

Effect of Hyperimmune Plasma on the Severity of Pneumonia Caused by *Rhodococcus equi* in Experimentally Infected Foals

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CLINICAL RELEVANCE

This study evaluated the prophylactic effectiveness of hyperimmune plasma (HIP) as an aid in the prevention of pneumonia caused by experimental infection with *Rhodococcus equi*. Thirty neonatal foals were administered *R. equi* HIP or saline at 2 days of age and were infected with virulent *R. equi* at 7 days. All foals developed signs or symptoms of respiratory disease. Radiographic scores on day 28 and neutrophil concentrations on day 49 were significantly greater in control foals, and time to respiratory effort score of 2 or higher was significantly shorter for control foals. Three foals, all in the principal group, died or were euthanized before the end of the study, but there was no significant difference in mortality between groups. VapA titers were significantly greater in principal foals. Administration of *R. equi* HIP decreased the severity of radiographic lesions and prolonged time to increased respiratory effort due to *R. equi*-induced pneumonia.

INTRODUCTION

Rhodococcus equi is a cause of severe pyogranulomatous pneumonia in foals and can result in high morbidity and mortality.¹⁻³ This

gram-positive facultative intracellular bacterium is a saprophytic inhabitant of soil and is present worldwide. Epidemiologic evidence indicates that most foals with spontaneous dis-

ease become infected as neonates.⁴ Clinical signs of disease, however, are not generally apparent until the foals are 1 to 2 months of age, and adults and foals older than 6 months are resistant to infection.^{1,4-6} The disease has an insidious onset, making early diagnosis difficult. The prevalence, high mortality rates, and prolonged, expensive treatment have a significant negative economic effect on breeding farms.^{3,7} Additionally, *R. equi* pneumonia may be detrimental to a foal's future athletic endeavors.⁸

R. equi isolates that cause disease in foals contain an 85- to 90-kilobase plasmid, which contains genes that encode the production of several virulence-associated protein antigens (i.e.,

ited range of isotypes until around 2 months of age.¹⁶ The role of antibody in preventing the development of rhodococcal pneumonia has also been documented by the protective effects seen with prophylactic administration of plasma harvested from horses hyperimmunized with *R. equi*.¹⁷⁻²⁰ Additionally, the work by Hooper-McGrevy and associates¹¹ suggests that immunoglobulin is the primary component of plasma that provides protection and that antibodies against VapA and VapC are protective against *R. equi* pneumonia.

Despite the expense and labor-intensive nature of plasma administration, it is the only prophylactic modality currently available proven to

Our findings underscore the limited usefulness of routine hematology as a screening aid for *R. equi* infection.

VapA and VapC–VapH).^{9,10} Although VapA is the most thoroughly studied of these virulence markers, its exact function is not known. Experimentally, VapA-specific antibody has been associated with protection of foals against infection with *R. equi*.¹¹

Development of immunity to *R. equi* seems to be dependent on humoral and cell-mediated responses.⁶ The important role of immunity in the development of this disease is demonstrated by the susceptibility of immunodeficient mice to *R. equi* pneumonia while immunocompetent mice clear infections within 21 days after respiratory challenge in experimental models.^{12,13} In addition, the majority of human patients infected with *R. equi* are immunocompromised.^{3,14}

Antibody plays a role in immunity to *R. equi*. Experimental evidence suggests that helper T type 1 (Th1) responses mediate immunity to *R. equi* in foals. These responses in part enhance production of opsonizing antibody isotypes.¹⁵ Foals appear to produce a lim-

prevent *R. equi* pneumonia or significantly reduce its severity.¹⁷⁻²² Treating infected foals with hyperimmune plasma (HIP) after infection has not been shown to be effective.²¹ Foals that received colostrum from mares vaccinated against *R. equi* during pregnancy were not protected from *R. equi* pneumonia in one study.²² In another study, however, foals from mares vaccinated with either killed *R. equi* or VapA protein antigen had increased anti-*R. equi* IgG levels and increased opsonization of the bacteria by polymorphonuclear leukocytes.²³ Even though there is evidence that HIP may be the best prophylactic measure available for preventing *R. equi* pneumonia, there also have been studies in which there was no difference between treated and nontreated foals.^{24,25} These results warrant further investigation of HIP and its ability to prevent pneumonia or reduce the severity of pneumonia caused by *R. equi* in foals.

This study is distinctive in that it is a blind, controlled design using an experimental

infection model. Our hypothesis was that the administration of *R. equi* HIP to foals before experimental infection would decrease the incidence and severity of pneumonia caused by *R. equi*.

■ MATERIALS AND METHODS

Animals

Thirty neonatal foals were used for this study: 14 Quarter Horses, eight draft–Quarter Horse cross or draft–American Paint Horse cross foals, four American Paint Horses, three Appaloosas, and one Mustang. Their average weight on day 7 was 63.1 kg (range, 35.9 to 90.5 kg). At birth, each foal was sequentially assigned a number and identified with a neck tag. A randomization table generated using statistical software (S-PLUS, version 6.2, Insightful Corporation, Seattle, WA) was used to assign foals to the principal (plasma; $n = 15$) or con-

Hyperimmune Plasma and Saline

On day 2 of life (at least 24 hours after birth), after a physical examination had been completed and blood samples collected, a 14-gauge catheter was placed in each foal's jugular vein. Each foal was administered 1 L of either commercial *R. equi* HIP (*Rhodococcus equi* Antibody, Mg Biologics, Ames, IA) or 0.9% saline solution based on the randomly assigned treatment group. Plasma or saline administration was performed either by an author (S. R. M.) or a veterinary technician, neither of whom participated in assessment of health parameters. The assessor of clinical signs (S. S. C.) remained blinded to the treatment status of foals until the completion of all observations.

Bacterial Preparation

Rhodococcus equi (American Type Culture Collection [ATCC] 33701+) was provided by

The prevalence, high mortality rates, and expensive treatment associated with R. equi pneumonia have a negative economic effect on breeding farms.

trol (saline; $n = 15$) group. Until foaling, mares were kept on pasture at a farm where horses had not previously been housed. Mares and foals were examined shortly after birth to ensure that the foals stood and nursed, and their navels were dipped in dilute 1:40 chlorhexidine (day 1). Mares and foals were kept on pasture until experimentally infected with *R. equi* on day 7 of life, after which they were housed at a different farm. After being infected, mares and foals were housed in a dry lot with free access to water, grass and alfalfa mixed hay, and a three-sided shelter. They were housed at this farm for the duration of the study. The study was approved by the Iowa State University Animal Care and Use Committee.

one of the coauthors (R. J. M.), and presence of the VapA-gene was verified as previously described.²⁶ The seed culture was stored at -80°C , and inoculum was freshly prepared for each day of experimental infection. Approximately one drop of the seed culture was removed, streaked on a 5% bovine blood agar plate, and incubated at 37°C for 48 hours. Plates were examined for contaminant bacteria based on colony morphology; if any was present, the plate was discarded. A sterile cotton-tipped swab was used to transfer five to six colonies of *R. equi* to 20 ml of brain–heart infusion broth with 10% newborn calf serum. This suspension was incubated at 37°C for 2 to 3 hours in a rotary shaking incubator at 220 rpm. The bacterial sus-

pension was adjusted to an optical density of 0.25 at 595 nm using a spectrophotometer, washed twice in 0.9% saline, and adjusted to a concentration of approximately 5×10^7 *R. equi*/ml in 0.9% saline. The suspension was stored on ice until used for experimental infection (within 1 to 2 hours). Following experimental infection, the remaining *R. equi* inoculum was kept on ice and returned to the laboratory to determine the actual dose. The inoculum was diluted serially 10-fold, and aliquots were plated on blood agar plates. The plates were incubated at 37°C for 48 hours, and then colonies were counted to determine the number of colony-forming units/ml.

Experimental Infection

On day 7 of life, the foals were sedated with xylazine (0.5 mg/kg) and anesthetized with ketamine (1 mg/kg) and diazepam (0.05 mg/kg) IV. They were placed in sternal recumbency. A 5-mm diameter endoscope was used to visually confirm placement of a 10-mm outer diameter and 6-mm inner diameter tube into the right mainstem bronchus. The tube was advanced until it lodged and then was retracted approximately 2 cm. A 2-ml suspension of virulent *R. equi* (ATCC 33701) that contained approximately 5×10^7 viable bacteria/ml was used for the experimental infection. The suspension was aerosolized into the lung using a nebulizer with oxygen flow at 20 psi and 5 L/min.

Clinical Observations and Thoracic Radiography

Between 24 and 48 hours after birth (day 2), a physical examination was performed on each foal. From day 2 until the completion of the study, each foal's temperature, heart rate, and respiratory rate were measured daily. In addition, scores for respiratory effort and general appearance were subjectively assigned as follows:

Respiratory Effort Score

- 0 = Normal respiratory effort at rest and during exercise
- 1 = Normal at rest; slight increased effort during exercise
- 2 = Slight increased effort at rest; moderate increased effort during exercise
- 3 = Moderate increased effort at rest; marked increased effort during exercise
- 4 = Marked to severe effort at rest and during exercise

General Appearance Score

- 0 = Normal
- 1 = Slight depression: Responsive; foal moves around without stimulus but is not bright and alert; suckes well; occasionally observed with ears down
- 2 = Moderate depression: Head and ears are down but foal responds to noise or stimulus; stands still without stimulus; suckling is adequate
- 3 = Severe depression: Foal is recumbent but slowly stands in response to stimulus
- 4 = Moribund: Foal is recumbent with little or no response to stimuli

Cough, ocular discharge, and nasal discharge were recorded as being absent or present. Scores were assigned by the same blinded observer (S. S. C.) each day, and monitoring was performed between 7:00 and 9:00 AM each morning.

On days 7, 28, and 49, the foals' body weights were obtained and left and right lateral thoracic radiographs were taken with the foals standing. A 10-cm radiopaque marker was placed on each cassette as a measurement reference. Foals were not included in the study if there was radiographic evidence of pneumonia before experimental infection on day 7. A board-certified radiologist (K. G. M.), who

was blinded to treatment groups, assigned a score of 0 to 4 to each radiograph as follows:

Radiographic Scores

- 0 = Normal
- 1 = Mild patchy structured or unstructured interstitial lung infiltrate
- 2 = Moderate patchy structured or unstructured interstitial lung infiltrate
- 3 = Severe structured interstitial pattern with or without scattered air bronchograms
- 4 = Severe interstitial or alveolar opacity with or without consolidation and with or without emphysematous changes

Clinical Pathology

A commercial IgG-ELISA test (SNAP Foal IgG, IDEXX Laboratories) was used on day 2 of life before plasma or saline administration to determine whether foals received adequate passive antibodies. Foals were not included in the study if their IgG concentration on day 2 was below 400 mg/dl. On days 2, 7, and 49 of life (or the last day of the study if the foal became severely affected and euthanasia was indicated), complete blood counts and fibrinogen concentrations were assessed. Serum was collected on day 2 before plasma or saline was administered and on day 7 before *R. equi* infection; samples were frozen at -20°C and later tested for VapA antibody titers. Serum VapA antibodies were determined by ELISA as previously described.²⁷ VapA antibody titers were also determined on an aliquot from each of four lots of HIP administered to the foals.

Necropsy

On day 49, or sooner if a foal exhibited signs of distress, foals were humanely euthanized with pentobarbital sodium and phenytoin sodium, and a postmortem examination was performed. The examination included gross

examination of all major organ systems, determination of lung weight, and collection of samples for histopathologic assessment, if deemed necessary. Samples were also collected from the lungs for microbiologic culture. Samples taken for culture were collected from the right lung in the area of termination of the mainstem bronchus and from areas in the lung lobes where lesions were present. If no lesions were present, a sample was taken from both the right and left lungs in the area of the mainstem bronchi. A total of two to three samples were taken at each necropsy. The cultures performed were not quantitative. Necropsy scores were assigned to the right, left, and pair of lungs based on gross appearance. The scores assigned were defined as follows based on the subjective estimation of the examiner:

Necropsy Scores

- 0 = No abscesses in lung(s)
- 1 = Less than 10 individual abscesses in the lung(s)
- 2 = Individual to coalescing abscesses in less than 20% of the lung(s)
- 3 = Diffuse abscessation in more than 20% but less than 50% of the lung(s)
- 4 = Severe, diffuse abscessation in more than 50% of the lung(s)

Statistical Analysis

Analyses included descriptive and inferential methods. For descriptive purposes, continuous data were summarized in tables and plots and categorical data in contingency tables. Because some continuous data appeared to have distributions that were non-Gaussian (non-normal), the Wilcoxon rank sum test was used to compare continuous data between the two study groups (principal and control foals).²⁸ For categorical data, the two groups were compared using the chi-square test or, when appropriate, Fisher exact test.²⁸ Because there were differ-

TABLE 1. General Appearance and Respiratory Effort Scores, Clinical Findings, Radiographic Scores, and Necropsy Results for Control and Principal Foals

<i>Parameter</i>	<i>Control Foals</i>	<i>Principal Foals</i>	<i>P value</i>
	<i>Median (range)</i>	<i>Median (range)</i>	
General appearance score			
Maximum	2 (1–3)	1 (0–3)	.2086
Age at maximum (days)	26 (14–49)	26 (10–42)	1
Respiratory effort score			
Maximum	2 (1–3)	1 (0–4)	.1661
Age at maximum (days)	26 (12–47)	27 (10–33)	.5305
Cough			
Proportion with a cough (%)	67	47	.2690
First day of cough (days old)	27 (17–42)	29 (15–43)	.6249
Ocular discharge			
Proportion with discharge (%)	40	20	.2399
First day of discharge (days old)	27 (22–35)	36 (10–46)	.5476
Nasal discharge			
Proportion with discharge (%)	100	100	1
First day of discharge (days old)	14 (7–20)	11 (8–41)	.1446
Body temperature			
Maximum (°F)	102.9 (102.0–104.7)	102.9 (102.0–105.1)	.9246
Age at maximum (days)	21 (2–35)	28 (2–48)	.4289
Radiography score			
Day 7	0 (0–0)	0 (0–0)	1
Day 28	3 (0–4)	2 (0–3)	.0203 ^a
Day 49	2 (0–4)	1 (0–4)	.4563
Necropsy results			
Body weight (kg)	109 (74–151)	95 (50–150)	.1583
Lung weight (kg)	1.95 (1.43–3.87)	1.85 (1.18–3.78)	.5337
Lung weight:body weight ratio	1.6 (1.1–4.5)	1.5 (1.2–5.8)	.8702

^aRepresents a significant difference versus controls ($P < .05$).

ences among foals in the duration of follow-up (because of death or censoring), survival analysis methods were used to compare the distribution of times to first detection of respiratory effort score greater than 2, general appearance score greater than 2, rectal temperature above 102.5°F, cough, nasal discharge, or ocular discharge. If a foal died, it was assumed to have a

positive result for each of these variables (e.g., a foal that died but did not have a respiratory effort score greater than 2 was assigned a score of greater than 2 on the day of death). Kaplan–Meier (product limit) survival curves were generated for the two treatment groups and were compared by the log-rank test.²⁹ Additionally, for variables that were observed on a

daily basis (e.g., rectal temperature), linear mixed-effects models were used to assess differences between the two groups in age-related changes.³⁰ For all analyses, a significance level of $P < .05$ was used. Analyses were performed using S-PLUS (version 6.2) statistical software.

RESULTS

Clinical Observations

There were no significant differences between the principal and control groups in the maximum value or age at which the maximum value was observed for general appearance score, respiratory effort score, and rectal temperature (Table 1). There was no significant difference between groups in the proportion of foals that developed a cough, nasal discharge, or ocular discharge or in the ages at which these clinical abnormalities were first detected. There was a significant ($P = .0195$) difference in the distribution of times to either respiratory effort score of 2 or higher or death (Figure 1): Foals in the control group were observed to have respiratory effort scores of 2 or higher at an earlier age than principal foals. The mean time to a respiratory effort score of 2 or higher was 30.9 days (SE, ± 3.1 days) for control foals and 41.9 days (SE, ± 2.5 days) for principal foals. There were no significant differences in the distributions of times to the specific event (or death) for the first observation of general appearance score of 2 or higher, nasal discharge, ocular discharge, or rectal temperature above 102.5°F.

Using linear mixed-effects models, the general appearance scores for all foals increased significantly ($P < .0001$) over time. General ap-

pearance data scores were higher for control foals than for principal foals, including the number of days that the appearance score was above 1, but the difference was not significant. The respiratory effort score for all foals increased significantly ($P < .0001$) over time, and respiratory effort scores were significantly ($P = .0216$) lower over time for principal foals than controls. The heart rates of foals decreased slightly but significantly ($P < .0001$) with age; however, treatment with HIP did not significantly ($P = .8038$) alter changes in heart rates over time. Respiratory rates decreased slightly but significantly ($P = .0061$) with age; treatment with HIP did not significantly ($P = .6573$) alter changes in respiratory rates over time.

Radiography

All foals had radiographic scores of 0 on day 7. The median radiographic score on day 28

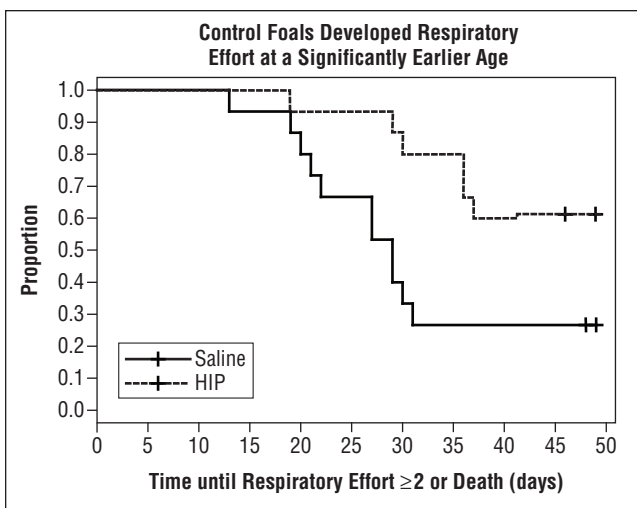


Figure 1. Kaplan-Meier plot of distribution of times to respiratory effort score ≥ 2 (or death) among 15 foals treated with hyperimmune plasma (HIP; dotted line) and 15 foals treated with saline (solid line) before experimental infection via the aerosol route with *Rhodococcus equi*. Cross-hatched marks (+) indicate censored values; proportion refers to the proportion free of either respiratory effort score ≥ 2 or death.

for control foals (median, 3; range, 0 to 4) was significantly ($P = .0203$) greater than that of principal foals (median, 2; range, 0 to 3). Considering these radiographic results as a dichotomous categorical variable (radiographic score above 2 versus a score of 2 or less), the proportion of control foals with scores above 2 (80%; 12 of 15) was significantly ($P = .0092$) greater than that of principal foals (29%; four of 14). One principal foal died or was euthanized before day 28. Assigning a radiographic score of 4 (maximal value) to this foal did not alter the statistical significance of results, re-

Neutrophil Concentration Data

Table 2 shows results of complete blood counts performed during the study. Neutrophil concentrations were significantly ($P = .0178$) higher for control foals (median, 10.7×10^3 cells/ μ l; range, 6.2 to 45.8×10^3 cells/ μ l) than principal foals (median, 7.6×10^3 cells/ μ l; range, 3.3 to 17.9×10^3 cells/ μ l) on day 49 (for the foal that was euthanized on day 39, the neutrophil concentration result for that day was used for day 49 as well). This difference remained significant ($P = .0367$) even when the principal foal that died at 22 days of age was

Foals in the control group were observed to have respiratory effort scores of 2 or higher at an earlier age than principal foals.

ardless of whether radiographic scores were considered as ordinal data or as a dichotomous categorical variable.

Clinical Pathology

Twenty-eight foals had IgG levels of 800 mg/dl or greater. Two foals (one control and one principal) had IgG concentrations between 400 and 800 mg/dl. There was no significant difference in serum VapA antibody titers ($P = .933$) between control (median, 80; range, 20 to 1,280) and principal (median, 80; range, less than 10 to 1,280) foals on day 2. Serum VapA antibody titers of principal foals were significantly ($P < .0001$) higher (median, 2,560; range, 2,560 to 5,120) than control (median, 40; range, 10 to 640) foals on day 7. The mean titer on day 7 was 3,754 for principal foals and 94 for control foals. HIP administered to foals came from four separate lots. The VapA antibody titers of each lot were above 10,240.

assigned a day 49 value that was equal to the maximal neutrophil concentration observed among other principal foals.

Band neutrophil concentrations were significantly ($P = .0393$) higher among principal foals than control foals at day 2 of life; there were no significant differences between groups for day 7 or 49. The proportion of control foals with any bands on day 2 of life (20%; three of 15) was not significantly different ($P = .0604$) than that of principals (60%; nine of 15). The continuous outcome of concentration differed between the two groups; the proportion that had any bands (bands observed = 1; no bands observed = 0) did not differ significantly but was higher among the principals, and the P value was close. Concentrations of lymphocytes, monocytes, basophils, and eosinophils did not differ significantly between control foals and principal foals on days 2, 7, or 49. The erythrocyte concentrations at day 7 were significantly ($P = .0441$) higher for control foals (median, 9.7;

range, 7.8 to 11.2) than for principals (median, 9.0; range, 5.3 to 10.3). Fibrinogen concentrations were significantly ($P = .0333$) higher for principal foals (median, 400; range, 300 to 800) on day 7 than for control foals (median, 300; range, 100 to 800).

Microbiologic Culture

There was no significant difference ($P = .11$) in concentrations of *R. equi* between bacterial suspensions used to infect control (median, 4.3×10^7 ; range, 2×10^7 to 8×10^7) and principal (median, 6.7×10^7 ; range, 3×10^7 to 6.8×10^8) foals, and all cultures were pure *R. equi*. Nine of 15 (60%) lung tissue samples taken at the time of necropsy from control foals grew *R. equi*. Samples from all other control foals (six foals; 40%) had no growth. Five of 15 (33%) lung tissue samples taken at the time of necropsy from principal foals grew *R. equi*. One foal from the principal group had a low growth of an α -hemolytic *Streptococcus*, and the remainder of the principal foals (nine foals; 60%) had no growth. The difference between groups in proportions of foals yielding growth of *R. equi* from lung specimens was not significant ($P = .2723$).

Necropsy

For right lung scores, there was no significant difference ($P = .9151$) between control (median, 2; range, 0 to 3) and principal (median, 1; range, 0 to 4) foals. Likewise for left lung scores, there was no significant difference ($P = .5392$) between control (median, 1; range, 0 to 2) and principal (median, 1; range, 0 to 4) foals. There was also no significant difference ($P = .7823$) between scores for both lungs for control (median, 2; range, 0 to 4) and principal (median, 1; range, 0 to 4) foals. Body weight, lung weight, and lung:body weight ratio results are shown in Table 1. There was no significant difference between groups for those values.

Outcome

One foal died and two foals were euthanized before the end of the study; all three were principal foals. There was no significant difference in mortality between principal and control groups ($P = .2241$). One foal was euthanized at 39 days of age and the other at 48 days. Both had stopped nursing or moving around the lot; they exhibited weight loss, depression, and severe respiratory effort. One foal was found dead at 22 days of age. The two foals that were euthanized were radiographed before euthanasia, and all three foals were weighed and underwent a postmortem examination.

DISCUSSION

The administration of HIP for prevention of *R. equi* pneumonia in foals has been studied in naturally occurring and experimentally induced disease, and some results have been contradictory.^{17-20,24,25,31} Development of the disease and providing protection from infection both involve several factors. Experimental infection was chosen in this study to reduce the variability seen with naturally occurring disease and to provide a more consistent basis to evaluate the *R. equi* HIP product.

All of the foals in our study exhibited at least some signs of disease, although in some foals the signs were mild and resolved rapidly. The general appearance scores of the control foals were worse than those of the principal foals. The degree of respiratory effort was monitored, and the time to respiratory effort score of 2 or higher was significantly shorter for control foals (i.e., control foals developed respiratory effort at a significantly earlier age). Respiratory effort among control foals was also significantly greater over time when analyzed using mixed-effects modeling. Our results are supportive of those in other experimental and clinical trials indicating that administration of HIP prevents or ameliorates the clinical pro-

TABLE 2. Results of Complete Blood Counts Performed on Days 2, 7, and 49

<i>Parameter</i>	<i>Control Foals</i>	<i>Principal Foals</i>	<i>P value^a</i>
	<i>Median (range)</i>	<i>Median (range)</i>	
Leukocyte concentration ($\times 10^3$ cells/μl)			
Day 2	7.5 (2.4–13.9)	6.2 (2.6–12.0)	.116
Day 7	10.1 (5.3–13.3)	9.9 (4.7–14.3)	.5897
Day 49	15.1 (11.2–51.5)	13.0 (7.1–22.4)	.0548
Neutrophil concentration ($\times 10^3$ cells/μl)			
Day 2	6.1 (1.2–11.7)	5.2 (1.3–9.6)	.137
Day 7	7.9 (2.9–10.4)	7.7 (3.1–12.8)	.5393
Day 49	10.7 (6.2–45.8)	7.6 (3.3–17.9)	.0178
Band neutrophil concentration ($\times 10^3$ cells/μl)			
Day 2	0 (0–0.37)	0.06 (0–0.62)	.0393
Day 7	0 (0–0.30)	0 (0–0.57)	.3258
Day 49	0 (0–1.54)	0 (0–2.70)	.7655
Lymphocyte concentration ($\times 10^3$ cells/μl)			
Day 2	1.2 (0.9–2.0)	0.9 (0.6–2.2)	.116
Day 7	2.1 (0.8–2.7)	1.7 (0.4–3.1)	.1485
Day 49	3.1 (0.2–6.6)	3.6 (1.4–7.4)	.5557
Monocyte concentration ($\times 10^3$ cells/μl)			
Day 2	0.1 (0–0.4)	0.1 (0–0.3)	.5874
Day 7	0.2 (0.1–0.7)	0.3 (0–1.6)	.5069
Day 49	0.3 (0.1–1.5)	0.3 (0–2.4)	.5853
Eosinophil concentration ($\times 10^3$ cells/μl)			
Day 2	0 (0–0.1)	0 (0–0.1)	.1458
Day 7	0 (0–0.3)	0 (0–0.1)	.7015
Day 49	0 (0–0.7)	0 (0–0.8)	.2693
Basophil concentration ($\times 10^3$ cells/μl)			
Day 2	0 (0–0.1)	0 (0–0.1)	.5229
Day 7	0 (0–0.2)	0 (0–0.1)	.1794
Day 49	0 (0–0.2)	0 (0–0.2)	.363
Total plasma protein concentration (g/dl)			
Day 2	5.9 (4.3–7.0)	5.9 (5.2–7.0)	.835
Day 7	5.9 (4.5–6.7)	6.3 (5.4–7.1)	.0844
Day 49	6.2 (5.7–7.5)	6.2 (5.5–6.8)	.9126
Fibrinogen concentration (mg/dl)			
Day 2	300 (100–600)	300 (200–400)	.8264
Day 7	300 (100–800)	400 (300–800)	.0333
Day 49	400 (200–800)	400 (200–700)	.8396

TABLE 2. Results of Complete Blood Counts Performed on Days 2, 7, and 49 (cont.)

<i>Parameter</i>	<i>Control Foals</i>	<i>Principal Foals</i>	<i>P value</i> ^a
	<i>Median (range)</i>	<i>Median (range)</i>	
Erythrocyte concentration ($\times 10^3$ cells/μl)			
Day 2	10.5 (8.7–12.0)	10.5 (8.4–11.7)	.6781
Day 7	9.7 (7.8–11.2)	9.0 (5.3–10.3)	.0441
Day 49	10.1 (8.3–12.2)	9.9 (5.0–11.2)	.1377
Hemoglobin concentration (mg/dl)			
Day 2	14.3 (12.4–16.4)	14.3 (12.0–16.6)	.8681
Day 7	12.9 (10.9–16.0)	12.4 (7.1–14.8)	.0812
Day 49	11.9 (10.2–14.4)	11.8 (6.6–13.1)	.1109
Hematocrit (%)			
Day 2	41.7 (35.8–42.8)	40.1 (37.8–42.7)	.9669
Day 7	37.7 (32.3–44.3)	35.2 (19.8–41.8)	.0969
Day 49	33.9 (29.4–38.1)	34.3 (18.1–37.5)	.1377
Mean corpuscular volume (fl)			
Day 2	39.6 (35.8–42.8)	40.1 (37.8–42.7)	.3609
Day 7	39.0 (35.1–42.3)	39.0 (37.2–42.2)	.8195
Day 49	34.4 (31.1–37.4)	34.7 (32.9–37.2)	.4847
Mean corpuscular hemoglobin (pg)			
Day 2	13.8 (12.2–15.1)	13.9 (13.1–14.8)	.7703
Day 7	13.6 (12.1–15.5)	13.6 (12.6–14.8)	.5744
Day 49	12.2 (11.3–13.4)	12.0 (11.5–13.2)	.7422
Mean corpuscular hemoglobin concentration (g/dl)			
Day 2	34.8 (33.4–36.5)	34.4 (33.4–36.0)	.5605
Day 7	34.5 (33.7–36.6)	35.1 (33.5–37.0)	.7711
Day 49	35.3 (33.5–38.2)	34.8 (33.9–36.4)	.3942
Platelet concentration ($\times 10^3$ cells/μl)			
Day 2	350 (254–474)	334 (274–471)	.3245
Day 7	216 (111–344)	215 (164–591)	.8518
Day 49	375 (260–552)	385 (229–666)	.6944

^aValues in bold represent a significant difference versus controls ($P < .05$).

gression of pneumonia caused by *R. equi* in foals.^{17–20}

Most foals began consistently exhibiting signs of illness between 3 and 4 weeks of age (2 to 3 weeks after infection). This is consistent with our finding that the control foals had radiographic scores on day 28 that were signifi-

cantly higher than those for principal foals. By day 49, however, the difference was not significant. It may be possible that some of the foals were able to control the infection to some degree. All foals in our study were followed for the first 49 days of life unless they were considered to be in severe distress (loss of appetite,

inability to nurse, recumbent and unable to rise, dyspnea, or neurologic signs). It has been reported elsewhere that some foals may recover spontaneously from *R. equi* pneumonia, including some with severe disease.^{17,31}

There was no significant difference between principal and control foals for many of the hematologic parameters measured, including concentrations of lymphocytes, total protein, platelets, and fibrinogen on days 2 and 49. Similar observations have been reported by others studying *R. equi* pneumonia,^{17,31,32} which underscores the limited usefulness of routine hematology as a screening aid for *R. equi* infection. Similarly, the finding that few foals were febrile during the course of the study is noteworthy in terms of the

ference between groups was not statistically significant, it was surprising in light of other study findings. A number of possible explanations for this finding exist. Foals were randomly assigned to a principal or control group, and it is possible that the principal foals that died or were euthanized may have had other concurrent systemic illness or sepsis before experimental infection, which may have rendered them more susceptible to disease. Evidence exists that the principal foals might have had an underlying illness with a systemic inflammatory response. Band neutrophil concentrations were significantly higher in principal foals than control foals on day 2, and all three foals that died or were eutha-

The median radiographic score on day 28 was significantly greater for control foals than for principal foals.

lack of sensitivity of body temperature for monitoring foals with experimental and possibly natural infection with *R. equi*.

Foals treated with HIP on day 2 had significantly higher antibody titers against VapA on day 7 than did control foals. This may be a very important finding for producers and consumers of *R. equi* immune plasma. Recently, it has been reported that administration of purified immunoglobulins specific for VapA and VapC alone reduced the severity of pneumonia in experimentally infected foals.²⁰ Production of VapA and VapC is encoded for by a large virulence plasmid. *R. equi* strains that do not express Vap are avirulent and unable to replicate in the macrophages of mice and foals in vitro.²⁰ In our study, we measured only levels of antibody against VapA.

All the foals that died or were euthanized because of *R. equi* infection before the end of the study were principals. Although this dif-

nized had band neutrophils on day 2 of life. The band neutrophil concentrations of two of these foals were among the four highest on day 2 (all four of the highest band concentrations were among principal foals). In addition, fibrinogen concentrations of principal foals were significantly higher than control foals at day 7. Blood samples on days 2 and 7 were taken before experimental infection and, thus, were not affected by experimental challenge. Foals with clinicopathologic abnormalities not deemed to be severe were included in the study unless they exhibited clinical or radiographic signs of illness on or before day 7.

It is also possible that some foals were individually more susceptible to infection and that more of these foals were assigned by chance to the principal group. Increasingly, evidence from our laboratory (Texas A&M University) and others indicates that there may be factors that render some foals more susceptible to infection.

Recently published work has shown that blood samples collected at 2 weeks of life from foals affected by *R. equi* pneumonia later in life had significantly lower concentrations of leukocytes, segmented neutrophils, lower proportions of EqCD4+T lymphocytes, and higher proportions of EqCD8+T lymphocytes compared with samples from unaffected foals.³² Finally, it is possible that administration of plasma increased the risk of death or severe disease in principals. In light of the preponderance of data from this experiment and others indicating that administration of HIP is safe and that clinical, hematologic, and radiologic findings can be ameliorated by transfusion of HIP, this explanation seems unlikely.¹⁷⁻²⁰

In our study, the administration of HIP before experimental infection with *R. equi* did not prevent development of pneumonia in all foals, but disease appeared to be more severe in foals that did not receive HIP. The results of previously published studies evaluating the use of HIP for prevention of *R. equi* pneumonia in foals have not been consistent.^{17-20,24,25,31} The 1995 study by Hurley and Begg²⁴ and the 2002 study by Giguere et al.²⁵ both found no significant difference in the incidence of pneumonia between HIP-treated and untreated foals. Both of these studies were clinical trials that investigated naturally occurring disease. There is probably a wide variability in the severity of naturally occurring disease, and prevalence of disease may vary greatly from year to year on the same farm.³³ In the study by Giguere et al.,²⁵ incidence rates were 19% and 30% for plasma-treated and nontreated foals, respectively, but the difference was not significant. The foals in our study were experimentally infected in an attempt to provide a consistent incidence rate. Neither of these other two studies^{24,25} used radiographic evidence of lung lesions to track the severity of the disease as we did in our study. It would be interesting to

know if our results showing a protective effect of HIP against radiographic changes remain consistent in a field study.

We administered 1 L of HIP to the foals in our study. Some studies evaluating the protective effects of HIP used 2 L of plasma.^{20,25} One group of investigators administered one dose during the first week of life and the second between months 1 and 2 of life.²⁵ There is some thought that a second dose should be administered as any maternal antibodies wane, which would dictate a second dose at around 1 month of age.^{1,25,31} The correct dose and timing of HIP administration to prevent *R. equi* pneumonia is not known. It is possible that we might have produced better results had we administered a second liter at a later time.

Another reason that it is hard to calculate a correct dose is that it is still unclear exactly how HIP administration protects against *R. equi* pneumonia. Protection is likely due in part to specific opsonizing antibodies and subsequent phagocytosis by alveolar macrophages and neutrophils.³⁴ In addition, other components of plasma, such as complement, interferon, and cytokines, may also offer some protection.^{17,31} However, protection was similar among foals that received either HIP or purified equine immunoglobulin specific for VapA and VapC before experimental infection with *R. equi*, indicating that specific immunoglobulins are the important protective factor in HIP.¹¹ These findings conflict with another recent report in which foals received either HIP or normal equine plasma before experimental challenge and researchers found no significant difference in mortality rates between groups.³¹ These differences may be related to study design. All foals in one study¹¹ were euthanized 14 days after infection, and the other study³¹ had strict euthanasia criteria. Some of the foals may have been able to control the disease without treatment.

CONCLUSION

This study and others indicate that HIP can be a valuable aid in the prevention and control of *R. equi* pneumonia in foals.^{17–20} Currently, HIP is the only prophylactic strategy proven to significantly reduce the incidence or severity of *R. equi* pneumonia in foals.^{17,18} Although HIP may not prevent infection in every foal, it increases VapA titers and provides enough protection to reduce the severity of radiographic lung lesions 28 days after infection as well as preventing increased respiratory effort, thereby reducing morbidity attributable to infection with *R. equi*.

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