Factors associated with likelihood of horses having a high serum *Streptococcus equi* SeM-specific antibody titer

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**Objective**—To identify factors associated with an increased likelihood that horses would have a serum *Streptococcus equi* SeM-specific antibody titer ≥ 1:1,600.

**Design**—Cross-sectional study.

**Animals**—188 healthy client-owned horses.

**Procedures**—A single serum sample from each horse was tested for SeM-specific antibody titer with an ELISA. Multivariate logistic regression was used to identify factors associated with having a titer ≥ 1:1,600.

**Results**—Age, breed, and vaccination status were significantly associated with the likelihood of having a titer ≥ 1:1,600. The odds of having a titer ≥ 1:1,600 increased by a factor of 1.07 with each 1-year increase in age. Quarter Horses and horses of other breeds were 4.08 times as likely as were Thoroughbreds and warmbloods to have a titer this high. Horses that had previously received an intranasal *S equi* vaccine were 4.7 times as likely as were horses without any history of vaccination to have a titer this high.

**Conclusions and Clinical Relevance**—Results indicated that older horses, horses other than Thoroughbreds and warmbloods, and horses that had been vaccinated with an attenuated-live intranasal *S equi* vaccine between 1 and 3 years previously had an increased likelihood of having a serum SeM-specific antibody titer ≥ 1:1,600. (*J Am Vet Med Assoc* 2009;235:973–977)

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Strangles is a highly contagious disease of the respiratory tract of horses caused by *Streptococcus equi* subsp *equi*. Vaccination against *S equi* is common, but the prevalence of secondary complications associated with currently available IM and intranasal *S equi* vaccines is perceived to be higher than prevalence for other vaccines commonly used in horses. A rare but serious possible sequela to vaccination against *S equi* is purpura hemorrhagica, an immune-mediated vasculitis that leads to a type III hypersensitivity reaction.⁴ The true incidence of purpura hemorrhagica secondary to strangles or vaccination against *S equi* is not known.¹ ⁵ However, in a retrospective study⁶ of 53 horses with purpura hemorrhagica, 4 reportedly had received an *S equi* M protein extract vaccine IM during the 2 weeks prior to admission. In addition, a study⁷ from 1990 reported an incidence of 2 cases of purpura-like disease for every 100,000 doses of attenuated-live intranasal *S equi* vaccine sold. Various authors have suggested that horses with high serum SeM-specific antibody titers may be predisposed to developing purpura hemorrhagica when vaccinated against *S equi*,¹ ⁶ and a recent consensus statement¹ from the American College of Veterinary Internal Medicine recommended both that horses with SeM-specific antibody titers ≥ 1:1,600 and that horses with SeM-specific antibody titers ≥ 1:3,200 not be vaccinated against *S equi*. It has been suggested that SeM-specific antibody titers be measured prior to instituting a vaccination program,¹ but the cost of testing an individual horse is approximately 4.5 times the cost of vaccinating the horse. Testing also requires an additional farm call fee. The purpose of the study reported here, therefore, was to identify factors associated with an increased likelihood that horses would have SeM-specific antibody titers ≥ 1:1,600.

**Materials and Methods**

**Study design**—The study was performed as a cross-sectional (prevalence) study. Study procedures were approved by the University of Pennsylvania Institutional Animal Care and Use Committee. Informed consent was obtained from the owner or agent of each animal included in the study.

**Horses**—A total of 188 healthy client-owned horses from 26 farms located in Chester, Delaware, and Lancaster counties in Pennsylvania; Cecil County in Maryland; and New Castle County in Delaware were included in the study. This represented approximately 20% of the New Bolton Center Field Service popula-
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Statistical analysis—Cases were defined as horses with a serum SeM-specific antibody titer \( \geq 1:1,600 \), and controls were defined as horses with a serum SeM-specific antibody titer \( \leq 1:800 \). Multivariate logistic regression analysis was used to identify factors (ie, exposures of interest) associated with having a serum SeM-specific antibody titer \( \geq 1:1,600 \). Herd size was represented either as a categorical variable coded into 5 categories (<3 horses, 20 to 29 horses, 30 to 39 horses, 40 to 90 horses, or >90 horses) or as a dichotomous variable (large herd [>30 horses] vs small herd [\( \leq 30 \) horses]).

The Pearson \( \chi^2 \) test was used to test for univariate associations between each factor and the outcome of interest (ie, serum SeM-specific antibody titer \( \geq 1:1,600 \) vs \( \leq 1:800 \)), and factors for which the \( P \) value was < 0.25 in this univariate analysis were included in the initial multivariate model, as described.\(^{1,10}\) Odds ratios were calculated for factors included in the initial multivariate model; 95% confidence intervals were calculated by use of the delta method.\(^{10}\)

The final multivariate model was determined by means of a combination of a purposeful backward model selection process and an automatic stepwise backward model selection process. The order in which the contributions of independent variables was evaluated during the purposeful selection process was based on the Wald statistic for each variable, with variables for which the absolute value of the Wald statistic was substantially < 2 evaluated first. After removal of each variable, the new model was compared with the old model by means of the likelihood \( \chi^2 \) test.\(^{10}\) The variable was permanently excluded from the model if the \( P \) value for the likelihood \( \chi^2 \) was > 0.15, unless its removal caused a > 50% alteration in the magnitude of the odds ratios for the remaining variables.\(^{10}\) Individual variables were also considered for removal when the interval estimates for their odds ratios were large.

After development of the multivariate model, clinically plausible interaction terms were identified and included in the model. Individual interaction terms were examined in a stepwise fashion and removed or retained by use of the same criteria used for the main effects variables. Model performance was evaluated by use of the Pearson \( \chi^2 \) test as a summary statistic of goodness of fit,\(^{10,11}\) the Hosmer-Lemeshow test, and the area under the calculated receiver-operator curve as a summary statistic for discriminating power of goodness of fit.

After summary statistics for goodness of fit of the final model were calculated, the contribution of each covariate pattern to these summary statistics was examined to verify that it was small relative to the error structure of the model and not systematic. Three diagnostic statistics were calculated for each covariate pattern: the change in the Pearson \( \chi^2 \) test statistic, the change in the deviance, and the change in the Prebigon influence statistic following the deletion of the covariate pattern. Covariate patterns were examined more thoroughly if the corresponding value for the change in the Pearson \( \chi^2 \) test statistic or the change in the deviance exceeded 4 or if the corresponding value for the change in the Prebigon influence statistic exceeded 1.\(^{10}\)

All analyses were performed with standard software.\(^{10}\) Values of \( P \leq 0.05 \) were considered significant.

Exposures of interest—Exposures of interest included signalment (age as a continuous variable and breed and sex as categorical variables); whether the horse had been vaccinated with an attenuated-live intranasal \( S \) equi vaccine, had been exposed to horses with strangles, or had ever had clinical signs of strangles; whether the farm had ever had an outbreak of strangles and, if so, how many years ago; and whether the farm had a history of respiratory tract diseases other than strangles. Additional exposures of interest included farm identification, herd size, and whether the farm had a low or high amount of traffic. For purposes of the present study, a low-traffic farm was defined as a farm that had not had any horses introduced to, or returned to, the herd within the 3 months prior to the present study, and a high-traffic farm was defined as a farm that had had at least 1 horse introduced to, or returned to, the herd within the 3 months prior to the present study. For horses that had been vaccinated against \( S \) equi, an additional exposure of interest was the total number of vaccine doses administered during the period for which records were available.

Revaccination—Seventy-five of the 89 horses that had been previously vaccinated were revaccinated with an attenuated-live intranasal \( S \) equi vaccine within 3.5 months after blood sample collection. Horses were subsequently followed up for a minimum of 1 year and a maximum of 2 years for any signs of purpura hemorrhagica.

All horses examined by the New Bolton Center Field Service during ambulatory calls between January 2006 and January 2007 were considered for inclusion in the study. Blood samples were collected into plain glass tubes, and serum was obtained within 16 hours after blood collection. Serum samples were stored at 25°C (77°F) and analyzed in batches by a commercial laboratory\(^9\) for SeM-specific antibody titers with an ELISA as described.\(^9\) Laboratory personnel were blinded to medical, exposure, and vaccination history of all horses.

Reactions at the time of the study. Horses were from a wide cross section of farm types, including boarding stables, breeding farms, and small private operations. All farms maintained open herds (ie, individual horses that left the property were allowed to return to the herd, and outside horses were occasionally added to the herd). All horses were at least 1 year old. Horses between 1 and 3 years old were included only if a complete medical history was available; horses >3 years old were included only if a minimum of 3 years of medical history was available. Medical history information was collected through interviews with owners of participating horses and searches of the computerized medical records of the New Bolton Center Field Service. Particular attention was paid to any history of exposure to or clinical infection with \( S \) equi, any farm history of \( S \) equi infection or other respiratory tract diseases, and \( S \) equi vaccination history. Horses were excluded from the study if they had been vaccinated against \( S \) equi <11 months prior to the time of study enrollment. In addition, horses with a history of natural infection during the preceding 24 weeks were excluded from the study on the basis of a previous suggestion\(^8\) that this is the minimum time after the onset of natural infection for the SeM-specific antibody titer to reach <1:1,600.

All horses examined by the New Bolton Center Field Service during ambulatory calls between January 2006 and January 2007 were considered for inclusion in the study. Blood samples were collected into plain glass tubes, and serum was obtained within 16 hours after blood collection. Serum samples were stored at 25°C (77°F) and analyzed in batches by a commercial laboratory\(^9\) for SeM-specific antibody titers with an ELISA as described.\(^9\) Laboratory personnel were blinded to medical, exposure, and vaccination history of all horses.
Results

Descriptive statistics—Of the 188 horses included in the study, 105 (56%) were geldings, 80 (43%) were mares, and 2 (1%) were stallions (sex of 1 horse was not recorded). Horses ranged from 1 to 35 years old (mean ± SD, 11.8 ± 7 years), with 94 (50%) of the horses being <11 years old, 72 (38%) being between 11 and 20 years old, and 22 (12%) being >20 years old. Fifty-six (30%) of the horses were Thoroughbreds, 56 (30%) were Quarter Horses and related breeds, and 20 (11%) were warmbloods. The remaining 56 horses represented 8 other breeds or were of unknown breed. Horses were used in 8 disciplines, with 53 (28%) used as hunter-jumpers, 23 (12%) used for breeding, 38 (20%) used as part of a school teaching herd, 18 (10%) used for trail-riding, 15 (8%) used for eventing, 13 (7%) used for dressage, 24 (13%) used for lesson programs, and 4 (2%) used for polo. Herd size ranged from <5 to 120 horses. Fifteen (8%) horses were in herds consisting of <5 horses, 25 (15%) were in herds consisting of 5 to 19 horses, 63 (34%) were in herds consisting of 20 to 29 horses, 41 (22%) were in herds consisting of 30 to 39 horses, 24 (13%) were in herds consisting of 40 to 90 horses, and 20 (10%) were in herds consisting of 91 to 120 horses. One hundred twenty-five (66%) horses were stabled at high-traffic farms, and 63 (34%) were stabled at low-traffic farms. Duration of medical history ranged from 1 to 15 years (median, 3 years).

Forty-two (22%) of the horses were confirmed to have a history of exposure to strangles, and 15 (8%) had a history of clinical strangles. Five of the 26 (19%) farms that had a history of clinical strangles had previously experienced an outbreak of strangles. These outbreaks had occurred 1, 4, 5, 5, and 8 years prior to collection of blood samples for the present study. None of the farms had a history of respiratory tract diseases other than strangles.

Ninety-nine (53%) horses had not been vaccinated against S equi during the 3 years prior to the present study. Eighty-nine (47%) horses had been vaccinated with an attenuated-live intranasal S equi vaccine between 11 and 13 months prior to the present study.

Serum SeM-specific antibody titers ranged from 0 to 1:3,200. Five (3%) horses had a titer of 0, 23 (12%) had titers <1:200, 54 (29%) had a titer of 1:400, 59 (31%) had a titer of 1:800, 40 (21%) had a titer of 1:1,600, and 7 (4%) had a titer of 1:3,200. In total, 47 (25%) horses had serum SeM-specific antibody titers ≥1:1,600. Thirty-five of the 89 (39%) previously vaccinated horses had serum SeM-specific antibody titers ≥1:1,600.

Logistic regression analysis—Eight variables were found in univariate analyses to be associated with having a serum SeM-specific antibody titer ≥1:1,600 (Table 1) and were included in the initial multivariate model. Three variables (age, breed, and vaccination status) were retained in the final multivariate model (Table 2). Potential interactions between age, breed, and vaccination status were examined, but inclusion of these interaction terms did not significantly improve the goodness of fit of the model. Therefore, they were removed. Goodness of fit of the final multivariate model was acceptable (area under the receiver-operator curve, 0.78). The odds of having a serum SeM-specific antibody titer ≥1:1,600 increased with age and

Table 1—Results of univariate analysis of factors potentially associated with having a serum SeM-specific antibody titer ≥1:1,600 in horses.

<table>
<thead>
<tr>
<th>Variable</th>
<th>OR</th>
<th>95% CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age *</td>
<td>1.06</td>
<td>1.01–1.12</td>
<td>0.022</td>
</tr>
<tr>
<td>Sex (mares vs geldings)*</td>
<td>0.53</td>
<td>0.26–1.06</td>
<td>0.072</td>
</tr>
<tr>
<td>Quarter Horse or other breed</td>
<td>4.54</td>
<td>1.98–10.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>(vs Thoroughbred or warmblood)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Previously vaccinated intranasally against S equi</td>
<td>4.70</td>
<td>1.84–11.97</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>High-traffic farm (vs low-traffic farm)</td>
<td>3.10</td>
<td>1.10–8.83</td>
<td>0.032</td>
</tr>
<tr>
<td>History of strangles on the farm</td>
<td>4.14</td>
<td>1.28–13.35</td>
<td>0.017</td>
</tr>
<tr>
<td>History of exposure to strangles</td>
<td>2.02</td>
<td>1.60–5.52</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>History of clinical strangles</td>
<td>2.12</td>
<td>0.99–4.51</td>
<td>0.051</td>
</tr>
</tbody>
</table>

Data represent results for 188 healthy client-owned horses. For each factor, the odds ratio represents the odds of having an antibody titer ≥1:1,600 among horses with the factor of interest versus the odds of that state in horses without the factor of interest or in the comparison group.

*Age was evaluated as a continuous variable; the odds ratio represents the increase in odds associated with each 1-year increase in age. NA single stallion in the study was removed from the analysis. CI = Confidence interval. OR = Odds ratio.

Table 2—Results of multivariate logistic regression analysis of factors potentially associated with having a serum SeM-specific antibody titer ≥1:1,600 in horses.

<table>
<thead>
<tr>
<th>Variable</th>
<th>No. of cases</th>
<th>No. of controls</th>
<th>Adjusted OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>NA</td>
<td>NA</td>
<td>1.07</td>
<td>1.01–1.14</td>
</tr>
<tr>
<td>Breed</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thoroughbred or warmblood</td>
<td>8</td>
<td>68</td>
<td>Referent</td>
<td>NA</td>
</tr>
<tr>
<td>Quarter Horse or other</td>
<td>39</td>
<td>73</td>
<td>4.08</td>
<td>1.70–9.90</td>
</tr>
<tr>
<td>Previously vaccinated intranasally against S equi</td>
<td>12</td>
<td>87</td>
<td>Referent</td>
<td>NA</td>
</tr>
<tr>
<td>No</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>35</td>
<td>54</td>
<td>4.7</td>
<td>2.12–10.34</td>
</tr>
</tbody>
</table>

NA = Not applicable. See Table 1 for key.
were higher for Quarter Horses and horses of other breeds (compared with the odds for Thoroughbreds and warmbloods) and for horses that had previously been vaccinated (compared with the odds for horses without any history of previous S equi vaccination).

Covariate analysis—Examination of diagnostic statistics indicated that there were no covariate patterns that the model fit poorly and that no covariate pattern exerted a disproportionate influence on parameter estimates.

Revaccination—Seventy-five of the 89 horses that had been previously vaccinated were revaccinated with an attenuated-live intranasal S equi vaccine within 3.5 months after blood sample collection. Twenty-one of these horses had a titer of 1:1,600, and 5 had a titer of 1:3,200. Horses were subsequently followed up for a minimum of 1 year and a maximum of 2 years for any signs of purpura hemorrhagica, with none seen.

Discussion

Results of the present study indicated that older horses, horses other than Thoroughbreds and warmbloods, and horses that had been vaccinated with an attenuated-live intranasal S equi vaccine between 1 and 3 years previously had an increased likelihood of having a serum SeM-specific antibody titer ≥ 1:1,600. Given previous recommendations that horses with high SeM-specific antibody titers not be vaccinated against S equi, this suggested that horses in these groups should be tested for SeM-specific antibody titer prior to revaccination. Forty-seven (25%) of the 188 horses in the present study had serum SeM-specific antibody titers ≥ 1:1,600, indicating that a substantial proportion of horses will have high serum SeM-specific antibody titers.

In the present study, horses that had previously received an attenuated-live intranasal S equi vaccine were significantly more likely to have a serum SeM-specific antibody titer ≥ 1:1,600, compared with horses that had not been previously vaccinated (odds ratio, 4.7). To our knowledge, there are no published data on the half-life of SeM-specific antibody titers following intranasal vaccination or the titer that can be expected 1 year after intranasal vaccination. Thus, it is possible that an SeM-specific antibody titer of 1:1,600 1 year after intranasal vaccination may be normal. Interestingly, in the present study, the likelihood of having a serum SeM-specific antibody titer ≥ 1:1,600 was not significantly associated with an increase in the weighted sum of the number of previous doses of the vaccine that had been received.

Five horses in the present study that had received an attenuated-live intranasal S equi vaccine between 11 and 13 months prior to blood sample collection had serum SeM-specific antibody titers < 1:200. This could have been due to an innate inability to respond to the vaccine, improper administration of the initial vaccination series, or improper administration of the vaccine. All 5 of these horses were ≤ 3 years old, and 4 of the 5 were from a university herd. None of the horses had been vaccinated by any of the authors of the previous study.

The other 2 factors significantly associated with an increased likelihood of having a serum SeM-specific antibody titer ≥ 1:1,600 in the present study were age and breed. Regardless of vaccination status, there was an estimated 7% increase in the odds of having a titer ≥ 1:1,600 with each 1-year increase in age. Authors of a previous retrospective study of 53 horses with purpura hemorrhagica suggested that repeated exposure to the S equi M protein may explain why older horses would be more likely to have purpura hemorrhagica. In the present study, the increased odds of having a high antibody titer associated with age were not attributable to the number of doses of the attenuated-live intranasal S equi vaccine that horses had received. Importantly, horses in the previous study had been vaccinated IM with a commercial vaccine containing an M protein extract and not with the attenuated-live intranasal S equi vaccine used in horses in the present study. Although a history of exposure to strangles was not found to be significantly associated with the likelihood of having a high serum SeM-specific antibody titer in the present study, it is possible that the increased odds associated with an increase in age reflected an association with exposure to strangles.

In the present study, horses other than Thoroughbreds and warmbloods were more likely to have a serum SeM-specific antibody titer ≥ 1:1,600 than were Thoroughbreds and warmbloods (odds ratio, 4.08). This may reflect differences in husbandry practices associated with these particular breeds. Within the study population, Thoroughbreds and warmbloods were more likely to be housed individually, which may have limited their exposure to strangles.

In univariate analyses in the present study, there was evidence for an association between sex and having a serum SeM-specific antibody titer ≥ 1:1,600. However, this factor was not retained in the multivariate analysis. We cannot explain this finding and suggest that it may be a reflection of the sample population and size. Similarly, univariate analysis provided evidence that horses housed at high-traffic farms were more likely than horses housed at low-traffic farms to have a serum SeM-specific antibody titer ≥ 1:1,600, although this factor was not found to be significant in the multivariate analysis. In general, high-traffic farms in the present study had a high turnover in their horse population or had horses that left the property to attend competitions, where they commingled with other horses. High-traffic farms were also more likely to host competitions, during which outside horses were stabled at the farm. Univariate analysis also provided evidence that horses with a history of exposure to strangles, horses with a history of clinical strangles, and horses from barns with a history of strangles outbreaks were more likely to have serum SeM-specific antibody titers ≥ 1:1,600. Surprisingly, none of these 3 factors were retained in the multivariate model. Horses were excluded from the study if they had had clinical strangles during the 24 weeks prior to study enrollment because the commercial laboratory we used to measure antibody titers has suggested that horses that have been infected with S equi within the past 2 years can have titers ranging from 1:800 to 1:1,600. We did not exclude horses that had had clinical strangles > 24 weeks prior to study enrollment because these horses represented a subpopulation that exists in ambulatory practice and that might be vaccinated after recovery from infection.
There was a low incidence of strangles among horses in the present study. Of the 26 farms included in the study, only 5 had a history of a previous outbreak of strangles. This low incidence of strangles limited the power of our statistical analyses, making it more difficult to identify factors associated with high serum S equi-specific antibody titers. In addition, there are limitations to the ELISA used to measure these titers because it does not differentiate between horses that have been exposed, horses that have recently been vaccinated, and horses that are carriers. Identification of carriers on these farms by means of bacterial culture of lavage fluid from the auditory tube diverticula (guttural pouches) or use of a PCR assay was beyond the scope of the present study. Additional research is necessary to determine factors associated with having a titer ≥ 1:3,200; however, only 7 of the 188 horses in the present study had a titer this high. Consequently, there was not enough power to examine this in the present study. Evaluation of other horse populations throughout the country may also provide more information.

Quantifying the risk of adverse effects following vaccination of horses with titers ≥ 1:1,600 or 1:3,200 is warranted, but would require vaccination of large numbers of horses with known titers. We examined this question to a limited extent in the present study, by following up 75 horses that had previously been vaccinated following revaccination. Twenty-one of these 75 horses had a titer of 1:1,600 and 5 had a titer of 1:3,200 at the time of revaccination, and none of these horses had any evidence of purpura hemorrhagica during the follow-up period. In addition, a search of the medical records for this population did not reveal any evidence of previous purpura hemorrhagica. On the other hand, given the reportedly low incidence of purpura hemorrhagica following vaccination, and none of these horses had any evidence of 1:1,600 and 5 had a titer of 1:3,200 at the time of revaccination. Twenty-one of these 75 horses had a titer ≥ 1:1,600, we believe that the current guideline that only horses with serum S equi-specific antibody titers < 1:1,600 be revaccinated may be too conservative. Further studies are needed to determine the antibody titer cutoff to be used when deciding whether to vaccinate horses against S equi.

References