Duration of serum antibody response to rabies vaccination in horses

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OBJECTIVE
To investigate the impact of age and inferred prior vaccination history on the persistence of vaccine-induced antibody against rabies in horses.

DESIGN
Serologic response evaluation.

ANIMALS
48 horses with an undocumented vaccination history.

PROCEDURES
Horses were vaccinated against rabies once. Blood samples were collected prior to vaccination, 3 to 7 weeks after vaccination, and at 6-month intervals for 2 to 3 years. Serum rabies virus–neutralizing antibody (RVNA) values were measured. An RVNA value of ≥ 0.5 U/mL was used to define a predicted protective immune response on the basis of World Health Organization recommendations for humans. Values were compared between horses < 20 and ≥ 20 years of age and between horses inferred to have been previously vaccinated and those inferred to be immunologically naïve.

RESULTS
A protective RVNA value (≥ 0.5 U/mL) was maintained for 2 to 3 years in horses inferred to have been previously vaccinated on the basis of prevaccination RVNA values. No significant difference was evident in response to rabies vaccination or duration of protective RVNA values between horses < 20 and ≥ 20 years of age. Seven horses were poor responders to vaccination. Significant differences were identified between horses inferred to have been previously vaccinated and horses inferred to be naïve prior to the study.

CONCLUSIONS AND CLINICAL RELEVANCE
A rabies vaccination interval > 1 year may be appropriate for previously vaccinated horses but not for horses vaccinated only once. Additional research is required to confirm this finding and characterize the optimal primary dose series for rabies vaccination. (J Am Vet Med Assoc 2016;249:411–418)

rabies is an acute, progressive encephalitis of mammals caused by the neurotropic rabies virus of the genus Lyssavirus, in the Rhabdoviridae family. The virus persists within carnivore or chiropteran reservoirs, and humans are typically exposed through an animal bite.

The incidence of rabies in horses is low, but because the disease is typically fatal and has considerable public health importance, rabies is recommended as a core vaccine for horses in the United States. Vaccines licensed for rabies prophylaxis in horses in the United States are inactivated (killed), tissue culture–derived products combined with an adjuvant. For adult horses, a single dose followed by annual revaccination is currently recommended by vaccine manufacturers and in the vaccination guidelines of the AAEP.

Research in several animal species indicates that the primary correlate of protection against rabies is the presence of neutralizing antibody in serum. The humoral immune response to rabies vaccination can be evaluated with the RFFIT or fluorescent antibody virus neutralization test, both of which are used for in vitro measurement of RVNA concentration in serum samples. The RFFIT is currently the gold standard and reference technique for most laboratories.

An RVNA titer of ≥ 0.5 U/mL is globally recognized as the threshold of seroconversion for humans. Values reported in this manner (U/mL) are titers expressed as standard units. This value of 0.5 U/mL, when obtained via RFFIT or fluorescent antibody virus neutralization, is also recognized by regulatory authorities in most rabies-free areas as proof of adequate response to vaccination for importation of cats and dogs. No general consensus currently exists regarding the RVNA titer needed to confer protection.

ABBREVIATIONS
AAEP American Association of Equine Practitioners
LOD Limit of detection
RFFIT Rapid fluorescent focus inhibition test
RVNA Rabies virus–neutralizing antibody

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in equids; however, it appears reasonable to extrapolate information from studies in humans and other species to provide logical guidelines for measurable correlates of immunity for horses.

In addition to vaccine-induced RVNA responses, other immunologic factors are likely to play a role in preventing rabies, and the ability to measure and interpret those factors is not well developed. For this reason and because of the potential zoonotic implications of rabies, it is recommended that evidence of circulating anti-rabies virus antibody in animals should not be used as a substitute for vaccination or for determining the need for booster vaccinations. On the other hand, the incidence of rabies transmission by horses is low, compared with that by wildlife or domestic small animals.

Limited data are available on long-term durability of anti-rabies virus antibody in horses. Findings of studies involving dogs and cats suggest that, for several pathogens, vaccine-induced immunity endures for at least 3 years after vaccination. Following these and other studies, some rabies vaccines for dogs, cats, and sheep that were previously licensed for annual administration became licensed for triennial administration. Therefore, if predicted protective RVNA titers can be demonstrated to persist for multiple years in horses, this would justify further research to determine optimal revaccination intervals.

Vaccination against rabies is undoubtedly an important aspect of equine preventive care as well as an important public health intervention to reduce the risk of zoonotic spread of the disease to humans. On the other hand, some equine practitioners and horse owners have expressed concern that current AAEP guidelines may result in horses being vaccinated more often than is necessary against some diseases, including rabies. All vaccines pose a small risk of causing a vaccine-associated adverse event. Potential complications associated with vaccination in horses range from pain, swelling, and infection at the injection site to life-threatening type I hypersensitivity reactions. In other species, vaccine-related chronic antigenic stimulation may also be linked to other conditions such as vaccine-associated sarcomas in cats or autoimmune diseases and type III hypersensitivity reactions; however, evidence to support some of these associations is not conclusive. In addition, the cost of vaccination is a burden for some horse owners. Therefore, it would be beneficial to determine whether annual revaccination of horses is truly necessary.

The equine population in the United States is evolving to include an increasing number of geriatric horses and additional research into the immune function of this subset is warranted. A decrease in immune function with increasing age, referred to as immunosenescence, has been reported for various animal species. In humans, evidence exists to both support and reject the hypothesis that response to vaccination can be affected by age. Some studies have shown that elderly people may be less effective than younger people at mounting antibody and cell-mediated immune responses after vaccination. Conversely, results of other studies suggest that healthy people of advanced age can respond to vaccination as well as younger people. No significant difference has been identified between immune responses of healthy elderly people and young people after vaccination against tetanus, diphtheria, and pneumococcal disease.

Age-related changes in immune function in horses have been described and may manifest in vivo as a decrease in responsiveness to vaccination or in vitro as diminished proliferative responses to mitogens. Aging in horses has been associated with a decrease in the immune response to vaccination against influenza. Healthy older horses reportedly generate a primary immune response to a killed rabies vaccine similar to that of younger horses but have a significantly lower anamnestic response to influenza vaccine. The mechanisms underlying this possible impaired immunologic response in older horses remain unclear and warrant further investigation, as does the effect of ageing on the duration of protective immunity following vaccination.

The primary purpose of the study reported here was to test the hypothesis that vaccine-induced RVNA titers would remain ≥ 0.5 U/mL for longer than the recommended revaccination interval of 1 year in horses. A secondary aim was to investigate the effect of horse age on the duration of RVNA values ≥ 0.5 U/mL after vaccination. We hypothesized that RVNA titers maintained in geriatric horses (≥ 20 years of age) would not differ significantly from those maintained in younger adult horses.

**Materials and Methods**

**Animals**

Forty-eight horses ≥ 3 years of age were included in the study. Horses were housed on 3 separate properties and were all members of herds of donated animals. Each horse’s age was determined as accurately as possible at the beginning of the study by physical examination of each horse was performed accurately as possible at the beginning of the study by review of facility records. Breeds included Quarter Horse, Thoroughbred, Standardbred, and Paint. A physical examination of each horse was performed prior to vaccination, and all horses were deemed healthy.

Horses were grouped by age, with 29 horses < 20 years (15 mares and 14 geldings) and 19 horses ≥ 20 years (7 mares and 12 geldings). Age groups were not significantly different in sex distributions. Rabies vaccination history for included horses was variable, with some horses having a reported, but undocumented, history of previous vaccination and others with unknown vaccination status. The study protocol was reviewed and approved by the University of California-Davis Institutional Animal Care and Use Committee.
Vaccination and blood collection protocol

Data collection for the study was performed between 2005 and 2012. All horses were vaccinated IM with a commercial inactivated rabies vaccine at the beginning of the study. Blood samples were collected from a jugular vein via 19-gauge needles into 8-mL serum separator tubes just prior to vaccination, 3 to 7 weeks after vaccination, and at approximately 6-month intervals (4 to 8 months) thereafter for 2 to 3 years. Follow-up was performed on all 48 horses for approximately 2 years (22 to 26 months) after vaccination. Forty of 48 (83%) horses had samples collected for approximately 2.5 years (28 to 32 months), and 36 (75%) horses had samples collected for approximately 3 years (34 to 38 months).

Laboratory analysis

After collection, all blood samples were allowed to clot and then immediately centrifuged. Serum was harvested and stored at −20°C pending analysis. For analysis, serum samples were allowed to thaw at room temperature (approx 20°C) and serum anti-rabies virus antibody titer was measured by use of an RFFIT. Serial serum dilutions were mixed with a standard amount of live rabies virus in 8-well chamber slides and incubated at 37°C in 2% to 5% CO₂, for 90 minutes. Tissue culture cells were added and incubated with the test sample and virus at 37°C in 2% to 5% CO₂ for 20 to 24 hours. After incubation, the cells were fixed on the slides and stained with fluorescein isothiocyanate–labelled anti–rabies nucleoprotein antibody. Slides were viewed with a fluorescence microscope for the presence of virus, and end point titer was calculated on the basis of the percentage of virus-infected areas within the wells containing the serial dilutions of the sample. The RVNA values were then calculated by comparing the titer in the measured sample with that of standard reference serum.

Interassay variability was evaluated, and results were within acceptable limits for comparing separate sample batches. The LOD of the assay was 0.1 U/mL, which was defined by use of human serum samples and extrapolated to apply to horse serum samples. The upper limit of measured RVNA values was 130 U/mL. For the purpose of determining whether a given RVNA value was protective against rabies, a cutoff of ≥0.5 U/mL was used. This cutoff was selected on the basis of World Organisation for Animal Health guidelines for pet export and World Health Organization guidelines for humans.

Reclassifications on the basis of serologic status

To evaluate the effect of vaccination history prior to the study on development of predicted protective RVNA titer (≥0.5 U/mL), horses were also grouped by their prevaccination RVNA titer: ≥0.1 U/mL (n = 32) and <0.1 U/mL (16). Horses with values ≥0.1 U/mL were considered likely to have been previously vaccinated, whereas horses with values < 0.1 U/mL were considered likely to have been immunologically naïve prior to vaccination in the study.

To further assess the potential impact of vaccination history prior to the study on postvaccination RVNA values, horses were also categorized on the basis of their response to vaccination at the start of the study. A large increase in RVNA potency 3 to 7 weeks after vaccination might be expected in horses with a history of previous vaccination against rabies (effective anamnestic immune response), whereas a more modest increase might be indicative of a lack of previous vaccination. Human data and the authors’ own observations on human and small animal rabies serologic test results (unpublished laboratory data) suggested that an increase in RVNA potency from less than the LOD of the assay before vaccination to >5 U/mL after vaccination is indicative of previous vaccination. With this criterion applied, horses were classified into 3 groups on the basis of whether they were inferred to have been previously vaccinated and already protected (RVNA value ≥0.5 U/mL) before vaccination against rabies in the study, likely previously vaccinated but without a protective RVNA value (ie, <0.5 U/mL) before vaccination against rabies in the study and with a value >5 U/mL 3 to 7 weeks after vaccination, or immunologically naïve (prevaccination RVNA value <0.5 U/mL) with a postvaccination RVNA value (<5 U/mL).

Statistical analysis

Mixed-effects linear regression modeling was performed to analyze the RVNA data collected for 2 to 3 years after vaccination, with horse age and RVNA assessment time point as fixed effects and horse identification and location as random effects. Response to vacc-

<table>
<thead>
<tr>
<th>Assessment time point</th>
<th>All horses</th>
<th>&lt; 20 years</th>
<th>≥ 20 years</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immediately prior to vaccination</td>
<td>29/48 (60)</td>
<td>16/29 (55)</td>
<td>13/19 (68)</td>
</tr>
<tr>
<td>1 mo</td>
<td>46/48 (96)</td>
<td>29/29 (100)</td>
<td>17/19 (89)</td>
</tr>
<tr>
<td>6 mo</td>
<td>42/47 (90)</td>
<td>25/28 (89)</td>
<td>17/19 (89)</td>
</tr>
<tr>
<td>12 mo</td>
<td>40/47 (85)</td>
<td>23/28 (82)</td>
<td>17/19 (89)</td>
</tr>
<tr>
<td>18 mo</td>
<td>41/46 (89)</td>
<td>24/27 (89)</td>
<td>17/19 (89)</td>
</tr>
<tr>
<td>24 mo</td>
<td>42/48 (88)</td>
<td>25/29 (86)</td>
<td>17/19 (89)</td>
</tr>
<tr>
<td>30 mo</td>
<td>35/40 (88)</td>
<td>24/28 (85)</td>
<td>11/12 (92)</td>
</tr>
<tr>
<td>36 mo</td>
<td>31/36 (86)</td>
<td>21/25 (84)</td>
<td>10/11 (91)</td>
</tr>
</tbody>
</table>

Table I—Proportions (%) of healthy adult (≥3 years of age) horses vaccinated once against rabies and followed for up to 3 years to assess duration of RVNA values (≥0.5 U/mL) predicted to be protective, overall and by age group.
cination and duration of RVNA values ≥ 0.5 U/mL were compared between horses < 20 and ≥ 20 years of age. For any RVNA values < 0.1 U/mL (the LOD), a value of 0.05 U/mL was used for analysis purposes. The same approach was used to compare duration of RVNA values ≥ 0.5 U/mL between horses with prevaccination RVNA values < 0.1 U/mL and those with prevaccination values ≥ 0.1 U/mL. Values of P < 0.05 were considered significant for all analyses.

**Results**

Significant associations between serum RVNA values and assessment points were identified, with RVNA values for all assessment points after vaccination of horses significantly (P < 0.001) greater than those before vaccination. More than 85% of horses maintained a predicted protective RVNA titer (≥ 0.5 U/mL) for all assessment points after vaccination ([Table 1; Figure 1](#table1)). A large percentage (88%) of horses maintained a predicted protective RVNA titer for 24 months (42/48) and 30 months (35/40). At 36 months, 86% (31/36) of horses had a predicted protective RVNA titer.

No significant association was identified between age and serum RVNA titer; therefore, no significant difference was identified between horses < 20 and ≥ 20 years of age in the response to vaccination or duration of protective RVNA titer ([Figure 2](#figure2)). Furthermore, no significant difference was detected between proportions of horses < 20 and ≥ 20 years of age with RVNA values ≥ 0.5 U/mL at each assessment point.

Nineteen horses had a serum RVNA value < 0.5 U/mL before vaccination at the beginning of the study, of which 16 had a value lower than the LOD of the assay (ie, < 0.1 U/mL). Those 16 horses were considered immunologically naïve prior to vaccination, and the remaining 32 horses were considered likely to have been previously vaccinated. Seven horses were classified as poor responders, defined as horses that never reached a protective RVNA value after vaccination or reached a predicted protective value but did not maintain it for a full year following vaccination. All of these poor responders were among those considered immunologically naïve prior to vaccination. A significant (P = 0.005) difference was identified over time between immunologically naïve horses and those considered likely to have been previously vaccinated ([Figure 3](#figure3)).

Reclassification of horses on the basis of likely previous vaccination history revealed that all 29 horses that were considered likely to have been previously vaccinated and were already protected against rabies before study vaccination (ie, prevaccination RVNA titer ≥ 0.5 U/mL) had a postvaccination RVNA titer that persisted at or above protective values for the duration of the study ([Figure 4](#figure4)). Eleven of 12 horses that were considered likely to have been previously vaccinated against ra-
Bies but did not have protective RVNA values before study vaccination developed protective values after vaccination and sustained those protective values for the duration of the study. The remaining horse in that group was considered a poor responder.

Only 1 of 7 horses in the group considered immunologically naïve (ie, with a prevaccination RVNA titer of < 0.1 U/mL and postvaccination titers of < 5 U/mL) was considered to have had a protective postvaccination RVNA value for the duration of the study. The 6 remaining horses in that group were considered poor responders. Horses in both previously vaccinated groups differed significantly ($P \leq 0.005$) from the immunologically naïve group in postvaccination RVNA values.

Three of the poor responders (1 considered previously vaccinated and 2 considered immunologically naïve) initially had an increase in the RVNA value ($\geq 0.5$ U/mL) after vaccination but developed values < 0.5 U/mL by 6 months after vaccination. The remaining 2 poor responders had an RVNA titer that was lower than the predicted protective value by 12 months after vaccination.

**Discussion**

In the present study, > 85% of adult horses maintained a protective degree of immunity against rabies as defined for humans by the World Health Organization at all blood sample collection points during a 2- to 3-year follow-up period. In addition, all of the horses that had RVNA values of $\geq 0.1$ U/mL prior to vaccination maintained a predicted protective degree of potency ($\geq 0.5$ U/mL) for this same period. These results supported the hypothesis that most horses would maintain a predicted protective RVNA titer for longer than the recommended revaccination interval of 1 year (for most horses, for 2 to 3 years after vaccination). Duration of protection was no different between horses < 20 and $\geq 20$ years of age. However, a prolonged duration of a protective immune response was not observed in horses considered likely to have been immunologically naïve prior to the study.

The US Code of Federal Regulations, Title 9, Section 113.209 states that to achieve FDA approval, rabies biologics must immunize $\geq 87\%$ of 25 or more test-vaccinated and virus-challenged animals, whereas $\geq 80\%$ of nonvaccinated, virus-challenged control animals must die as a result of that challenge. Viral challenge of horses was not performed in the present study; therefore, the findings reported here do not meet those requirements.
However, the percentage of horses with RVNA values \( \geq 0.5 \text{ U/mL} \) remained \( > 85\% \) at all assessment points. This indicated that a predicted protective RVNA titer was achieved in those horses after rabies vaccination, although the relationship between RVNA values and true protection against viral challenge remains uncertain.

Serologic data in the present study assumed a typical antibody response curve. As expected, a large increase in RVNA values attributable to stimulation of the humoral immune response was observed 3 to 7 weeks after vaccination. Nineteen horses had RVNA values \( < 0.5 \text{ U/mL} \) prior to the study, of which 16 had values lower than the LOD of the assay (0.1 U/mL). Low or undetectable RVNA values likely represented horses that were unvaccinated or vaccinated only once in the past, vaccinated a long time ago, or poor responders to previous rabies vaccination. Only those horses with an RVNA value \( < 0.1 \text{ U/mL} \) prior to vaccination were classified as poor responders.

Significant differences in the duration of a protective immune response were observed between horses that were considered likely to have been previously vaccinated and those that were considered likely to have been immunologically naïve, suggesting that naïve horses did not have the same protective effect of vaccination that was identified in previously vaccinated horses. In addition, when horses were further classified on the basis of prevaccination RVNA values and magnitude of the immune response 3 to 7 weeks after vaccination, significant differences were again identified between horses that were likely previously vaccinated and those that were likely immunologically naïve.

The identification of poor responders among horses classified as immunologically naïve or likely previously vaccinated but without a protective RVNA value prior to vaccination indicated that protection may be inadequate in most horses following primary vaccination with a single dose and calls into question the current AAEP rabies vaccine guidelines for adult horses. In another study, a considerable booster effect was identified in horses given a second rabies vaccine 4 weeks after the first. Reports of dog and cat responses to rabies vaccination support the finding that persistence of a protective immune response is less prolonged after primary vaccination with a single dose of rabies vaccine than after subsequent administration of booster doses. This may also be the situation in horses and may account for many of the poor responders in the present study.

Individual variation in response to rabies vaccination of other species has been reported. For humans, dichotomous responses to rabies vaccination have been reported, with results classified as pertaining to high or good responders and poor or low responders. Research into the serologic responses of dogs and cats to rabies vaccination has revealed substantial individual variation, with 4% to 5% of animals having less than the required antibody threshold of \( \geq 0.5 \text{ U/mL} \) and a small proportion having no detectable antibody despite repeated vaccination. Interestingly, in the present study, approximately 4% (2/48) of horses had RVNA values \( < 0.5 \text{ U/mL} \) at all postvaccination assessment points.

The present study had several limitations. The rabies vaccination history of included horses was unknown. Many horses were likely to have been routinely revaccinated annually in the past; however, some could have had a long revaccination interval and others were likely immunologically naïve. The manufacturers of any rabies vaccines administered prior to the study were also unknown. Use of prevaccination RVNA values and serologic responses 3 to 7 weeks after vaccination to infer the prior vaccine history was potentially misleading because vaccinated horses may have had RVNA values that were lower than the LOD of the assay used, and unvaccinated horses may have had cross-reactive antibodies or other nonspecific inhibitors of virus that can mimic specific antibody in laboratory testing. In addition, interpretation of serologic data to determine grouping may have introduced some degree of bias.

The criteria used to group horses by serologic status were not intended as definitive means of determining previous vaccination but as an indicator for the purposes of analysis. Complete medical records containing vaccination histories would certainly have been preferable; however, we believed the variation in vaccination status at the beginning of the study reflected a commonly encountered situation in equine clinical practice, whereby an individual horse’s serologic status and vaccination history are often unavailable. Additional research involving horses with accurately documented vaccination histories is warranted. In addition, nonvaccinated control horses were not used in the present study to account for increases in RVNA values related to natural infection; such horses are often used in vaccination efficacy trials. This was believed to have been unnecessary, however, because of the low likelihood that any of the study horses had prior rabies exposure given the low prevalence and fatal nature of the infection.

A cutoff of \( \geq 0.5 \text{ U/mL} \) was used to indicate a protective RVNA titer in the present study. Data are lacking on the correlation between RVNA values and protection of horses against rabies. One study involving experimental infection with the rabies virus revealed that clinical signs of rabies still developed in vaccinated horses after viral challenge (although RVNA values in these horses were \( < 0.5 \text{ U/mL} \) and information on the vaccine used was not provided). Five of 21 horses in a rabies case series were reported to have been vaccinated, but RVNA values and accurate vaccine history were unavailable. Additionally, affected horses in that study were young and their primary response to rabies vaccination may have been subject to interference by maternally derived antibody. These studies and others highlight the fact that our understanding of correlations of protective immunity to ra-
bies virus infection in horses is incomplete and that additional research is needed to determine the relationship between serologic findings and protection.

Individualized vaccination programs should consider the health and circumstances of each animal, the probability of exposure to an infectious agent, susceptibility to disease, severity of the corresponding disease, efficacy and safety of the vaccines, potential concerns regarding public health, and preference of the owner. Serologic testing is used in human medicine in the United States to measure response to rabies vaccination, and this also plays an important role in determining adequate rabies vaccination in small animals prior to importation into rabies-free areas. Use of serologic testing for antibodies against various diseases has also been suggested for small animals to aid decision making in the ongoing debate over vaccine booster frequency. Serologic testing is not a perfect means of assessing immunity and can be expensive; therefore it is not feasible in all situations. Decisions regarding individual vaccination protocols should be based on sound epidemiological principles, including risk assessment and laboratory data.

Results of the present study suggested that annual rabies vaccination may be more frequent than is necessary to maintain a protective degree of immunity in horses that have received primary and subsequent booster vaccine doses, but this finding should not be applied for protection of horses that have received only a single primary vaccine dose. Additional research is required to characterize optimal administration intervals for a primary dose series for rabies vaccination of horses.

For horses with previous vaccination, the efficacy of triennial vaccination, as is performed now for dogs, cats, and sheep, should be investigated. This is particularly important when considering that horses are considerably less likely to pose a public health risk for rabies transmission than are small domestic animals. In addition, triennial vaccination protocols reportedly result in an increased number of dogs and cats vaccinated against rabies, compared with the number vaccinated through annual administration protocols. Data from the 2005 National Animal Health Monitoring System indicate that only 33.1% of equine operations had at least 1 equid to which a rabies vaccine had been administered within the previous 12 months. Introduction of a triennial rabies vaccination regimen for horses could potentially result in improved rabies vaccination rates and therefore better individual horse immunity and public health protection.

Whereas it would be unwise to advocate extension of the revaccination interval beyond the 1 year recommended for USDA-licensed rabies vaccines until a prolonged duration of protection can be confirmed, the results reported here may inform decision making when practitioners treat horses with a history of unacceptable vaccine-associated adverse reactions, provided those horses have been vaccinated multiple times in the past. No evidence was obtained to support a prolonged duration of protective immunity against rabies after a single vaccine dose.

Acknowledgments

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Footnotes

a. Imrab-3, Merial, Duluth, Ga.
b. Vacutainer, Becton-Dickinson, Rutherford, N.J.
c. Kansas State Veterinary Diagnostic Lab, Kansas State University, Manhattan, Kan.
e. Stat/IC, version 13.1, StatCorp LP, College Station, Tex.

References


