Pneumonia caused by *Rhodococcus equi* remains an important cause of disease and death in foals on horse breeding farms throughout the world. Results of previous studies conducted to evaluate the airborne concentration of virulent *R equi* have provided important information in regard to the epidemiology of *R equi*. These findings include an increase in the airborne concentration of virulent *R equi* as ambient temperature increases; higher concentrations of virulent *R equi* in air samples collected from barns, compared with that in air samples collected from paddocks; and a positive correlation between the airborne concentration of virulent *R equi* and the prevalence of *R equi* pneumonia in horses on breeding farms. However, in a study conducted on 2 horse breeding farms in central Kentucky that had histories of foals affected by *R equi* pneumonia, the concentration of virulent *R equi* in air samples collected from stalls did not differ significantly from that in air samples collected from paddocks, and the airborne concentration of virulent *R equi* did not vary significantly by month. In contrast, results of a study that was conducted at another horse breeding farm in central Kentucky indicated that virulent *R equi* was more likely to be detected in air samples collected from stalls versus paddocks and earlier (January and February) versus later (May and June) during the foaling season. (Am J Vet Res 2012;73:1603–1609)
from stalls used to house mares and neonatal foals than in air samples collected from paddocks used to confine those same mares and foals.

The objectives of the study reported here were to determine whether the concentrations of virulent \textit{R equi} in air samples collected from horse breeding farms in central Kentucky were associated with the location (stall vs paddock) from which the samples were obtained and whether airborne concentrations of virulent \textit{R equi} varied by month during the foaling season (January through June). Additionally, the association between the number of virulent \textit{R equi}-positive air samples obtained from stalls and the cumulative incidence of foals with \textit{R equi} pneumonia at horse breeding farms was determined.

**Materials and Methods**

**Farms**—In 2008, horse breeding farms were recruited for the study from the clientele of Hagyard Equine Medical Institute in Lexington, Ky, by 2 investigators (NMS and SEB). To be included in the study, a farm had to have at least 15 mares expected to reside and foal on the farm during January through June 2009. Efforts were made to recruit farms that did not screen foals for early detection of \textit{R equi} pneumonia by use of ultrasonography or other methods. Also, farm management had to grant permission for 2 other investigators (JLS and RSS) to collect air samples monthly from January through June 2009. Thirty farms met the eligibility requirements and were enrolled in the study.

**Sample collection**—At each farm, 1 stall in a barn that was used to house mares and neonatal foals and 1 paddock that was used to confine the same group of mares and foals were identified for sampling. Because evidence indicates that foals are most susceptible to becoming infected with \textit{R equi} early in life,1⁰,¹¹ air samples were collected from each stall and paddock monthly from January through June 2009, the period during which most foals would be born and reach 1 month of age on these farms. All farms were sampled within the first 4 days of each month and all air samples were collected between 8 am and noon. Mares and foals were present in the stalls and paddocks at all sampling times. Air samples obtained from paddocks were collected within 9 m of the gate because that was the area of the paddock where mares and foals tended to congregate.

Air samples were collected as described³ by use of a portable air sampling device⁴ and petri dishes (diameter, 100 mm) containing NANAT, a selective culture media commonly used for the detection of \textit{R equi} in epidemiological studies.⁸,⁹,¹²,¹³ To approximate the methods used for air sample collection in other studies,⁸,⁹,¹³ the air sampling device was positioned to collect air at approximately 10 cm above the stall floor or paddock ground. Prior to each air sample collection, the sieve of the air sampling device was disinfected with an isopropanol wipe.⁴ For each sample, 500 L of air was aspirated onto a modified NANAT culture plate at a rate of 100 L/min. At the time of each sample collection, another handheld device⁴ was used to measure and record ambient temperature, relative humidity, and wind speed.

**Modified colony immunoblot assay**—The modified NANAT culture plates were chilled and transported in insulated containers with icepacks to the Equine Infectious Disease Laboratory at Texas A&M University. Upon receipt at the laboratory, the culture plates were incubated at 37°C for 48 hours. The plates were then visually inspected for the presence of bacterial colonies with morphological characteristics consistent with those of \textit{R equi}.

For modified NANAT culture plates that had at least 1 bacterial colony with the morphological characteristics of \textit{R equi}, a modified colony immunoblot assay was performed as described¹⁰,¹¹ to determine the concentration of virulent \textit{R equi}. Briefly, the modified immunoblot assay detects virulence-associated protein A, which allows for the quantification of virulent \textit{R equi} within a background of bacterial and fungal contamination.¹⁰,¹¹ For each plate assayed, a nitrocellulose membrane was placed on the modified NANAT culture medium until it was completely saturated. The nitrocellulose membrane was then removed from the culture medium, air-dried at room temperature (approx 22°C) for 30 minutes, baked in a hybridization oven at 100°C for 1 minute, and incubated in a 5% nonfat dry milk solution diluted in TBSS at 37°C for 1 hour to block unbound sites. Then, the nitrocellulose membrane was washed 3 times (10 min/wash) in TBSS with 0.05% Tween 20. Membranes were incubated at 4°C overnight (approx 18 hours) on a rocking platform with monoclonal antibody diluted 1:10,000 in a 5% nonfat milk solution and then washed 3 times (10 min/wash) with fresh TBSS at 37°C. Horseradish peroxidase-conjugated goat IgG fraction against mouse IgG was diluted 1:2,000 in 5% nonfat dry milk solution and added to the membrane, which was then incubated at 37°C for 1 hour. The membrane was washed 3 times (10 min/wash) with fresh TBSS at 37°C and then washed once with citrate-EDTA buffer (10mM sodium citrate and 10mM EDTA [pH, 5.0]) at 37°C for 5 minutes, once with citrate-EDTA buffer and 1% dextran sulfate at 37°C for 10 minutes, and 3 times (5 min/wash) with citrate-EDTA buffer at 37°C. The substrates 3,3′,5′-tetramethylbenzidine and hydrogen peroxide were added to the membrane, and the membrane was then incubated at 37°C for 30 minutes or until development of the positive control membrane was considered sufficient. The substrate was discarded and distilled water was added to the membrane to stop development. Virulent \textit{R equi} colonies appeared blue, whereas colonies of avirulent \textit{R equi} and contaminant bacteria remained colorless.

For each batch of modified colony immunoblot assays performed, a positive and a negative control specimen were included. The positive control was obtained from a pure culture of virulent \textit{R equi} (ATCC strain 33701) grown on a modified NANAT culture plate, and the negative control was obtained from a pure culture of avirulent \textit{R equi} (ATCC strain 33703) grown on a modified NANAT culture plate. The pure culture strains had been grown in \textit{R equi} minimal media,⁶ and 10-fold serial dilutions were performed with PBS solution.⁶ For both the positive and negative controls, 100 mL of the dilution (approx 10⁶ CFUs of \textit{R equi}/mL) was inoculated onto a modified NANAT culture plate. The culture
plates containing the control specimens were incubated and processed in the same manner as the plates containing the air samples.

Airborne concentrations of virulent *R. equi* were expressed as CFUs/m$^3$ of air and calculated as ($T \times X$) / ($F \times t$), where *T* is the number of CFUs of *R. equi* counted on the membrane, *t* is time (5 minutes) during which the air sample was collected, and *F* is the rate of airflow (100 L/min) through the sampling device.13

**Data collection**—The following data were collected from each participating farm for 2009: number of mares residing at the farm, number of foals residing at the farm, and number of foals that developed *R. equi* pneumonia. For this study, *R. equi* pneumonia was diagnosed if a foal developed clinical signs consistent with pneumonia (ie, cough, dyspnea, fever, lethargy, nasal discharge, or tachypnea) between 3 and 24 weeks of age and had at least 2 of the following diagnostic findings: evidence of pulmonary consolidation or abscess formation as determined by ultrasonography, isolation of *R. equi* from a tracheobronchial aspirate or postmortem lung specimen via microbiologic culture, or evidence of gram-positive, intracellular coccobacilli as determined by cytologic evaluation of a tracheobronchial aspirate.

**Data analysis**—The primary outcome of interest was the airborne concentration of virulent *R. equi*, which was measured and expressed as integers with values $\geq 0$. Most of the air samples yielded no isolates of virulent *R. equi* (ie, the data contained many zeros), and a random-effects zero-inflated Poisson model did not provide a good fit for the data. Therefore, a dichotomous outcome variable was created such that results of the assayed air samples were classified as either positive or negative for virulent *R. equi*. The proportion of virulent *R. equi*-positive air samples obtained from stalls was compared with the proportion of virulent *R. equi*-positive air samples obtained from paddocks by means of a $\chi^2$ test. Because the $\chi^2$ test did not account for correlation among samples obtained from a given location on a given farm, the association of location (stall vs paddock) with the presence of virulent *R. equi* in air samples was evaluated with a random-effects logistic regression model, in which farm was modeled as a random effect and location was modeled as a fixed effect.17

Random-effects logistic regression was also used to evaluate the association of month of air sample collection with the presence of virulent *R. equi* in air samples. Farm was again modeled as a random effect. Instead of analyzing month of collection as a 6-level categorical variable, it was blocked on the basis of similar mean daily temperatures (for 2009) into three 2-month periods (January and February, March and April, and May and June) to enhance the power to detect an association. Post hoc pairwise comparisons among the 2-month periods were performed via the method of Sidak.19

A generalized linear model was used to evaluate the association between the number of air samples obtained from the stall that were positive for virulent *R. equi* on a farm and the number of foals with *R. equi* pneumonia and the cumulative incidence of foals with *R. equi* pneumonia (number of foals with *R. equi* pneumonia/number of foals that resided on the farm during 2009). Each farm was classified into 1 of 3 categories ($\leq 1$, $2$, or $\geq 3$) in accordance with the number of air samples obtained from stalls that were positive for virulent *R. equi*. A logarithmic transformation was applied to the data for the number of foals with *R. equi* pneumonia to normalize its distribution; 0.1 was added to all values of 0 so the logarithmic transformation could be performed. All analyses were performed with the use of statistical software,$^9$ and values of $P < 0.05$ were considered significant.

**Results**

**Farms**—The 30 study farms were located in 5 counties in central Kentucky near Lexington. The farms ranged in size from 28 to 647 hectares (median, 192 hectares). Horses had been housed on the farms for a median of 24 years (range, 1 to 130 years). During 2009, 2,956 mares and 2,064 foals were resident on the 30 farms. The number of mares on each farm ranged from 15 to 350 (median, 70) and the number of foals ranged from 14 to 150 (median, 58). Transthoracic ultrasonography was used on 5 of 30 (16.7%) farms to screen foals for *R. equi* pneumonia. *Rhodococcus equi* pneumonia was diagnosed in accordance with the study criteria in at least 1 foal on 20 of 30 (66.7%) farms. On those 20 farms, the number of foals affected with *R. equi* pneumonia ranged from 1 to 11 (median, 3) and the cumulative incidence of foals with *R. equi* pneumonia ranged from 0.7% to 25.0% (median, 5.3%). Virulent *R. equi* was not identified in any air sample (stall or paddock) collected from 2 farms; however, *R. equi* pneumonia was diagnosed in 1 foal on each of those farms during the study period, which resulted in cumulative incidence rates for *R. equi* pneumonia on those farms of 0.7% (1/150) and 1% (1/105).

**Association of airborne virulent *R. equi* and location from which air sample was obtained**—Virulent *R. equi* was identified in 49 of 180 (27.2%) of air samples collected from stalls and in 13 of 180 (7.2%) air samples collected from paddocks (Table 1). On 2 farms, virulent *R. equi* was not detected in any air sample (stall or paddock) collected. On 1 farm, virulent *R. equi* was identified more frequently from air samples obtained from paddocks than from stalls. Virulent *R. equi* was more frequently isolated from air samples collected from paddocks than from stalls. Airborne *R. equi* was isolated from 13 of 180 (7.2%) air samples collected from stalls and in 13 of 180 (7.2%) air samples collected from paddocks (Table 1). On 2 farms, virulent *R. equi* was not detected in any air sample (stall or paddock) collected. On 1 farm, virulent *R. equi* was identified more frequently from air samples obtained from paddocks than from stalls. Airborne *R. equi* was isolated from 13 of 180 (7.2%) air samples collected from stalls and in 13 of 180 (7.2%) air samples collected from paddocks (Table 1). On 2 farms, virulent *R. equi* was not detected in any air sample (stall or paddock) collected. On 1 farm, virulent *R. equi* was identified more frequently from air samples obtained from paddocks than from stalls.

**Table 1—Number (percentage) of virulent *Rhodococcus equi*-positive air samples obtained from stalls and paddocks on 30 horse breeding farms in central Kentucky by month from January through June 2009.**

<table>
<thead>
<tr>
<th>Month</th>
<th>No. (%) of air samples from stalls positive for virulent <em>R. equi</em></th>
<th>No. (%) of air samples from paddocks positive for virulent <em>R. equi</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>January</td>
<td>10 (33)</td>
<td>2 (7)</td>
</tr>
<tr>
<td>February</td>
<td>15 (50)</td>
<td>1 (3)</td>
</tr>
<tr>
<td>March</td>
<td>9 (30)</td>
<td>3 (10)</td>
</tr>
<tr>
<td>April</td>
<td>9 (30)</td>
<td>2 (7)</td>
</tr>
<tr>
<td>May</td>
<td>4 (13)</td>
<td>3 (10)</td>
</tr>
<tr>
<td>June</td>
<td>2 (7)</td>
<td>2 (7)</td>
</tr>
</tbody>
</table>

For each farm, an air sample was collected approximately 10 cm above the ground surface from 1 stall located in a barn used to house mares and neonatal foals and 1 paddock used to confine those same mares and foals; air samples were collected monthly from the same stall and paddock throughout the study. Virulent *R. equi* was identified in air samples via a modified colony immunoblot assay.
from the paddock than from air samples obtained from the stall. The proportion of air samples that yielded positive results for virulent \textit{R. equi} was significantly \((P < 0.001)\) greater for stalls than that for paddocks as determined by \(\chi^2\) analysis. When a random-effects logistic regression model was used to account for correlation among repeated measures within location on farm, virulent \textit{R. equi} was more likely to be identified in an air sample obtained from a stall than from an air sample obtained from a paddock \((\text{OR, 5.2; 95\% CI, 2.1 to 13.1; } P < 0.001)\). The mean concentration of virulent \textit{R. equi} was 4.3 CFUs/m\(^3\) (95\% CI, 3.2 to 5.4 CFUs/m\(^3\)) in air samples obtained from stalls and 2.5 CFUs/m\(^3\) (95\% CI, 1.9 to 3.0 CFUs/m\(^3\)) in air samples obtained from paddocks. The airborne concentration of virulent \textit{R. equi} was significantly \((P < 0.001)\) greater in samples obtained from stalls than that in samples obtained from paddocks as determined by a generalized linear model.

Association of airborne virulent \textit{R. equi} with the 2-month period during which the air sample was collected—Statistical analysis to determine whether identification of virulent \textit{R. equi} in an air sample obtained from a paddock was associated the 2-month period during which the air sample was collected was not performed because only 13 air samples obtained from paddocks yielded positive results for virulent \textit{R. equi}. Subjectively, the proportion of virulent \textit{R. equi}–positive air samples obtained from paddocks did not vary much by month (Table 1), whereas the proportion of virulent \textit{R. equi}–positive air samples obtained from stalls appeared to be lowest during May and June. The odds of virulent \textit{R. equi} being identified in an air sample obtained from a stall in May and June were 78\% less than that in an air sample obtained from a stall in January and February \((\text{OR, 0.22; 95\% CI, 0.08 to 0.63; } P = 0.005)\) as determined by use of random-effects logistic regression (Table 2). Although the proportion of virulent \textit{R. equi}–positive air samples collected from stalls was lower for the months of March and April (30\%) than that for January and February (42\%), the 2 proportions did not differ significantly \((P = 0.269)\). Similarly, the proportion of virulent \textit{R. equi}–positive air samples collected from stalls was lower in May and June (10\%) than that in March and April (30\%), and these 2 proportions did not differ significantly.

The odds of virulent \textit{R. equi} being identified in an air sample were also affected by an interaction between location and the 2-month period during which an air sample was collected. Virulent \textit{R. equi} was significantly \((P = 0.041)\) more likely to be identified in an air sample obtained from a stall versus an air sample obtained from a paddock during January and February, whereas during May and June, the likelihood of identifying virulent \textit{R. equi} did not differ between an air sample obtained from a stall and an air sample obtained from a paddock.

Random-effects linear regression revealed that the airborne concentration of virulent \textit{R. equi} in January and February \((2.2 \text{ CFU/m}^3; 95\% \text{ CI, 1.5 to 2.9 CFU/m}^3)\) was significantly higher, compared with that for March and April \((1.2 \text{ CFU/m}^3; 95\% \text{ CI, 0.5 to 1.9 CFU/m}^3; P = 0.048)\) and that for May and June \((0.3 \text{ CFU/m}^3; 95\% \text{ CI, 0 to 1.3 CFU/m}^3; P < 0.001)\). Although the airborne concentration of virulent \textit{R. equi} for May and June was lower than that for March and April, the difference in airborne virulent \textit{R. equi} concentrations between these 2 periods was not significant.

Association of airborne virulent \textit{R. equi} with environmental conditions at the time of sample collection—The association of virulent \textit{R. equi} with wind speed at the time air samples were collected from stalls was not evaluated because the wind speed at the time of sample collection was 0 for all except 3 samples. The mean \(\pm\) SD humidity at the time of air sample collection from stalls was similar for virulent \textit{R. equi}–positive air samples (56.7 \(\pm\) 11.9\%) and virulent \textit{R. equi}–negative air samples (59.6 \(\pm\) 13.0\%). Likewise, mean \(\pm\) SD temperature at the time of air sample collection from stalls did not differ between virulent \textit{R. equi}–positive air samples \((13.2 \pm 6.08^\circ \text{C})\) and virulent \textit{R. equi}–negative air samples \((15.1 \pm 6.48^\circ \text{C}; P = 0.251)\) as determined via random effects logistic regression.

Association of airborne virulent \textit{R. equi} with cumulative incidence of \textit{R. equi} pneumonia in foals—During the study period, \textit{R. equi} pneumonia was not diagnosed in any foal on the farm from which virulent \textit{R. equi} was more frequently identified in air samples obtained from the paddock than in air samples obtained from the stall. Thirteen farms were classified as having \(\leq 1\) virulent \textit{R. equi}–positive air samples obtained from stalls; 10 farms each had 2 virulent \textit{R. equi}–positive air samples obtained from stalls, and 7 farms each had \(\geq 3\) virulent \textit{R. equi}–positive air samples obtained from stalls. Two farms had 0 virulent \textit{R. equi}–positive air samples obtained from stalls, and no farms had \(> 3\) virulent \textit{R. equi}–positive air samples obtained from stalls. For farms classified as having \(\leq 1\) virulent \textit{R. equi}–positive air samples obtained from stalls, the mean \(\pm\) SD number of foals that developed \textit{R. equi} pneumonia during the study period was \(1.4 \pm 1.4\), whereas that for farms with \(2\) virulent \textit{R. equi}–positive air samples obtained from stalls was \(2.1 \pm 4.0\) and that for farms with \(\geq 3\) virulent \textit{R. equi}–positive air samples obtained from stalls was \(3.6 \pm 3.0\). The number of foals with \textit{R. equi} pneumonia did not differ significantly among the farm classification groups. For farms classified as having \(\leq 1\) virulent \textit{R. equi}–positive air samples obtained from stalls, the mean cumulative incidence rate of \textit{R. equi} pneumonia in foals was 1.0\% (95\% CI, 0.2\% to 3.4\%), whereas that for farms with \(2\) virulent \textit{R. equi}–positive air samples obtained from stalls was 1.2\% (95\% CI, 0.2\% to 6.0\%) and that for farms with \(\geq 3\) virulent \textit{R. equi}–positive air samples obtained from stalls was 2.8\% (95\% CI, 0.6\% to 6.48\%)

<table>
<thead>
<tr>
<th>2-month period</th>
<th>OR</th>
<th>95% CI</th>
<th>(P) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>January–February</td>
<td>Referent</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>March–April</td>
<td>0.53</td>
<td>0.17–1.67</td>
<td>0.269</td>
</tr>
<tr>
<td>May–June</td>
<td>0.22</td>
<td>0.08–0.63</td>
<td>0.005</td>
</tr>
</tbody>
</table>

— = Not applicable.
See Table 1 for remainder of key.

Table 2—Odds ratio for the presence of virulent \textit{R. equi} in air samples obtained from stalls used to house mares and neonatal foals on 30 horse breeding farms in central Kentucky by each 2-month period as determined by random-effects logistic regression.
In contrast to the results of another study that was conducted by our laboratory group on 2 other horse breeding farms in Kentucky. The reason results vary among farms in central Kentucky, each of which was classified into 1 of 3 categories (≤ 1 [n = 13], 2 [10], or ≥ 3 [7]) in accordance with the number of virulent *R. equi*-positive air samples that were obtained from stalls from January through June 2009. From each farm, an air sample was obtained approximately 10 cm above the floor surface of 1 stall located in a barn that was used to house mares and neonatal foals; air samples were obtained monthly from the same stall throughout the study. Virulent *R. equi* were identified in air samples via a modified colony immunoblot assay. The criteria for a diagnosis of *R. equi* pneumonia were a foal between 3 and 24 weeks of age that developed clinical signs consistent with pneumonia (ie, cough, dyspnea, fever, lethargy, nasal discharge, or tachypnea) and that had at least 2 of the following diagnostic findings: evidence of pulmonary consolidation or abscess formation as determined by ultrasonography, isolation of *R. equi* from a tracheobronchial aspirate or postmortem lung specimen via microbiological culture, or evidence of gram-positive, intracellular coccobacilli as determined by cytologic evaluation of a tracheobronchial aspirate. For each farm, the cumulative incidence of *R. equi* pneumonia in foals was calculated as the number of foals that developed *R. equi* pneumonia on the farm in 2009 divided by the number of foals that resided on the farm in 2009. Each box represents the interquartile range (25th to 75th percentiles), the horizontal line in each box represents the median, and the vertical line and brackets (whiskers) demarcate 1.75 times the interquartile range.

As the number of virulent *R. equi*-positive air samples obtained from stalls increased, the cumulative incidence of *R. equi* pneumonia in foals also increased (Figure 1); however, the cumulative incidence of *R. equi* pneumonia in foals was calculated as the number of foals that developed *R. equi* pneumonia on the farm in 2009 divided by the number of foals that resided on the farm in 2009. Each box represents the interquartile range (25th to 75th percentiles), the horizontal line in each box represents the median, and the vertical line and brackets (whiskers) demarcate 1.75 times the interquartile range.

Figure 1—Box-and-whiskers plot of the cumulative incidence of *Rhodococcus equi* pneumonia in foals on 30 horse breeding farms in central Kentucky, which each of which was classified into 1 of 3 categories (≤ 1 [n = 13], 2 [10], or ≥ 3 [7]) in accordance with the number of virulent *R. equi*-positive air samples that were obtained from stalls from January through June 2009. From each farm, an air sample was obtained approximately 10 cm above the floor surface of 1 stall located in a barn that was used to house mares and neonatal foals; air samples were obtained monthly from the same stall throughout the study. Virulent *R. equi* were identified in air samples via a modified colony immunoblot assay. The criteria for a diagnosis of *R. equi* pneumonia were a foal between 3 and 24 weeks of age that developed clinical signs consistent with pneumonia (ie, cough, dyspnea, fever, lethargy, nasal discharge, or tachypnea) and that had at least 2 of the following diagnostic findings: evidence of pulmonary consolidation or abscess formation as determined by ultrasonography, isolation of *R. equi* from a tracheobronchial aspirate or postmortem lung specimen via microbiological culture, or evidence of gram-positive, intracellular coccobacilli as determined by cytologic evaluation of a tracheobronchial aspirate. For each farm, the cumulative incidence of *R. equi* pneumonia in foals was calculated as the number of foals that developed *R. equi* pneumonia on the farm in 2009 divided by the number of foals that resided on the farm in 2009. Each box represents the interquartile range (25th to 75th percentiles), the horizontal line in each box represents the median, and the vertical line and brackets (whiskers) demarcate 1.75 times the interquartile range.

Discussion

Results of the present study indicated that the concentration of virulent *R. equi* was higher in air samples that were collected from stalls than in air samples that were collected from paddocks at the horse breeding farms in central Kentucky that were evaluated. These results were consistent with results from a study that was conducted on 3 horse breeding farms in Ireland and a study that was conducted by our laboratory group on 1 horse breeding farm in Kentucky but were in contrast to the results of another study that was conducted by our laboratory group on 2 other horse breeding farms in Kentucky. The reason results vary among these studies is unclear, but 1 possible explanation is that factors (eg, barn ventilation) specific to each farm caused variations in the airborne concentration of virulent *R. equi*. Indeed, even among the farms of the present study, 1 farm differed from the rest of the study farms in that virulent *R. equi* was identified more frequently in air samples obtained from the paddock than from air samples obtained from the stall. Another explanation for the conflicting results among studies is that the cumulative incidence of *R. equi* pneumonia in foals varied among farms, which likely was a reflection of the various criteria used to diagnose *R. equi* pneumonia as well as the environmental concentration of *R. equi* on each farm. Because foals generally become infected with virulent *R. equi* via inhalation, the fact that virulent *R. equi* was identified more frequently in air samples obtained from stalls than in air samples obtained from paddocks suggests that young foals may be at greater risk of becoming infected with *R. equi* when they are housed in a barn than when they are confined in a paddock. Thus, strategies that limit the amount of time that foals spend in stalls in barns or that reduce the airborne concentrations of virulent *R. equi* in stalls should decrease the incidence of *R. equi* pneumonia in foals.

In the present study, the odds of air samples yielding positive results for virulent *R. equi* was significantly lower in the 2-month period of May and June relative to the 2-month period of January and February; the odds of air samples yielding positive results for virulent *R. equi* was also lower in the 2-month period of March and April relative to the 2-month period of January and February, but this result was not significant. The reasons for these findings are unknown. In Australia, a warmer ambient temperature was associated with an increased concentration of virulent *R. equi* in air samples that were collected from outdoor areas (ie, paddocks and lanes). In the present study, virulent *R. equi* was more frequently identified in air samples obtained from stalls within barns. As the temperature became warmer, it is likely that windows and doors within the barns were opened, which likely improved ventilation and air exchange in the barns so that there were fewer dust particles on which *R. equi* could become airborne. Alternatively, it is probable that the number of mares and foals housed in the barns decreased as the foaling season progressed such that the concentration and activity of horses and farm personnel in the barns were reduced, which in turn reduced the concentration of airborne virulent *R. equi* in stalls. Unfortunately, information regarding the proportional occupancy of horses in the study barns was not collected; however, anecdotally, mares and foals spend less time in barns during May and June than during January and February on breeding farms in central Kentucky. The results of the present study indicate that foals are at greatest risk of being exposed to virulent *R. equi* during the earlier months of the year (January and February); therefore, the implementation of management practices that reduce the environmental concentration of virulent *R. equi* during that time is important for reducing the risk of *R. equi* pneumonia in foals.

An interaction was identified between location (stall or paddock) and the 2-month period during which air samples were collected. During January and
February, virulent *R equi* was more likely to be identified in an air sample obtained from a stall than in an air sample obtained from a paddock; however, during May and June, virulent *R equi* was just as likely to be identified in an air sample obtained from a stall as from an air sample obtained from a paddock. The implication of this interaction on the control and prevention of *R equi* pneumonia remains to be determined and further research is necessary to determine whether such an interaction is consistent on all horse breeding farms.

To our knowledge, only 1 study had been conducted to evaluate the association between airborne concentrations of virulent *R equi* on horse breeding farms and the cumulative incidence of *R equi* pneumonia in foals on those farms. In that study, air samples were obtained from outdoor areas (ie, paddocks and lanes) only, and the results indicated that the cumulative incidence of *R equi* pneumonia in foals increased as the airborne concentration of virulent *R equi* increased. Results of the present study suggested a similar (albeit nonsignificant) association between the airborne concentration of virulent *R equi* and the cumulative incidence of *R equi* pneumonia in foals. Further study to address this important issue is warranted.

It was not possible to make any inferences about the temporal pattern for the detection of virulent *R equi* in air samples obtained from stalls and the cumulative incidence of *R equi* pneumonia in foals of the present study because of the study design. In retrospect, it would have been advantageous to have collected the data necessary to calculate the monthly incidence rate of *R equi* pneumonia in foals for each farm; however, that information would have provided limited additional evidence of a causal relationship between *R equi* exposure and the development of pneumonia because of the unknown incubation period for *R equi*. Moreover, a causal relationship between a pathogen and a disease would be better evaluated at the individual animal level rather than at the farm level.

The present study had a number of limitations. One of those limitations was that only a single stall and a single paddock were sampled at each farm. Unfortunately, sampling all stalls and paddocks at a large number of farms would have required funding beyond what was available for the study reported here. Another study limitation was that the incidence of *R equi* pneumonia in foals was reported to study investigators by the farms' veterinarians and was not validated. It is possible some foals were misclassified as either having or not having *R equi* pneumonia, and that misclassification may have resulted in under- or overreporting of the cumulative incidence of *R equi* pneumonia in foals. Some misclassification of disease is inevitable; however, we believe the case definition and extensive clinical experience of the farms' veterinarians minimized the impact of misclassification in the present study. Additionally, the median cumulative incidence of *R equi* pneumonia in foals (5%) on affected farms in the present study was similar to that (7%) reported by investigators of a study that was conducted on horse breeding farms in Texas, which suggested that cumulative incidence rate of *R equi* pneumonia in foals for the present study was not under- or overreported.

Exposure of foals to *R equi* was estimated by the collection of air samples approximately 10 cm above the ground surface. This sampling method was used to approximate those used in other studies. It could be argued that air samples collected at the nares of foals might be a more relevant sample. Air sampling devices that can be fitted to halters and harnesses exist, but we did not have the resources or consent to apply such devices to the foals at the study farms. Young foals spend a substantial proportion of time (approx 32% to 39%) recumbent; therefore, collecting air samples approximately 10 cm above the ground surface was deemed clinically and biologically relevant. Nevertheless, it would have been informative to have compared the concentration of *R equi* in air samples collected at 10 cm above the ground surface with that in air samples collected at the approximate height of the nares of a standing neonatal foal.

Although we quantified the concentration of virulent *R equi* in all air samples, the data were analyzed as having a dichotomous outcome (the presence or absence of virulent *R equi*). The *R equi* concentration data was dichotomized because a random-effects Poisson regression model did not fit the raw *R equi* concentration data well, whereas a random-effects logistic regression model provided a better fit to the dichotomous *R equi* concentration data and results that were consistent with the observed data. A larger sample size might have improved the power of the present study and made repeated-measures analyses of zero-inflated data possible, but that was beyond the scope of the study. Because virulent *R equi* was identified in only a few air samples obtained from paddocks, inferences about the exposure of foals to *R equi* in paddocks could not be made. In our experience, increasing the volume of air sampled does not improve the likelihood of obtaining positive *R equi* results because of contamination of the culture plates by other bacteria, yeast, and fungi, despite the use of selective media. Also, the significant difference in the concentration of virulent *R equi* in air samples obtained from stalls, compared with that in air samples obtained from paddocks that was determined via the generalized linear model, should be interpreted with caution because, although the model fit the data well, the large number of 0 values resulted in residuals that were not normally distributed, a violation of one of the underlying assumptions required for the model.

The present study did not have enough power to identify a significant association between the number of virulent *R equi*–positive air samples obtained from the stall on each farm and the number of foals with *R equi* pneumonia or the cumulative incidence of *R equi* pneumonia in foals, respectively. The results of the present study suggested that the incidence of *R equi* pneumonia in foals increased as the number of virulent *R equi*–positive air samples obtained from stalls increased, and these results were consistent with those of a previous study; however, further research is needed to confirm this association. Additionally, evidence to definitively prove that a causal relationship exists between exposure of foals to airborne virulent *R equi* and subsequent development
of R equi pneumonia in those foals will require studies in which individual foals are the observational units rather than farms.

Five farms in the present study screened foals for R equi pneumonia via ultrasonography. On those 5 farms, foals with ultrasonographic evidence of pulmonary consolidation or abscess formation were treated with an antimicrobial regardless of whether they had clinical signs of pneumonia and without obtaining a tracheobronchial aspirate for culture and cytologic evaluation (ie, subclinical cases). At these 5 farms, the number of foals that developed R equi pneumonia in accordance with the study criteria and the number of foals that were treated for subclinical R equi pneumonia were recorded. To assess the impact of the 5 farms that screened foals for R equi pneumonia, 3 analyses were performed. First, an analysis was performed that included data from all 30 farms but only used data from the foals that fulfilled the study criteria for diagnosis of R equi pneumonia (3/5 farms that performed screening reported that at least 1 foal developed clinical signs), and the results from this analysis are those reported here. Second, an analysis was performed that included data from all foals (ie, those with clinical and subclinical R equi pneumonia) on all 30 farms. Third, the analysis was repeated after excluding the cumulative incidence data from the 5 farms that screened foals for R equi pneumonia. Regardless of the data included in each analysis, the direction, magnitude, and significance of the respective associations did not differ. However, similar studies conducted in the future should be designed to appropriately account for the ultrasonographic screening of foals for R equi pneumonia.

In the present study, air samples obtained from stalls were more likely to test positive for virulent R equi than were air samples obtained from paddocks on 29 of 30 horse breeding farms in central Kentucky. Air samples obtained from stalls were more likely to test positive for virulent R equi during the 2-month period of January and February than during May and June. It remains to be determined whether exposure of foals to higher concentrations of virulent R equi in stalls is associated with an increased risk for those foals to develop R equi pneumonia and whether management practices designed to decrease the concentration of virulent R equi in stalls will consequently reduce the incidence of R equi pneumonia in foals.

References