tr>
</tbody>
</table>

*ELISA values were significantly different between the groups ($P < .05$).
The extent of pulmonary disease was rated normal (0), unilateral (1), or bilateral (2) after examination of the ventrodorsal radiographs. A total radiographic score was the sum of the bronchointerstitial, alveolar, and/or abscessation and ventrodorsal scores.

For foals that survived to the end of the study, the parameters listed above were measured for 48 to 102 days (mean 73.5 days) after infection. Foals that died during the study had complete clinical evaluations performed until the time of death (mean = 23 days, range = 15 to 32 days after infection).

If body temperature exceeded 38.8°C, heart rate 100 beats per minute, respiratory rate 60 breaths per minute, white blood cell count 12,000/µl, total protein 6.0 g/dl, hematocrit 40%, fibrinogen 400 mg/dl, bronchial score 2, alveolar score 2, and total radiographic score 2, these variables were considered elevated. In the hospital conducting the study, these values are used as clinical indicators of inflammation and/or infection for foals less than 3 months of age. The day any of these variables became elevated and the duration of the elevated value were recorded for each foal. Weight gain was determined by subtracting the original weight on the day of infectious challenge from the weight on approximately Day 42 (Day 41 to 43).

The criterion for euthanasia required that three of the following conditions be present: body temperature 39.4°C or higher for 5 consecutive days; respiratory rate more than 100 breaths/min for 24 hours; inability or unwillingness to rise when prompted; radiographic evidence of severe pneumonia with more than 50% of the lung involved; weight loss for 5 consecutive days; and a hematocrit value greater than 50%. Some foals died before the need for euthanasia could be determined using these criteria, even with close observation of foals by experienced handlers four times daily. All foals that died were examined postmortem, including histopathology and cultures of four areas of the lungs (both the right and left cranial and caudal lung lobes) and the mediastinal lymph node.

ELISA Technique

The sandwich-ELISA technique described by Hietala and coworkers21 was modified (California Animal Health and Food Safety Laboratory System, Davis, CA) to detect antibodies to R. equi in the serum of horses. Plates coated with R. equi antigen were used as an indirect ELISA. The R. equi antigen was prepared by streaking a blood agar plate with an R. equi isolate and incubating at 37°C for 48 hours. A positively identified individual colony of R. equi then was cross-streaked onto Mueller-Hinton agar and incubated at 37°C for 48 hours. The R. equi was then harvested by washing each plate with PBS and suspending the colonies. The suspensions were shaken at 120 rpm in a water bath (42°C) for 2 hours and then centrifuged for 15 minutes at 2000 rpm. The supernatant was harvested and centrifuged a second time, harvested again, and an aliquot collected and stored at –20°C until needed. The antigen was thawed and diluted in coating buffer (sodium carbonate, sodium bicarbonate; pH 9.6), and 100 µl of diluted antigen was added to each well of a 96-well plate with the exception of wells A-1 thru A-3. The dilution factor was determined by checkerboard titration, and each test was optimized.25 The plates were incubated overnight at room temperature. The contents of the R. equi-coated ELISA plates were discarded and washed five times with ELISA wash buffer (sodium chloride, potassium chloride, disodium phosphate, potassium phosphoric acid, Tween® 20; pH 7.4).

Control sera were tested by methods previously described21 and consisted of a negative (serum from foals that had not ingested
colostrum) and a strong positive (serum from the donor horse from which the HI plasma was harvested) sample. The controls and test sera were tested in triplicate. Only conjugate and substrate were placed in three wells, and positive control sera without antigen were placed in three wells. Test and control serum were diluted to 1:2000 with buffer 3 (0.15 M sodium chloride, 1 mM sodium EDTA, 0.05 M trishydrochloric acid, 0.1% bovine serum albumin, 0.05% Tween® 20; pH 7.4), and 100 µl of diluted serum samples and controls were added to the appropriate wells. Buffer 3 was also added to the three wells that did not contain serum. The plates were incubated for 30 minutes at 37°C. Horseradish peroxidase (HRP)-conjugated antihorse IgG was diluted with buffer 3 to a concentration determined by checkerboard titration. One hundred µl of the HRP conjugate was added to each well and incubated for 30 minutes at 37°C. The plates were washed five times with ELISA wash buffer and three times with distilled water, and 100 µl of the substrate 3, 3', 5, 5' tetramethylbenzidine was added to each well and incubated at room temperature for 20 minutes. The reaction was stopped by adding 100 µl of 4 N sulfuric acid. The optical density (OD) was read immediately with the ELISA automated plate reader set to a wavelength of 450 nm. The results were reported as a percent of the positive control. For the assay to be valid, the negative control average had to have an OD less than 0.200, the strong-positive control average had to have an OD of at least 1.8, and the average strong-positive control OD divided by the average negative control OD had to be greater than eight.

Statistical Analysis

Analyses were performed for data generated from the day of challenge to 48 days after challenge because all foals that survived had observations until that time. The 95% confidence intervals (CI) were computed for mortality data. A commercial statistical analytical program (Statistix for Windows; Analytical Software, Tallahassee, FL) was used for all other analyses. Two-tailed Wilcoxon’s rank-sum tests were performed to determine if the groups differed on the day before or the day of challenge with respect to body temperature, respiratory rate, heart rate, weight, white blood cell count, total protein, fibrinogen, hematocrit, ELISA, and bronchial and alveolar radiographic scores as well as to determine if the severity of disease differed. Severity of the disease was determined by the time to the onset of an increase; duration of the increase; and the highest value for temperature, pulse, respiratory rate, white blood cell count, total protein, fibrinogen, hematocrit, and bronchial and alveolar radiographic score; and weight gain. Differences were declared significant when \( P \leq .05 \).

Wilcoxon’s signed-rank test was used to confirm whether all foals had higher ELISA titers after receiving normal equine plasma as a colostrum replacement. Tests were two-sided at 1 and 14 days of age and one-sided thereafter. The justification for this change in procedure was that groups were expected to be equivalent before treatment; however, after intervention, the hypothesis being tested was that HI plasma conferred a benefit over normal equine plasma.

RESULTS

The ELISA titers of foals in the first day of life before the transfusion of normal plasma and again at 24 hours of age after administration of normal plasma are reported in Table 1. There was no statistical difference between the ELISA titers of the foals in either group before or after the normal equine plasma administration during the first 24 hours of life (\( P = .8 \)). Also, titers were higher in all foals after receiving normal plasma on Day 1 (\( P \leq .0001 \)).
ELISA titers were significantly higher ($P = .0005$) in the foals that received HI plasma (43%) than the foals given normal equine plasma (21%) on the day of experimental challenge (Table 1). There were no other significant differences ($P \geq .10$) detected between the two groups of foals on the day they were experimentally infected.

The mortality rate (33%) was not significantly different ($P = 1.0$) between foals given normal equine plasma and those given hyperimmune $R.\ equi$ plasma (30%). Foal 3 fit the criteria for euthanasia at Day 25 and was considered a nonsurvivor. Four other foals died between 15 and 32 days after infection (mean = 23 days). The postmortem examination of all foals revealed multiple small abscesses throughout all lung fields or a single large pulmonary abscess and enlarged mediastinal lymph nodes. No other gross findings were noted in postmortem examinations.

Histopathologic findings included a mediastinal lymphadenitis as well as subacute and severe bronchopneumonia with an inflammatory response consisting of neutrophils, histiocytes, and fibrin. One foal had multinucleated giant cells. Streptococcal organisms in chains were seen intracellularly in all foals necropsied, although these organisms were not isolated. In all instances, cultures of the right and left cranial and caudal lung lobes as well as the mediastinal lymph nodes grew moderate to many $R.\ equi$ organisms. All foals had congestion of the liver with centrilobular degeneration.

Nearly 70% of the foals (11 of 16) survived without any treatments, made a clinical recovery, and were normal and healthy as yearlings. All of these 11 survivors had been considered severely affected based on their physical examination, hematology findings, and radiographic data within 48 days after challenge. Thoracic radiographs of a foal in the group that received HI plasma demonstrated the severe radiographic changes (20 days after infection) followed by improvement in radiographic changes 56 days after infection (Figures 2A to 2D).

There were no significant differences ($P > .07$) in the time to event, duration of event, or maximum value of body temperature, weight gain, hematocrit, total protein, white blood cell count, fibrinogen, bronchial interstitial score, alveolar and/or abscessation score, or in total radiographic scores (Table 2).

**DISCUSSION**

Hyperimmune $R.\ equi$ plasma has been shown to be the best therapeutic intervention for $R.\ equi$ pneumonia by decreasing the incidence and severity of both natural and experimental infections. The intent of the present study was to determine whether HI plasma was more efficacious than normal plasma in protecting against $R.\ equi$ pneumonia. To further explore this potential efficacy, a negative control group, either another group of foals given saline or a group for which plasma was not administered at 14 days of age, would have been necessary. Results suggest that $R.\ equi$-specific antibodies are not solely responsible for the protection that HI plasma provides. Other protective constituents of equine plasma might be fibronectin, interferon, complement factors, cytokines, and other proteins, several of which aid in immunologic defense and provide some protection. This might explain why administration of HI plasma is beneficial in reducing the incidence of $R.\ equi$ pneumonia, whereas vaccination of mares and provision of high-antibody colostrum is not. In contrast, one recent study showed that foals given HI plasma or purified equine immunoglobulin specific for VapA and VapC had less severe pulmonary lesions and had fewer $R.\ equi$ organisms cultured from the lungs than those given saline, supporting a protective effect of $R.\ equi$-specif-
Figure 2A. Lateral thoracic radiograph 20 days after challenge with *R. equi* showing severe pulmonary infiltrates. The radiographic score was 2 for both bronchial interstitial and alveolar/abscessation changes.

Figure 2B. Ventrodorsal thoracic radiograph 20 days after challenge with *R. equi*. The radiographic score was 2.

Figure 2C. Lateral thoracic radiograph 56 days after challenge with *R. equi*. The radiographic score was 1 for a bronchial interstitial score and 0 for alveolar/abscessation changes.

Figure 2D. Ventrodorsal thoracic radiograph 56 days after challenge with *R. equi*. The radiographic score was 0.

Figure 2. Radiographs taken 20 (A and B) and 56 (C and D) days after challenge for a surviving foal treated with hyperimmune plasma containing *Rhodococcus equi* antibodies and challenged with *R. equi* 7 days later.
TABLE 2. Physical, Hematologic, and Radiographic Variables for Foals Challenged with *Rhodococcus equi* 1 Week After Treatment with Either Normal Equine Plasma or Hyperimmune *R. equi* Antibody Plasma

<table>
<thead>
<tr>
<th>Variable</th>
<th>Normal Equine Plasma</th>
<th>Hyperimmune R. equi Antibody Plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of Foals</td>
<td>Median</td>
</tr>
<tr>
<td><strong>Physical Examination</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart Rate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time to reach 100 beats/min (days)</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>Days rate was ≥100 beats/min</td>
<td>6</td>
<td>20</td>
</tr>
<tr>
<td>Maximum value (beats/min)</td>
<td>6</td>
<td>126</td>
</tr>
<tr>
<td>Respiratory Rate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time to reach 60 breaths/min (days)</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Days rate was ≥60 breaths/min</td>
<td>6</td>
<td>25</td>
</tr>
<tr>
<td>Maximum value (breaths/min)</td>
<td>6</td>
<td>111</td>
</tr>
<tr>
<td>Body Temperature</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time to reach 38.9°C (days)</td>
<td>6</td>
<td>12</td>
</tr>
<tr>
<td>Days temperature was ≥38.9°C</td>
<td>6</td>
<td>13</td>
</tr>
<tr>
<td>Maximum value (°C)</td>
<td>6</td>
<td>40.2</td>
</tr>
<tr>
<td>Weight gain (kg)</td>
<td>4</td>
<td>21</td>
</tr>
<tr>
<td><strong>Hematology</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hematocrit</td>
<td>4</td>
<td>16</td>
</tr>
<tr>
<td>Days value was ≥40%</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Maximum value (%)</td>
<td>4</td>
<td>44</td>
</tr>
<tr>
<td>Total protein</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>Days value was ≥6.0 g/dl</td>
<td>6</td>
<td>26</td>
</tr>
<tr>
<td>Maximum value (g/dl)</td>
<td>6</td>
<td>7.1</td>
</tr>
<tr>
<td>White blood cell count</td>
<td>5</td>
<td>14</td>
</tr>
<tr>
<td>Time to reach ≥12 × 10³/µl</td>
<td>5</td>
<td>24</td>
</tr>
<tr>
<td>Days value was ≥12 × 10³/µl</td>
<td>5</td>
<td>34.6</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>6</td>
<td>10</td>
</tr>
<tr>
<td>Time to reach ≥400 mg/dl</td>
<td>6</td>
<td>21</td>
</tr>
<tr>
<td>Days value was ≥400 mg/dl</td>
<td>6</td>
<td>950</td>
</tr>
<tr>
<td><strong>Radiography</strong></td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Bronchial score</td>
<td>6</td>
<td>40</td>
</tr>
<tr>
<td>Days score was ≥2</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>Alveolar/abscessation score</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time to reach score ≥2 (days)</td>
<td>6</td>
<td>14</td>
</tr>
<tr>
<td>Days score was ≥2</td>
<td>6</td>
<td>18</td>
</tr>
<tr>
<td>Maximum score</td>
<td>6</td>
<td>2</td>
</tr>
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</table>

NA = not applicable (no horses in the group treated with *R. equi* plasma developed a hematocrit value above 40%).
ic VapA and VapC antibodies. The disparity between results of the earlier study, indicating that specific antibodies may be important, and the present study, suggesting they may not, would most likely be related to the period that the foals were evaluated. Strict euthanasia criteria were followed in our study, and despite the foals becoming severely affected, many survived, whereas the foals in the earlier study were euthanized 14 days after infection.

Specific \textit{R. equi} antibody might contribute to immunity of the disease by blocking the initial stages of cellular infection, altering the route by which bacteria enter the macrophage, and/or decreasing the bacterium's ability to inhibit phagosome-lysosomal fusion. VapA appears to be important in development of immunity to \textit{R. equi} and stimulates an effective Th1-based immune response. Furthermore, plasma rich in VapA antibodies prevented disease due to \textit{R. equi} in immunocompromised rats. The present study is the first to compare the disease in foals having low \textit{R. equi} antibody titers (treated with normal equine plasma) with the disease in foals having high VapA and ELISA titers for \textit{R. equi} antibodies (treated with HI plasma). There were no differences between the groups in severity of the disease or survival rates.

The 70% survival rate for foals in this study suggests that plasma (either normal or HI) has some protective effect and is useful not only for the prevention of \textit{R. equi} pneumonia but also for improving the prognosis for recovery in foals that develop \textit{R. equi} infection. It is recognized that a saline control group with a higher mortality rate might have provided more information. The lack of differences between foals given normal equine plasma and those treated with HI \textit{R. equi} plasma suggests two possibilities: \textit{R. equi}-specific and VapA antibodies play an insignificant role in the protection against \textit{R. equi} pneumonia as compared with other factors in equine plasma, or there is a protective effect from the very small amount of \textit{R. equi} and VapA antibody in the normal equine plasma.

Mares vaccinated with various \textit{R. equi} antigens have not been provided increased protection against natural or experimental \textit{R. equi} infections. All foals were colostrum-deprived in the present study; therefore, we cannot comment on the role of maternally derived colostral antibodies as protectors against \textit{R. equi}. However, many foals survived without antibiotic therapy and all foals were colostrum-deprived, despite having received normal equine plasma on the first day of life and achieving IgG levels above 800 mg/dl. The effects of the normal equine plasma given on the first day of life on the survival of foals in this study are unknown because there was no group for this comparison. It may be that collostral antibodies to \textit{R. equi} have some protective value but not enough to determine in small field trials. The contribution of collostral antibodies plus plasma with \textit{R. equi} antibodies (HI plasma) and other nonspecific opsonins might provide additive protection against \textit{R. equi}.

It was surprising that 70% of the foals in this study survived without any medical intervention. A similar finding was noted in experimentally infected foals that received \textit{R. equi} plasma. The plasma-treated foals in that study developed moderate-to-severe clinical signs of \textit{R. equi}, yet 100% survived until 3 months of age, when they were euthanized, and no significant \textit{R. equi} pathology was noted on postmortem examination. It is difficult to compare the survival percentages of the saline-treated control foals and the plasma-treated foals in that previous study because most of the control foals were euthanized 3 months post-infection. One severely ill control foal recovered spontaneously. The survival rates in foals receiving plasma in the earlier study and in the present study were greater.
than that experienced in foals with naturally occurring infections before the widespread use of erythromycin and rifampin for the treatment of rhodococcal pneumonia substituting for the 72%, >80%, 88%) of that seen today might be that experimental infection does not mimic that occurring naturally in the field. Foals chosen for experimental inoculation may be less susceptible to the disease. The means of inducing infection as a one-time inoculum, the quantity and quality of the inoculum, and other environmental factors may play a role in susceptibility. For example, pony foals were used in all of the experimental studies, and ponies might be more resistant to R. equi. To the author’s knowledge, R. equi has not been reported in ponies (although this lack of occurrence might be due to epidemiologic reasons and not necessarily a genetically derived resistance to R. equi). In the field, foals might develop subclinical R. equi infections and clear the organism rapidly; however, foals seen with clinical disease due to R. equi may be inherently more susceptible and less likely to respond or recover without aggressive therapy.

It is unknown exactly when plasma should be administered to foals to protect them against R. equi pneumonia. Recommendations are to give plasma before exposure to R. equi; this recommendation would imply a plasma transfusion in the first 1 to 2 weeks of life. In theory, the decline of passively transferred antibodies to nonprotective levels (especially when foals are born early in the foaling season and the exposure to R. equi is highest during the warm, dry months) supports a second plasma transfusion 25 to 30 days later. In past studies investigating protective effects of HI plasma (1 to 2 L), plasma was administered only once to foals 1 to 60 days of age. The benefits of repeated plasma transfusions had not been investigated until the present study and another recent study performed by Giguère and coworkers. Those authors administered two doses of HI plasma (the first dose during the first week of life, and the second dose between 30 and 50 days), but they did not observe a significant difference between the incidence of R. equi pneumonia in foals that received HI plasma versus those that did not. Many of the foals developed R. equi pneumonia prior to the second dose of HI plasma; therefore, recommendations would be to give HI plasma to foals in the first week of life and then again as early as 14 days or up to 35 days of age. However, the optimal time for the second dose may vary from farm to farm.

In addition to the need to determine the optimal timing of administering plasma, the minimum dose of plasma needed to prevent R. equi pneumonia is unknown. Commercially available plasma products are packaged in 500- and 950-ml bags; therefore, research has focused on treating foals with doses that provide 25 to 35 ml/kg. Because the component(s) of the plasma that imparts protection to the foal has not been isolated, a true and meaningful dose cannot be recommended at this time. The recommendation of the authors, however, is to transfuse an entire bag (950 ml) to a foal that is at risk of developing disease caused by R. equi.

### CONCLUSIONS

Until an effective vaccine is available for R. equi, practitioners will continue to use early detection of R. equi cases, farm management, and immune prophylaxis to control and prevent disease caused by R. equi. Because HI plasma and/or normal plasma is safe to administer and may be protective against mortality from R. equi, either HI plasma or normal plasma should be administered to foals at risk. However, additional studies are needed to identify the protective component(s) in the plasma.
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