Rhodococcus equi, a Gram-positive facultative intracellular pathogen, can cause a severe form of pneumonia in foals worldwide.1,2 Virulent strains of R equi express the virulence-associated protein A (VapA),3,4 which is necessary for this bacterium to replicate in macrophages and cause disease in mice and foals.5–8 To the authors’ knowledge, no licensed vaccine is available for preventing rhodococcal foal pneumonia. In the US, control and prevention are based on transfusion of plasma from donor horses hyperimmunized against R equi.9 While most experimental and field studies9–18 have demonstrated reduced incidence or severity of rhodococcal pneumonia following transfusion of R equi hyperimmune plasma (REHIP), conflicting evidence exists19,20 and efficacy is only partial. The component(s) of REHIP that mediate protection remains ill defined. As recently reviewed,9 evidence exists that antibodies...
targeting rhodococcal antigens, particularly VapA, are critical for protection. It is possible that the variable effects of REHIP are attributable to differences in the activity of anti-\( R\ equi \) antibodies among plasma products.\(^1\) Differences in subtypes of immunoglobulin G (IgG) in REHIP also might contribute to variable clinical effectiveness. Evidence\(^{20–24}\) indicates higher IgG\(_2\) activity is important for REHIP-mediated protection, but conflicting evidence\(^{25}\) exists that IgG\(_{4/7}\) might be important.

Some evidence also suggests that factors other than antibodies might mediate protection. Foals of mares vaccinated against \( R\ equi \) or VapA develop increased antibody activities against these antigens in their serum and colostrum that are transferred to foals, but their foals are not protected.\(^{26,27}\) No difference in incidence or severity of pneumonia was detected after intrabronchial infection at age 21 days in colostrally deprived foals transfused with standard commercial plasma at birth followed 14 days later by transfusion with either REHIP or standard plasma.\(^{28}\) Opsonization with either standard commercial plasma or REHIP increased phagocytosis and decreased intracellular survival of \( R\ equi \), indicating that \( R\ equi \)-specific antibodies were not essential for these processes.\(^{29}\) Complement is important for mediating opsonophagocytic killing of \( R\ equi \) and opsonization of \( R equi \) is important for intracellular killing of \( R equi \).\(^{29,31–35}\) Individual plasma donors with similar IgG concentrations can vary in their complement-mediated opsonizing capacity.\(^{31}\) Foals have been reported to have decreased opsonic capacity relative to adults.\(^{31,33}\) Although evidence is conflicting,\(^{34}\) it is possible that variation in complement activity between plasma products or among foals contributes to the variable clinical efficacy of REHIP observed under field and experimental conditions.\(^{23,29–35}\) However, the effects of transfusion of REHIP to foals on serum concentrations of complement have not been reported. Thus, the objectives of our study were 2-fold. First, we wanted to compare serum concentrations of complement component 1q (C1q) in foals before and after transfusion with REHIP. We hypothesized that C1q concentrations would be increased following transfusion. We chose to investigate C1q because its functions include initiating the classical complement pathway when it binds to antibody-antigen complexes.\(^{36}\) Second, we wanted to determine whether the serum concentrations of C1q and the levels of anti-VapA IgG\(_1\) or IgG\(_{4/7}\) before and after transfusion were associated with the odds of developing rhodococcal pneumonia in foals transfused with REHIP. We hypothesized that after transfusion lower concentrations of C1q and IgG\(_1\) but not IgG\(_{4/7}\) would be associated with increased odds of rhodococcal pneumonia.

### Methods

#### Ethics statement

The study was approved by the Texas A&M University Institutional Animal Care and Use Committee and the Clinical Research Review Committee of the College of Veterinary Medicine & Biomedical Sciences (AUP No. 2021-0041 CA). All methods were performed in accordance with relevant guidelines and regulations for animal use and for laboratory practices including environmental health, occupational safety, and biosafety (Texas A&M University Infectious Biohazard Committee IBC No. 2017-105).

#### Study population and sample collection

Foals born at 2 breeding farms with a history of \( R equi \) foal pneumonia during the preceding 5 years in the Saratoga Springs, NY, area that received care from a coauthor (PF-A) were recruited. Eligible foals were born healthy during 2022 (beginning January 1, 2022) and transfused with REHIP within 48 hours after birth. Foals were excluded from the study if they had health disorders (such as neonatal isoerythrolysis, diarrhea, or sepsis), they were not transfused with REHIP, serum was not available to test for both activity of anti-VapA antibodies and C1q concentrations at each time point (ie, before and after transfusion), or they were treated for pneumonia in the absence of clinical signs of pneumonia (ie, had subclinical pneumonia identified by thoracic ultrasonography). We targeted a population of 200 foals based on calculations using the following assumptions: (1) binary outcome of pneumonia; (2) an OR of at least 2 for association of the binary outcome of pneumonia in the lower quartile relative to the upper quartiles among foals with activity of IgG\(_1\) against the \( R\ equi \) protein VapA; (3) variance of VapA relative optical densities (ODs) of 0.2 based on prior unpublished data using frozen serum samples sent to investigators in Texas by 2 coauthors (PF-A; SA); (4) statistical power of 80%; and (5) statistical significance of \( P < .05 \). The rationale for powering our study to detect differences in IgG\(_1\) was the results of prior publications\(^{22–24}\) (including work from our laboratory) indicating this subisotype is important for mediating protection against rhodococcal pneumonia.

All eligible foals were transfused with REHIP from a single manufacturer (Mg Biologics). An IV catheter was placed in a jugular vein, and plasma was transfused as is standard practice at the participating farms. Foals were transfused with either 1 or 2 L of REHIP, according to standard practices for the participating farm. Blood samples (4 mL) were collected immediately before transfusion through the IV catheter placed routinely for transfusion and immediately after transfusion from the jugular vein contralateral to the side used for transfusion. Blood was placed in serum separator tubes (Becton, Dickinson, and Co) labeled with the date and foal study identification number and then refrigerated at 4°C until shipped from NY to the Equine Infectious Disease Laboratory (EIDL) at Texas A&M University; chilled serum samples were shipped using overnight delivery twice weekly. Serum samples received in the EIDL were aliquoted and frozen at −80°C until use.

Foals were monitored through weaning (≈ 5 months of age; up to December 1, 2022) by farm veterinary medical and technical staff at least twice daily for signs of pneumonia (including lethargy,
coughing, depressed attitude, or increased respiratory rate (> 60 breaths/min) or effort (abdominal lift, flaring nostrils) and extrapulmonary manifestations of R. equi pneumonia (such as polysynovitis and uveitis). Foals with clinical signs had CBCs and thoracic ultrasonography performed. Foals were diagnosed with presumed R. equi pneumonia if they had all the following findings: (1) cough; (2) fever (rectal temperature > 102.5 °F); (3) lethargy, tachypnea, or dyspnea; and (4) ultrasonographic evidence of pulmonary abscesses or consolidations ≥ 2 cm in maximal diameter. At the discretion of the farm managers and attending veterinarians, a small number of foals diagnosed with R. equi pneumonia underwent transendoscopic tracheobronchial aspiration to confirm cytologic evidence of septic pneumonia and to isolate R. equi. Daily medical records were maintained that included reports of all clinical findings and treatments. All foals that developed pneumonia were treated per the high standard of care for the veterinarians and farms participating in this study.

At the time of transfusion, data collection was initiated using a study data form that included information entered on the day of transfusion (identifier for mare and foal, date of birth, age at transfusion, and volume of REHIP transfused) and follow-up information through weaning about the foal’s health. After all data had been collected for all foals and their forms completed, forms were scanned and transmitted to investigators at Texas A&M University. Data forms were inspected for aberrant or suspicious values, and investigators worked with the person who completed the data forms (PF-A) to resolve errors. Data were then entered into a computerized database for analysis.

C1q ELISA

Serum samples were tested by ELISA for concentrations of C1q using a 96-well commercial kit (Horse Complement 1Q ELISA Kit; MB5026276; MyBioSource). The aliquots used for complement had not been thawed before testing. Assays were performed using undiluted serum samples in duplicate in accordance with the manufacturer’s protocol. Standards, reagents, stop solution (dilute sulfuric acid), wash solution (PBS containing 0.05% Tween 20), Chromogen Solution A (hydrogen peroxide solution), and Chromogen Solution B (tetramethylbenzidine) came preformulated and premeasured for the amount and concentration needed for a complete 96-well kit. Six individual 0.5-mL vials of C1q standards at concentration gradients of 25, 50, 100, 200, 400, and 800 μg/mL of C1q, respectively, were included in each kit. A volume of either 50 μL of foal serum or each of the 6 standards was added to wells in duplicate for all ELISA plates; 4 wells on each plate were left empty as blanks. The horseradish peroxidase (HRP) conjugate reagent (100 μL) was then added to every well except the blank wells. Plates were then incubated for 1 hour at 37 °C. Plates were washed 4 times with 1 volume of provided wash solution that was diluted with 19 volumes of deionized water. Chromogen Solution A (50 μL) followed by Chromogen Solution B (50 μL) was added to each well, including blank wells. Plates were gently mixed and then incubated for 15 minutes at 37 °C. The reaction was then stopped by adding 50 μL of the provided stop solution. The ODs were measured at 450 nm using a photometric microplate reader. Results of C1q serum concentration were calculated based on the respective standard curves for each plate. The average C1q concentration of the duplicates of a given foal’s serum was used for data analysis.

Anti-VapA IgG1 and IgG4/7 ELISA

Serum samples from study foals were tested by ELISA for relative activities against VapA of IgG1 and IgG4/7 subisotypes. Serum samples had not been thawed before analysis. ELISA plates (Maxisorp; Thermo Scientific) were coated with purified VapA (1.0 μg/mL) diluted in sensitization buffer (0.04 M PO4; pH 7.2) overnight at 4 °C. Plates were washed 6 times with PBS containing 0.05% Tween 20, blocked with 150 μL of PBS containing 1% skim milk for 1 hour at 37 °C, and washed again. Foal serum samples (100 μL) were added in duplicate to wells of the ELISA plate and incubated for 1 hour at 37 °C. Pretransfusion serum samples were initially diluted in the incubation buffer (PBS with 1% skim milk and 0.05% Tween 20) to 1:20 for IgG1 and 1:80 for IgG4/7, posttransfusion serum samples were diluted to 1:500 for IgG1 and 1:1,000 for IgG4/7. A sample of serum from a REHIP donor was included in each ELISA plate as a positive control, using 5 serial 2-fold dilutions beginning at 1:160 for IgG1 and 1:10,240 for IgG4/7. Plates were washed again, and then 100 μL per well of anti-horse IgG1 or IgG4/7 conjugated to HRP (Bethyl Laboratories) were added to the wells. Plates were incubated for 1 hour at room temperature and then washed. Peroxidase substrate (SeraCare) was added to the wells for 20 minutes. The reaction was stopped by adding sulfuric acid solution to the wells. Optical densities were determined at 450 nm using a microplate reader. The mean of the 2 replicates of a given foal’s serum sample was used for analysis. The relative activity of IgG1 and IgG4/7 for each sample was calculated by dividing the mean OD value for a foal’s serum sample by the OD value of the positive control (for a prespecified dilution) on the same plate.

Data analysis

All analysis was performed using R statistical software (version 4.3.1; R Foundation for Statistical Computing). Data were described using tables and figures. Pearson correlation coefficient (r) analysis was performed for data with normal distribution, whereas Spearman correlation coefficient (ρ) analysis was performed for data with nonnormal distribution. Correlation for r or ρ was defined as negligible (|0 to 0.10|), low (| > 0.10 to 0.39|), moderate (|0.4 to 0.69|), high (|0.7 to 0.89|), or very high (|0.90 to 1.0|). Proportions were compared between variables using χ2 tests. For birth months, a binary variable for birth during April or May versus earlier months (ie, January, February, or March) was included on the basis of previous experience at these farms37 and other reports. The difference in age at...
transfusion between farms and C1q concentrations after transfusion (ie, posttransfusion concentration minus pretransfusion concentration) were tested for statistical significance using a generalized linear model with a Gaussian link.

The association of the binary outcome of diagnosis of rhodococcal pneumonia with individual variables was made using $\chi^2$ tests as described. The association of the binary outcome of diagnosis of rhodococcal pneumonia with multiple independent variables (ie, pre- and posttransfusion C1q concentrations, pre- and posttransfusion IgG1 and IgG4/7 concentrations, birth month, and farm) was performed using multiple logistic regression; variables associated with pneumonia at $P < .15$ in bivariable analysis were included in multivariable modeling. Because we did not expect that either C1q concentrations or anti-VapA activities would be linear in the logit scale, we created binary variables for C1q concentrations and IgG subtype distributions for logistic regression analysis before inferential data analysis based on exploratory data analysis. For C1q, low concentration was defined as a value ≤ median; for IgG1 and IgG4/7, low activity was defined as ≤ 25th percentile (ie, the lowest quartile). Farm was included in modeling irrespective of statistical significance to account for any differences in measured or unmeasured variables between farms; modeling was also conducted excluding farm. All pair-wise interactions between independent variables included in modeling were evaluated for statistical significance. Significance for all analyses was set at $P < .05$.

**Results**

**Study population**

A total of 107 and 106 foals were born at farms 1 and 2, respectively, between January 13, 2022, and May 30, 2022. Four foals at each farm were excluded. At farm 1, 3 foals were excluded because serum samples were not available to test for C1q (2 pretransfusion and 1 posttransfusion), and 1 foal was excluded because it was treated for subclinical pneumonia at the request of the farm manager. At farm 2, 1 foal was excluded because insufficient serum was available posttransfusion to test for both antibody subtypes and C1q, and 3 foals were excluded because they were treated for subclinical pneumonia at the farm owner’s request. The reason some foals had inadequate serum was that some samples with discrepant ELISA results were retested, depleting serum available to test for C1q from these foals. Thus, a total of 103 and 102 foals were included from farms 1 and 2, respectively. Of the 205 foals, 202 (98.5%) were transfused with 1 L. The mean age at transfusion was 15.6 hours (SD, 5.01 hours). The ages at which foals were transfused at farm 1 (mean, 16.7 hours; 95% CI, 15.7 to 17.7 hours) were significantly ($P = .0026$) greater than those of foals at farm 2 (mean, 14.5 hours; 95% CI, 13.1 to 15.9 hours). The distribution of birth months did not differ significantly ($P < .2424$) between farms (Table 1). Although the proportion of foals born in April and May was higher for farm 1 (64%; 64/103) than farm 2 (49%; 50/102), this difference was not significant ($P = .0802$). Of the 205 foals, 53 (26%) developed *R equi* pneumonia; the proportion was similar for farm 1 (25%; 25/103) and farm 2 (26%; 27/102).

**Serum C1q concentrations before and after transfusion**

Serum concentrations of C1q before and after transfusion varied considerably (Figure 1). Serum concentrations of C1q before and after transfusion were highly correlated (Supplemental Figure S1; $r = 0.87$; $P < .0001$). Serum concentrations were significantly lower after transfusion than before (Figure 2); the mean difference in serum C1q after transfusion was $−18 \mu g/mL$ (95% CI, $−18$ to $−12 \mu g/mL$; $P < .0001$).

**Activities of anti-VapA IgG1 and IgG4/7 before and after transfusion**

Activities of IgG1 and IgG4/7 recognizing VapA were expressed as a percentage of the positive control. Pretransfusion activities of IgG1 (Figure 3) ranged from < 0.1% to 120.5% of the positive control (mean, 12.7%; SD, 18.0%; median, 6.7%; 25th percentile, 2.0%), and pretransfusion activities of IgG4/7 ranged from < 0.1% to 173.2% of the positive control (mean, 17.2%; SD, 24.8%; median, 8.2%; 25th percentile, 2.7%). Posttransfusion activities of IgG1 (Figure 4) ranged from 1.6% to 36.8% of the positive control (mean, 14.5%; SD, 7.8%; median, 13.3%; 25th percentile, 7.9%), and posttransfusion activities of IgG4/7 ranged from 20.2% to 177.4% of the positive control (mean, 80.8%; SD, 25.7%; median, 80.8%; 25th percentile, 67.8%). Before transfusion, the data for both subtypes were skewed toward lower values, and after transfusion, the data for IgG1 were skewed toward lower values. Direct comparison of pre- and posttransfusion values of IgG subtypes was not possible because testing was performed at different dilutions; however, samples posttransfusion had to be diluted more than pretransfusion samples because the concentration of IgG of both subtypes was markedly higher after transfusion. Pretransfusion activities of IgG1 and IgG4/7 were highly correlated ($r = 0.85$; $P < .0001$). Posttransfusion activities of IgG1 and IgG4/7 activities were significantly but only moderately correlated ($r = 0.67$; $P < .0001$), whereas they were highly correlated before transfusion. Pre-...
Figure 1—Box-and-whisker plots of serum concentrations of complement component 1q (C1q) before (A) and after (B) transfusion of Rhodococcus equi hyperimmune plasma in 205 foals from 2 farms in New York born between January 13, 2022, and May 30, 2022. The bold line bisecting the box is the median; the top line of the box represents the 75th percentile, and the bottom line of the box represents the 25th percentile; the box spans the IQR. The whiskers extend from the boxes by a multiple of 1.5 of the IQR (eg, 3rd quartile + 1.5 [IQR]). Each black dot represents the C1q concentration for an individual foal included in the study.

Figure 2—Boxplot of the mean difference in concentration of C1q after transfusion (post minus pre) for 205 foals born between January 13, 2022, and May 30, 2022, transfused with R equi hyperimmune plasma at 2 farms in New York. The mean difference was −18 µg/ml, which was significantly (P < .0001) < 0 µg/ml. The red line at 0 indicates no difference between concentrations of C1q before and after transfusion. The top line of the box represents the 75th percentile. The bold line bisecting the box is the median; the top line of the box represents the 75th percentile, and the bottom line of the box represents the 25th percentile; the box spans the IQR. The whiskers extend from the boxes by a multiple of 1.5 of the IQR (eg, 3rd quartile + 1.5 [IQR]). Each black dot represents the C1q concentration for an individual foal included in the study. Note that ≈75% of foals have a difference < 0.
posttransfusion anti-VapA IgG₁ activities were significantly correlated, but the estimated correlation coefficient was less strongly correlated than C1q (ρ = 0.38 [low]; P < .0001). Similarly, pre- and posttransfusion anti-VapA IgG₄/₇ activities were significantly correlated but less highly than C1q (ρ = 0.28 [low]; P < .0001).

**Association of C1q concentrations and anti-VapA antibody activities with odds of pneumonia**

The proportion of foals that developed pneumonia among those with pretransfusion serum C1q concentrations ≤ 250 µg/mL (the population median concentration) was significantly (P = .0189) greater (33%; 35/105) than that among foals having concentrations > 250 µg/mL (18%; 18/100). The proportion of foals that developed pneumonia among foals that had posttransfusion serum C1q concentrations ≤ 218 µg/mL (the population median) was not significantly (P = .3349) different (24%; 24/102) than that among foals having concentrations > 218 µg/mL (28%; 29/103). The proportion of foals that developed pneumonia among foals that had pretransfusion IgG₁ values in the lower quartile (36%; 14/39) was not significantly different (P = .1649) than that among foals in the other 3 quartiles (23%; 39/166). The proportion of foals that developed pneumonia among foals that had posttransfusion IgG₁ values in the lower quartile (27%; 19/52) was not significantly different (P = .9836) than that among foals in the other 3 quartiles (25%; 34/153); however, this met our a priori criterion for inclusion in multivariable logistic regression modeling. The proportion of foals that developed pneumonia among foals that had posttransfusion IgG₄/₇ values in the lower quartile (27%; 14/52) was not significantly different (P = .9836) than that among foals in the other 3 quartiles (25%; 39/153). The proportion of foals that developed pneumonia among foals that had posttransfusion IgG₄/₇ values

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**Figure 3**—Violin plots of IgG₁ (A) and IgG₄/₇ (B) activities against the virulence-associated protein A (VapA) of *R equi* pretransfusion for 205 foals transfused with hyperimmune plasma at 2 farms in New York born between January 13, 2022, and May 30, 2022, and monitored through weaning (age ≥ 5 months). The white area reflects the kernel probability density of the data. Values are markedly skewed toward lower values.

**Figure 4**—Violin plots of IgG₁ (A) and IgG₄/₇ (B) activities against VapA of *R equi* posttransfusion for 205 foals transfused with hyperimmune plasma at 2 farms in New York that were born between January 13, 2022, and May 30, 2022, and monitored through weaning (age ≥ 5 months). The white area reflects the kernel probability density of the data. Values are skewed toward lower values but less so than pretransfusion values (Figure 3); skewing was more pronounced for IgG₁ (A) than IgG₄/₇ (B). Please note that the scale of the y-axis differs between IgG₁ and IgG₄/₇ because the latter is more abundant in serum and plasma.
in the lower quartile (25%; 13/52) was not significantly different ($P = 1.0000$) than that among foals in the other 3 quartiles (26%; 40/153). The proportion of foals that developed pneumonia among those born during April or May (31%; 35/114) was not significantly ($P = .1065$) greater than that among foals born in January, February, or March (20%; 18/91); however, this met our a priori criterion for inclusion in multivariable logistic regression modeling.

Using multivariable logistic regression, concentrations of pretransfusion concentration of C1q ≤ 250 μg/mL, being in the lower quartile of posttransfusion activity of IgG1, and birth during April or May were significantly associated with higher odds of developing pneumonia, irrespective of whether farm was included in the model (Table 2 and Supplemental Table S1); the rationale for forcing farm in the model was to account for differences that existed between farms. Although pre- and posttransfusion C1q concentrations were significantly correlated, posttransfusion C1q concentrations were not significantly associated with pneumonia using multivariable regression.

### Table 2—Results of logistic regression modeling of factors associated with rhodococcal pneumonia in a study of 205 Thoroughbred foals from 2 farms in New York born between January 13, 2022, and May 30, 2022, and monitored through weaning (age ≥ 5 months); 53 (26%) of these foals developed pneumonia.

<table>
<thead>
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<th>Variable</th>
<th>OR (95% CI)</th>
<th>$P$ value</th>
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</thead>
<tbody>
<tr>
<td>Pretransfusion C1q ≤ 250 μg/mL</td>
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<td>.0330</td>
</tr>
<tr>
<td>Lowest quartile IgG1</td>
<td>3.3 (1.4 to 7.5)</td>
<td>.0051</td>
</tr>
<tr>
<td>posttransfusion</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Birth month of April or May</td>
<td>3.0 (1.3 to 6.7)</td>
<td>.0074</td>
</tr>
<tr>
<td>Farm 2</td>
<td>1.3 (0.6 to 2.5)</td>
<td>.4800</td>
</tr>
</tbody>
</table>

### Discussion

Transfusion of foals with REHIP significantly decreased serum concentrations of C1q. The cause of this decrease was not determined. After observing this finding, we measured the concentration of C1q in a liter of REHIP from the manufacturer of the plasma transfused to the foals in this study; this REHIP was produced 1 year after that used for the foals included in this study. The concentration of C1q in the REHIP was approximately 280 μg/mL. Thus, it is unlikely that the REHIP directly diluted concentrations of C1q. We speculate that an increase in plasma oncotic pressure resulting from transfusion of 2 L REHIP for most (98.5%) foals led to a shift of fluid from cells or interstitial spaces into the vasculature resulting in a dilutional effect. Although complement in REHIP would likely have increased the total mass of C1q in plasma, it does not appear that transfusion of REHIP markedly increases plasma concentrations of C1q in foals.

Lower pretransfusion concentration of C1q was significantly associated with increased odds of pneumonia. It is unclear whether this association was causal (ie, did increased concentration of C1q prevent pneumonia or was C1q simply a marker of other factors such as innate immune function that contributed to disease resistance), but complement is known to play an important role in antibody-mediated killing of *R equi* 

The finding that pretransfusion concentrations were important indicates that transfusion of REHIP was not essential for this effect; the pretransfusion differences in concentration of C1q among foals likely reflect differences in endogenous production of C1q by the foal and transferred via colostrum. The finding that lower posttransfusion concentration of C1q in foals was not associated with increased odds of developing rhodococcal pneumonia was surprising because pre- and posttransfusion concentrations were highly correlated (Supplemental Figure S1). It is possible that we simply lacked the power to detect a difference that existed or that the dilutional effects of transfusion of REHIP somehow masked the effect. The latter seems unlikely because lower posttransfusion concentrations of C1q were not associated with increased odds of rhodococcal pneumonia irrespective of whether we used the median of the pre- or posttransfusion concentrations of C1q to define the cut-off point for analysis. Further investigation is warranted to determine the validity of our results.

Lower activity levels of IgG, but not IgG4/7 recognizing VapA were associated with increased odds of pneumonia in this study. Although some conflicting evidence exists, results of this study support other evidence that the IgG1 subisotype, although lower in absolute concentration both in REHIP and foal serum, is particularly important for mediating protection against rhodococcal pneumonia. Currently, the potency of REHIP is assessed for licensure by the USDA by the activity of total IgG recognizing VapA. Assessing the activity of anti-VapA IgG1 activity might be a more appropriate measure of the potency of REHIP. IgG1 activity against VapA might also be useful for assessing rhodococcal vaccines.

Foaling during April or May was associated with increased odds of rhodococcal pneumonia in this study. This finding corroborates prior results including foals born at the farms included in this study. The reasons for this association are unknown. The density of mares and foals is increased later in the foaling season, and increased density of mares and foals has been associated with increased odds of rhodococcal pneumonia. Greater numbers of mares and foals later in the season might lead to greater environmental contamination with virulent *R equi*. Finally, warmer ambient temperatures in later months might favor either growth of *R equi* in the environment at horse farms or conditions that increase airborne virulent *R equi*, leading to greater risk of inhalation of virulent bacteria.

Although farm was not significantly associated with rhodococcal pneumonia, we elected to include it in our final model to account for farm effects. Results were similar, however, when farm was not included.

This study had a number of limitations. Only a single breed of horses at 2 farms in a single region...
were studied. The extent to which our results can be generalized to other breeds and regions is unknown and cannot be assumed. Nevertheless, our findings are consistent with reports we have cited from other regions in other breeds.\textsuperscript{1,2,11,12,16-21,46,48} Foals were transfused at varying ages, and the median age at which foals were transfused is younger than the age at which colostral absorption is considered to end. Although this likely contributed to some of the variability in anti-VapA antibody activities observed in this study, we do not believe it vitiates the significant associations observed in this study for the following reasons. First, posttransfusion samples had to be considerably diluted for ELISA testing indicating that anti-VapA activities of IgG\textsubscript{1} and IgG\textsubscript{4/7} were markedly higher after transfusion than those delivered via colostrum, suggesting that additional colostral antibody absorption was unlikely to markedly change posttransfusion concentrations. More importantly, for this factor to have affected the association between antibody activity levels and disease there would have had to be a differential bias such that foals that did not develop pneumonia were more likely to have been transfused earlier than foals that developed pneumonia. However, the age at transfusion for foals that did not develop pneumonia (median age, 14.0 hours; range, 8 to 41 hours) did not differ significantly (\(P = .8756\); Wilcoxon rank-sum test) from that of foals that developed pneumonia (median age, 14.75 hours; range, 9 to 32 hours). We collected serum samples immediately after transfusion. Collecting samples longer after transfusion would have allowed for equilibration of fluid shifts and circulation of transfused proteins. Collecting samples immediately after transfusion was dictated by convenience for sampling. Thus, we do not know how long concentrations of C1q remained decreasing following transfusion in most foals. The effect of transfusion on C1q concentrations was relatively small: the mean difference in C1q concentration of undiluted serum samples after transfusion was 8% of the population average (18 μg/dL/218 μg/dL), and nearly 25% of foals in fact had an increase in serum C1q concentration (Figure 2). In contrast, we had to dilute serum samples severalfold after transfusion to measure anti-R\textit{equi} antibody concentrations, indicating that the effects of transfusion on C1q concentrations were relatively small. Moreover, C1 concentrations before and after transfusion values were highly correlated indicating that pretransfusion values were the principal determinant of posttransfusion concentrations. Regarding the association of C1q concentrations with disease, the difference between C1q concentrations before and after transfusion did not differ significantly between foals that developed pneumonia and those that did not (data not shown), whereas pretransfusion concentrations of C1q were significantly associated with the odds of pneumonia developing.

We did not evaluate other components of complement. It is possible and plausible that, for example, serum concentrations of C3 also are associated with rhodococcal pneumonia. We lacked the resources to test additional complement proteins, but we hope to be able to do this in the future. Anti-VapA activity levels were relative and not absolute. This precluded us from directly determining the magnitude of the increase in concentration of each IgG subisotype after transfusion of REHIP. Experiments we conducted to establish dilutions used for testing sera in this study indicated that posttransfusion samples had to be diluted considerably relative to pretransfusion samples, indicating higher concentrations of both subisotypes after transfusion. Diagnosis of \textit{R equi} pneumonia was not confirmed by microbiologic culture and cytologic evaluation of tracheobronchial aspirate fluid from all foals; however, \textit{R equi} was isolated from a small number of foals (n = 7), and the 2 farms have a history of confirmed rhodococcal pneumonia. It is also unclear whether misclassification would have been differential for foals with lower serum concentrations of C1q or serum activity of anti-VapA IgG\textsubscript{1} that would confound our findings. Both C1q concentrations and anti-VapA antibody activities were highly variable in our study. This high degree of variation could reflect differences between lots or batches of plasma in antibody activity\textsuperscript{43} or C1q concentrations or technical factors such as extent of hemolysis, sample handling and processing, conditions and technique during ELISA testing, and variation in antigen coating on plates. We have observed similar variation in prior studies\textsuperscript{49,50} of antibody activities. We did not evaluate other isotypes of IgG or subisotypes of IgG, such as IgG\textsubscript{4/7}. Prior results\textsuperscript{13,22-29,41-43} led us to focus on these subisotypes. Ideally, our study would have included a group of foals that were not transfused for comparative purposes, particularly for the association of pretransfusion complement C1q concentrations with pneumonia. The farms participating in this study, however, were not willing to withhold plasma transfusion from foals. The small magnitude of the effect of transfusion on C1q concentrations and the finding that pre- and posttransfusion concentrations were highly correlated indicate that the association of pretransfusion concentrations with C1q is not spuriously influenced by the effects of transfusion.

Despite the limitations of this study, it provides further evidence that complement contributes to protection against \textit{R equi} and that IgG\textsubscript{1} appears to play a more important role than IgG\textsubscript{4/7} in protecting foals against rhodococcal pneumonia. This information is useful both for producing and assessing the potency of REHIP and possibly for evaluating immune responses to rhodococcal vaccines. It also indicates that the inherent resistance of some foals to \textit{R equi} might be mediated by complement or other immune factors coassociated with C1q concentration.

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References

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Supplementary Materials

Supplementary materials are posted online at the journal website: avmajournals.avma.org