

Evaluation of a commercially available hyperimmune plasma product for prevention of naturally acquired pneumonia caused by *Rhodococcus equi* in foals

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Objective—To determine efficacy of a commercially available hyperimmune plasma product for prevention of naturally acquired pneumonia caused by *Rhodococcus equi* in foals.

Design—Randomized clinical trial.

Animals—165 foals.

Procedure—Foals were randomly assigned to 1 of 2 groups (hyperimmune plasma or nontreated controls). Foals with failure of passive transfer (FPT) of immunity were treated with hyperimmune plasma and evaluated as a third group. Foals that received plasma were given 950 ml between 1 and 10 days of age and between 30 and 50 days of age. A tracheobronchial aspirate was obtained from foals with clinical signs of respiratory tract disease for bacteriologic culture.

Results—A significant difference in incidence of pneumonia caused by *R equi* in foals with adequate passive transfer was not detected between foals that received plasma (19.1%) and nontreated foals (30%). Of 13 foals without FPT that received plasma and developed pneumonia caused by *R equi*, 12 developed disease prior to administration of the second dose of hyperimmune plasma. Incidence of undifferentiated pneumonia of all causes was not different between groups.

Conclusion and Clinical Relevance—Intravenous administration of the commercially available hyperimmune plasma was safe, and the product contained high concentrations of anti-*R equi* antibodies. However, within this limited foal population, the difference in incidence of pneumonia caused by *R equi* observed between foals that received plasma and control foals was not significant. (*J Am Vet Med Assoc* 2002;220:59–63)

Rhodococcus equi, a gram-positive facultative intracellular pathogen, is the most devastating cause of pneumonia in foals between 3 weeks and 5 months of age. Other less common clinical manifestations of *R equi* infections in foals include ulcerative enterocolitis,

colonic or mesenteric lymphadenopathy, immune-mediated synovitis and uveitis, osteomyelitis, and septic arthritis.¹ *Rhodococcus equi*, a saprophytic inhabitant of soil, is widespread in the environment of horse breeding farms. Although *R equi* is present in the environment of most horse farms and antibodies to *R equi* are widespread in the horse population, the clinical disease is unrecognized or sporadic on some farms but enzootic and devastating on others, with morbidity rates sometimes exceeding 40%.^{2,3} On farms where the disease is enzootic, costs associated with veterinary care, early diagnosis, long-term treatment, and mortality of foals may be extremely high. In addition to substantial immediate costs, pneumonia caused by *R equi* has a long-term detrimental effect on the equine industry, because foals that recover from the disease are less likely to race as adults.⁴

The farm-to-farm variation in incidence of the disease likely reflects differences in environmental and management conditions as well as differences in the virulence of isolates. Unlike most environmental *R equi*, isolates from pneumonic foals typically contain an 80- to 90-kilobase plasmid encoding a family of 7 closely related virulence-associated proteins (Vap) designated VapA and VapC to VapH.⁵⁻¹⁰ Plasmid-cured derivatives of virulent *R equi* strains lose their ability to replicate and survive in macrophages and fail to induce pneumonia in foals, confirming the importance of the large plasmid for the virulence of *R equi*.^{11,12} Control of *R equi* on farms with enzootic organisms depends on strategies aimed at decreasing the size of infective challenge and promoting earlier recognition and treatment of affected foals.¹³ Intravenous administration of hyperimmune plasma obtained from horses vaccinated against *R equi*, using various antigens, has also proven effective in substantially reducing the incidence of pneumonia caused by *R equi* in foals after both experimental and natural challenge in some studies.^{3,14-16} However, other studies failed to identify substantial differences in the incidence of naturally acquired pneumonia caused by *R equi* between hyperimmune plasma recipients and control foals,^{17,18} suggesting that the efficacy of a particular hyperimmune plasma product cannot be extrapolated to another product prepared in a similar but not identical fashion. In North America, most horse farms that rely on hyperimmune plasma for prevention of pneumonia caused by *R equi* use commercially available hyperimmune plasma products. However, to the authors' knowledge, the efficacy of these plasma products has never been critically inves-

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Supported by Lake Immunogenics Inc.

The authors thank John Franks, Phil Hronel, and John Davies for providing farm access and Juan Reyes for technical assistance.

tigated under field conditions of heavy environmental challenge. The purpose of the study reported here was to investigate the efficacy of a commercially available plasma product for the prevention of naturally acquired pneumonia caused by *R equi*.

Materials and Methods

Foals—The study was conducted on a Thoroughbred farm^a located in Marion County, Florida. All 165 foals owned by the farm and born during the year 2000 breeding season were included in the study. In 1999, incidence of respiratory tract disease in foals < 6 months of age on the farm was approximately 25%, and 4 foals died of pneumonia caused by *R equi*, which was confirmed at necropsy. The true incidence of *R equi*-induced pneumonia on the farm prior to year 2000 was not known, because bacteriologic culture of tracheobronchial aspirates was not performed. All mares on the farm were vaccinated against equine herpesvirus type 1 during pregnancy and treated with ivermectin at time of foaling. Foals were given initial anthelmintic treatment at 6 to 8 weeks of age and again at 4- to 8-week intervals. Vaccination with tetanus, eastern and western equine encephalitis, influenza, rhinopneumonitis, and rabies vaccines was started at approximately 12 weeks of age.

Hyperimmune plasma—Hyperimmune plasma was prepared by a commercial firm.^b The product is categorized as *Rhodococcus equi* antibody by the United States Department of Agriculture and is commercially available under that product name. Eleven donor horses were immunized with *R equi* antigens by a proprietary method until antibody titers $\geq 80\%$ were obtained by use of a described ELISA method.^{19,c} Plasma was collected by machine plasmapheresis, frozen at -20°C , and specific *R equi* ELISA values were determined on an aliquot from each serial prior to distribution into individual units. Aliquots from each donor were also assayed for antibody titers against VapA, using a described ELISA.^{20,d} The VapA ELISA titer was expressed as the last serum dilution giving a reading twice the optical density of a negative control serum diluted 1:10. Aliquots from each plasma collection had negative culture results for aerobic and anaerobic bacteria and fungi and were found free of antibodies against equine infectious anemia virus. Aliquots from each plasma collection also had negative culture results for equine viral arteritis virus.

Study design and hyperimmune plasma administration—Prior to birth, foals were randomly assigned to 1 of 2 treatment groups (hyperimmune plasma or nontreated controls) on an alternate basis according to their predicted date of birth. Serum was obtained from blood collected from all foals between 12 and 24 hours of age to verify adequate passive transfer of immunity by use of either the zinc sulfate turbidity test or a commercially available ELISA kit^e for semi-quantitative measurement of equine IgG. The IgG concentration was considered adequate if ≥ 800 mg/dl. Foals with an IgG concentration < 800 mg/dl were administered hyperimmune plasma immediately for treatment of failure of passive transfer of immunity, regardless of the treatment group to which they were originally assigned. Foals with failure of passive transfer of immunity were therefore considered in a separate group. Foals that received transfusions were given 2 complete 950-ml doses of freshly thawed hyperimmune plasma IV through a filtered blood administration set attached to a 14-gauge catheter during a period of 3 to 10 minutes by applying manual pressure to the administration bag. The first 950-ml dose of hyperimmune plasma was given between 1 and 10 days of age, and the second dose was given between 30 and 50 days of age. Foals that received transfusions and

nontreated control foals were kept together in small groups on pasture with the mares according to time of foaling. Foals were not segregated into treatment groups or identified as such. They were rotated from pasture to pasture in such a way that they were exposed to many areas on the farm during the study period.

Clinical observation, sample collection, and treatment of foals—Foals were monitored daily for clinical signs of illness by experienced farm personnel. In addition, for the first 6 months of life, blood samples were obtained from each foal at 4-week intervals for WBC counts and measurement of fibrinogen concentrations. Foals with clinical signs of illness received a complete physical examination, including thorough auscultation of the lungs, by the farm veterinarian (CM). A tracheobronchial aspirate was obtained from every foal with clinical signs of disease of the lower portion of the respiratory tract, such as cough, bilateral nasal discharge, tachypnea, fever, and abnormal lung sounds. Foals with a WBC count $\geq 14,000$ cells/ μl or a fibrinogen concentration ≥ 600 mg/dl also were examined by the farm veterinarian, even in the absence of clinical signs of respiratory tract disease. Foals without clinical signs of respiratory tract disease but with WBC counts between 13,000 and 14,000 cell/ μl or with fibrinogen concentrations between 500 and 600 mg/dl had a follow-up blood sample analyzed 7 days later. Diagnosis of undifferentiated pneumonia was made on the basis of clinical signs and lung auscultation by the farm veterinarian. Diagnosis of *R equi* pneumonia was made on the basis of culture of the organism from a tracheobronchial aspirate. Tracheobronchial aspiration was performed by passing a sterile double-guarded aspiration catheter^f through the biopsy channel of a 1.2-m fiberoptic endoscope, as described.²¹ Bacteriologic culture of tracheobronchial aspirates obtained by use of this technique correlates closely with the transtracheal approach.²¹ After each use, the endoscope was disinfected with 70% alcohol, soaked for at least 15 minutes in a 0.2% chlorhexidine solution,^g and thoroughly rinsed with sterile water. This disinfection protocol was found adequate for complete inhibition of *R equi* growth from the endoscope in preliminary experiments. The tracheobronchial aspirate fluid was kept refrigerated until submitted to a local laboratory^h for aerobic bacteriologic culture between 30 minutes and 5 hours after collection. Tracheobronchial aspirate fluid was inoculated onto standard bacteriologic culture media (blood agar and MacConkey agar) as well as on *R equi*-selective nalidixic acid-novobiocin-actidione (cycloheximide)-potassium tellurate medium.²² Bacterial pathogens were identified by use of standard identification procedures. Immediately after collection of the tracheobronchial aspirate, oral administration of erythromycin ethylsuccinate (25 mg/kg [11.4 mg/lb] of body weight, q 8 h) and rifampin (5 mg/kg [2.23 mg/lb], q 12 h) was initiated and continued for a minimum of 3 weeks. During treatment, WBC counts and fibrinogen concentrations were measured every second week. Criteria for discontinuation of treatment included resolution of clinical signs and hematologic values within reference ranges on at least 2 occasions. Foals that died during the study were examined via complete postmortem examination.

Statistical analysis—Incidence of undifferentiated pneumonia and culture-confirmed pneumonia caused by *R equi* between hyperimmune plasma recipients and foals that did not receive hyperimmune plasma was compared by use of the Fisher exact probability test. The Mann-Whitney *U* test was used to analyze differences in age of foals at time of diagnosis and in duration of treatment between hyperimmune plasma recipients and nontreated control foals. Linear associations (correlation coefficients [*r*]) between anti-VapA antibody titers of the serial administrations of hyperimmune

plasma and incidence of pneumonia caused by *R equi* were assessed by use of simple linear regression analysis. Results were considered significant if the value of *P* was < 0.05.

Results

Mean \pm SD *R equi* ELISA titer of the hyperimmune plasma was $85 \pm 4\%$ (range, 80 to 100%). The anti-VapA ELISA titers of the hyperimmune plasma ranged between 2,560 and 20,480. One hundred sixty-five foals were born on the farm between Jan 31 and May 30, 2000. Of these 165 foals, 3 died of problems unrelated to infection with *R equi* and were excluded from the study. Fourteen foals had failure of passive transfer of immunity and were considered in a separate group. Of the 148 foals without failure of passive transfer of immunity, 68 foals received transfusions with hyperimmune plasma, and 80 foals served as nontreated controls. Mean \pm SD age of foals at the time of plasma administration was 5.3 ± 1.8 days for the first transfusion and 40.4 ± 3.7 days for the second transfusion. No adverse reactions were noticed during or after administration of hyperimmune plasma.

Difference in incidence of pneumonia caused by *R equi* between foals without failure of passive transfer of immunity that received transfusions with hyperimmune plasma and nontreated controls was not significant (*P* = 0.09; Table 1). Difference in incidence of pneumonia caused by *R equi* between foals that received transfusions, with and without failure of passive transfer of immunity, was not significant. Incidence of undifferentiated pneumonia in foals without failure of passive transfer of immunity was not significantly different between foals that received transfusions and control foals. Similarly, the difference in incidence of undifferentiated pneumonia between foals that received transfusions, with and without failure of passive transfer of immunity, was not significant. There was no correlation between anti-VapA antibody titers in the hyperimmune plasma and incidence of pneumonia caused by *R equi* (*r* = 0.24).

Streptococcus equi subspecies *zooepidemicus* (*n* = 8), non- β -hemolytic *Streptococcus* spp (14), *Klebsiella* spp (3), and *Escherichia coli* (1) were cultured either alone or in various combinations from the 18 foals with pneumonia from which *R equi* was not cultured from the tracheobronchial aspirate. *Streptococcus zooepidemicus* was also isolated in combination with *R equi* from 6 foals. At the time of diagnosis of pneumonia caused by *R equi*, the age of foals that received transfusions (median, 36.5 days; range, 29 to 51 days) was not significantly different from that of the nontreated control foals (median, 38.0 days; range, 28 to 94 days). Of the 13 foals without failure of passive

transfer of immunity that received transfusions and developed pneumonia caused by *R equi*, 12 developed the disease prior to administration of the second dose of hyperimmune plasma. Duration of antimicrobial treatment in foals that received transfusions and had culture-confirmed pneumonia caused by *R equi* (median, 46 days; range, 27 to 80 days) was not significantly different from that of the control foals (median, 46 days; range, 37 to 63 days). Only 1 foal died of pneumonia caused by *R equi* during the study; that foal had complete failure of passive transfer of immunity (IgG < 200 mg/dl).

Discussion

Although administration of commercially available hyperimmune plasma products for prevention of pneumonia caused by enzootic *R equi* on farms in North America is common practice, we are unaware of controlled field studies evaluating the efficacy of such commercial products. Results of studies evaluating the efficacy of various experimentally produced hyperimmune plasma preparations have been contradictory.^{3,14-18} This suggests that various factors such as the method of immunizing plasma donors, the amount of hyperimmune plasma used, the timing of administration of hyperimmune plasma, management conditions, and the number of virulent bacteria in the environment may influence the effectiveness of a particular hyperimmune plasma product. In our study, the difference in incidence of the disease between foals that received transfusions and control foals (19.1 vs 30%, respectively) was not significant. Failure of the hyperimmune plasma to result in a significant (*P* < 0.05) reduction in incidence of pneumonia may either reflect ineffectiveness of the product as administered in our study or, alternatively, may be a result of the low statistical power of the study (46%). To determine that an incidence of 19% was significantly different from an incidence of 30% with an 80% probability at *P* \leq 0.05, a sample size of 188 foals/group would have been necessary.

The mechanisms by which hyperimmune plasma confers protection are not completely understood. The list of possible effector molecules includes antibodies and nonspecific factors such as fibronectin, complement components, collectins, cytokines, and acute-phase proteins. Opsonization of *R equi* with a specific antibody promotes phagocytosis and killing by alveolar macrophages, suggesting that antibodies may be a critical component of hyperimmune plasma.²³ In some studies, plasma donors were immunized with whole-cell vaccines^{3,15} or a mixture of several soluble antigens,¹⁴ making it impossible to determine the role of antibodies against defined antigens of *R equi*. Recent studies have focused more specifically on the role of antibodies against plasmid-encoded Vap. A monoclonal antibody against VapA and serum from horses immunized with partially purified VapA increases phagocytosis of *R equi* by macrophages in vitro.^{9,24} Moreover, purified immunoglobulins obtained from horses vaccinated with partially purified VapA protect mice against intraperitoneal challenge with virulent *R equi*, compared with mice administered immunoglobulins from nonimmunized horses.²⁵ More recently, IV

Table 1—Incidence (%) of *Rhodococcus equi* pneumonia and undifferentiated pneumonia in foals that received transfusions with hyperimmune plasma (*n* = 68), nontreated control foals (80), and foals that received transfusions and had failure of passive transfer of immunity (14)

Group	Undifferentiated pneumonia (%)	<i>R equi</i> -induced pneumonia (%)
Hyperimmune plasma	33.8	19.1
Nontreated control foals	40	30
Failure of passive transfer	50	35.7

administration of purified immunoglobulins obtained from horses immunized with recombinant VapA and VapC to foals reduced the severity of pneumonia after heavy experimental challenge with *R equi*.²⁶ In the same study, the degree of protection conferred by purified anti-VapA and anti-VapC immunoglobulins was similar to that provided by hyperimmune plasma.²⁶ The hyperimmune plasma product used in the study reported here was found to have high anti-VapA antibody titers. There was, however, no correlation between anti-VapA antibody titers in the units of hyperimmune plasma used and development of pneumonia caused by *R equi*.

The ideal time for administration of hyperimmune plasma is not known. In a recent study, application of Sartwell's model of the distribution of incubation periods to age at onset and age at death of foals with *R equi* pneumonia was consistent with the hypothesis that foals become infected with *R equi* within the first several days of life.²⁷ However, this hypothesis is not widely accepted.²⁸ Administration of hyperimmune plasma after infection with *R equi* is not protective.²⁹ However, administration too early may result in decline of passively transferred antibodies to nonprotective concentrations at times when foals are still susceptible to *R equi*.³⁰ In an attempt to overcome these problems, we chose to administer hyperimmune plasma within the first 10 days of life, followed by a second administration between 30 and 50 days of age. The age for the second dose of hyperimmune plasma was selected on the basis of results of a study indicating that anti-*R equi* antibody is maintained at high concentrations for > 30 days after administration in most foals that receive transfusions.¹⁸ Of the 13 foals without failure of passive transfer of immunity that received transfusions and developed pneumonia caused by *R equi*, 12 developed the disease prior to administration of the second dose of hyperimmune plasma. The only foal that developed pneumonia caused by *R equi* after administration of 2 doses of hyperimmune plasma developed disease only 5 days after the second administration. Because the incubation period of experimentally induced pneumonia caused by *R equi* varies between 7 and 15 days, depending on the challenge dose,^{11,12} this foal was probably infected at the time of the second transfusion. Median age at diagnosis of *R equi*-induced pneumonia on this particular farm was 36 days. Therefore, administration of the second dose of hyperimmune plasma at an earlier age may have been more beneficial. These findings suggest that the ideal time for hyperimmune plasma administration may vary from farm to farm, depending on the age at which most foals on the farm develop the disease. Whether or not earlier administration of the second dose of the hyperimmune plasma product would result in a significant reduction in the incidence of *R equi*-induced pneumonia remains to be determined.

Diagnosis of *R equi*-induced pneumonia in our study relied on culture of the organism from a tracheobronchial aspirate. The exact sensitivity of bacteriologic culture of a tracheobronchial aspirate for the diagnosis of *R equi*-induced pneumonia is unknown. Combining the results of 3 separate studies, 24 of the 28 (86%) foals with positive *R equi* culture results at

necropsy were previously found to yield *R equi* on culture of an antemortem tracheobronchial aspirate.^{16,31,32} In a recent study, culture was less sensitive than nucleic acid amplification by use of polymerase chain reaction (PCR) for detection of *R equi* in tracheobronchial aspirates.³³ However, in other studies, results of culture of a tracheobronchial aspirate compared closely with that of PCR, suggesting that it is a sensitive and reliable method of diagnosing *R equi*-induced pneumonia.^{34,35} In our study, the possibility that a few foals with negative culture results may have had *R equi*-induced pneumonia cannot be totally excluded.

Intravenous administration of the commercially available hyperimmune plasma appears to be safe, and the product contains high concentrations of anti-*R equi* antibody. However, within the limited foal population in our study, the difference in incidence of *R equi*-induced pneumonia between foals that received transfusions and control foals was not significant. If used for control of *R equi* on farms with enzootic infections, administration of hyperimmune plasma should always be combined with other control strategies, such as attempts at decreasing the size of infective challenge and early identification and treatment of infected foals.

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^bLake Immunogenics Inc, Ontario, NY.

^cCalifornia Animal Health & Food Safety Laboratory System, Davis, Calif.

^dLaboratory of Dr. John F. Prescott, Department of Pathobiology, University of Guelph, Guelph, ON, Canada.

^eCite Foal IgG test kit, Idexx Laboratories Inc, Westbrook, Me.

^fDarien Microbiology Aspiration catheter, Mill-Rose Laboratories Inc, Mentor, Ohio.

^gNolvasan solution, Fort Dodge Animal Health, Fort Dodge, Iowa.

^hMid-Florida Veterinary Laboratory, Ocala, Fla.

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