Neutalizing antibodies against vesicular stomatitis viruses (serotypes New Jersey and Indiana) in horses in Costa Rica

Maren Blickwede, Gaby Dolz, Marco V. Herrero, Sarah M. Tomlinson, Mo Salman

Abstract. Serum samples were collected from domestic horses in 4 different regions of Costa Rica to detect antibodies against vesicular stomatitis viruses, serotypes New Jersey (VSV-NJ) and Indiana (VSV-IN). A total of 214 samples were tested by the virus neutralization test. The sampling regions were identified as low North Pacific dry area (1), low Middle Atlantic humid area (2), low South Pacific humid area (3), and the highlands (4). In region 1, 97.1% of horses were positive for VSV-NJ and 16.5% were positive for VSV-IN. The mean antibody titer and its standard deviation after logarithmic transformation were 5.86 ± 0.9 for VSV-NJ and 3.55 ± 1.66 for VSV-IN for region 1. In region 2, 40.7% of horses were positive for VSV-NJ and 32.2% were positive for VSV-IN. The mean antibody titer in region 2 was 4.33 ± 1.82 for VSV-NJ and 3.47 ± 1.73 for VSV-IN. In region 3, 20.79% of horses were positive for VSV-NJ and 27.6% were positive for VSV-IN. The mean antibody titer in region 3 was 4.39 ± 1.89 for VSV-NJ and 3.47 ± 1.82 for VSV-IN. In region 4, 91.3% of horses were positive for VSV-NJ and 73.9% were positive for VSV-IN. The mean antibody titer in region 4 was 5.77 ± 1.10 for VSV-NJ and 4.85 ± 1.63 for VSV-IN. This is the first published report of the detection of virus-neutralizing antibodies against VSV-NJ and VSV-IN in horses in Costa Rica.
tralizing antibodies to VSV-NJ and VSV-IN. Eight serum samples were heat inactivated (56°C for 30 minutes), diluted from 1:100 to 1:6,400 (4-fold dilutions) with Dulbecco minimum essential medium in duplicate wells of tissue culture plates, and 100–200 median tissue culture infective doses of VSV-NJ (Greentree strain) and VSV-IN (Cuhayo strain) were added to each well. After incubation of the plates at 37°C and 5% CO2 for 1 hour, Vero E6 cells were added as indicator cells (10,000 cells/well). Cell control, virus control, positive control, and negative control serum samples were included in each neutralization assay. All plates were read in 48 hours, and each sample for each serotype (New Jersey or Indiana) was determined to be positive if a confluent monolayer of Vero cells appeared at a dilution of 1:8 or greater.

The percentage of seropositive horses was defined as the number of positive samples for each serotype, divided by the number of samples for that region. The log-likelihood chi-square test was used to compare the proportion of seropositive horses between regions (Table 1). Antibody titers were transformed by taking the natural log of the end titer dilution. The mean and the standard deviation for the transformed titer data were calculated. The 4-fold dilutions used for the VNT results in the titer data were considered non-continuous. Therefore, the Kruskal–Wallis test, which performs an analysis of the ranks of the data, was used to compare the mean titers of VSV-NJ and VSV-IN between regions. The results of these comparisons are provided in Table 2. All statistical tests were conducted with statistical software.

Previous studies in Costa Rica reported that VSV-NJ infections occur much more frequently than VSV-IN infections in dairy cattle. Our findings concur, with 70.6% of horses serologically positive for VSV-NJ and 28.5% positive for VSV-IN.

Table 1. Characteristics and percent seropositive for vesicular stomatitis virus, serotype New Jersey (VSV-NJ) and serotype Indiana (VSV-IN) for horses sampled in 4 regions of Costa Rica.

<table>
<thead>
<tr>
<th>Region</th>
<th>Total farms</th>
<th>Total horses</th>
<th>No. males</th>
<th>No. females</th>
<th>No. horses ≤5 years</th>
<th>No. horses &gt;5 years</th>
<th>% positive VSV-NJ</th>
<th>% positive VSV-IN</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3</td>
<td>103</td>
<td>32</td>
<td>71</td>
<td>23</td>
<td>81</td>
<td>97.1</td>
<td>16.5</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>59</td>
<td>30</td>
<td>29</td>
<td>14</td>
<td>45</td>
<td>40.7</td>
<td>32.2</td>
</tr>
<tr>
<td>3</td>
<td>7</td>
<td>29</td>
<td>20</td>
<td>9</td>
<td>3</td>
<td>26</td>
<td>20.7</td>
<td>27.6</td>
</tr>
<tr>
<td>4</td>
<td>7</td>
<td>23</td>
<td>13</td>
<td>10</td>
<td>0</td>
<td>23</td>
<td>91.3</td>
<td>73.9</td>
</tr>
<tr>
<td>Totals</td>
<td>19</td>
<td>214</td>
<td>95</td>
<td>119</td>
<td>40</td>
<td>175</td>
<td>70.6*</td>
<td>28.5†</td>
</tr>
</tbody>
</table>

* $\chi^2 = 99.7, P < 0.0001$.
† $\chi^2 = 30.9, P < 0.0001$. 

Figure 1. Costa Rican regions for sampling horses for evidence of vesicular stomatitis virus (note: 1, 2, 3, and 4 refer to study regions; see Table 1).
Table 2. Means of transformed virus-neutralization antibody titers for vesicular stomatitis virus, serotype New Jersey (VSV-NJ) and serotype Indiana (VSV-IN) in horses in 4 regions of Costa Rica.

<table>
<thead>
<tr>
<th>Region</th>
<th>VSV-NJ mean titer (SD)*</th>
<th>VSV-IN mean titer (SD)†</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5.86 (0.90)</td>
<td>3.55 (1.66)</td>
</tr>
<tr>
<td>2</td>
<td>4.33 (1.82)</td>
<td>3.47 (1.73)</td>
</tr>
<tr>
<td>3</td>
<td>4.39 (1.89)</td>
<td>3.47 (1.81)</td>
</tr>
<tr>
<td>4</td>
<td>5.77 (1.10)</td>
<td>4.85 (1.63)</td>
</tr>
</tbody>
</table>

* Kruskal–Wallis test for VSV-NJ: χ² = 25.5, P < 0.0001.
† Kruskal–Wallis test for VSV-IN: χ² = 7.09, P < 0.07.

VSV-IN. Previous research also proposed the existence of 2 foci of VS, 1 located at a dry, low elevation (region 1) and the other at a higher elevation (region 4).9 We found that in regions 1 and 4, more than 90% of horses tested had virus-neutralizing antibodies to VSV-NJ. In regions 2 and 3, respectively, 40.7 and 20.7% of horses had virus-neutralizing antibodies to VSV-NJ. The percentage positive between regions was statistically different for VSV-NJ (χ² = 99.7, P < 0.0001). The greatest and least percent positive for VSV-IN were found in region 4 (73.9%) and region 1 (16.5%), respectively. In regions 2 and 3, respectively, 32.2 and 27.6% of horses had virus neutralizing antibodies to VSV-IN. The percentage positive between regions was statistically different for VSV-IN (χ² = 30.9, P < 0.0001). Differences in percent positives among the regions may be associated with the different ecological conditions found in each region. Variations in temperature, total precipitation, timing of precipitation, elevation, and vegetation all effect diversity, density, and activity of insect species. Region 4, which had high numbers of seropositive horses for both VSV-NJ and VSV-IN, is premontane wet forest with an average annual rainfall of >2,000 mm and temperatures ranging from 15 to 30 C. Phlebotomine sand flies, known vectors of VSVs, have been collected from farms in this region.3 Although a reservoir of VSVs has yet to be identified, a small mammal is the most likely reservoir of VSV. Regional variations in ecologies would result in differences in populations of small mammals, both in density and species in each region. Research that focuses on the prevalence, population, and requirements of specific vectors in each region is warranted to understand the importance of insects and rodents in the transmission of VS throughout Costa Rica.

This report describes an exploratory study to determine the presence of virus-neutralizing antibodies against VSV-NJ and VSV-IN in horses within different regions of Costa Rica. Premises included in this study may not be representative of all premises with horses in the regions selected. The relationship of farm factors (management, location, land use, and other animal species), host factors (age, breed, gender, geographic origin, and travel history), ecologic factors (precipitation, temperature, and humidity), and insect vector and rodent factors (presence and prevalence of speculated VSV transmitters) to the presence of antibodies were not examined in this study. Further examination of these factors may explain the significant difference in frequency of VSV-NJ and VSV-IN antibody titers and lead to the identification of those factors that contribute to the variability between regions.

All previous studies of VS in Costa Rica have focused on the disease in cattle.1,2,7,8,10,13 Horses, pigs, and humans live in the same areas as cattle and all may be exposed to similar risk factors. More specific studies are required to determine the serologic status of other domestic animals and their role in the transmission of VSV in endemic areas.

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Sources and manufacturers

a. SAS Version 8, SAS Institute, Cary, NC.

References

A 6-week-old female dromedary (Camelus dromedarius) calf with a history of acute diarrhea died 5 days after the initial symptoms. According to the owner, the calf was born in Missouri on a pasture that was also inhabited by zebras. The camel nursed from its mother for 1 week after birth. At 1 week of age, the calf was separated from its mother and shipped to a farm in Wisconsin. Upon arrival, the calf was contained indoors and initially fed approximately 1 liter of warm calf milk replacer containing oxytetracycline and neomycin 4 times per day. The amount of milk replacer was gradually increased to approximately 2 liters per feeding. Clover grass hay was available at all times. The calf had contact with miniature horses, zebras, and reindeers that were kept in the same barn in different stalls. The calf was healthy for approximately 4 weeks. Its weight was approximately 75 kg. Five days before death, the calf developed watery diarrhea after initially being bloated. The owner administered penicillin (approximately 20,000 units/kg) intravenously and received electrolytes per os because it was still drinking. At the second visit 2 days later, the rectal temperature was approximately 35°C (reference range: 40–50 beats/minute). The white blood cell count was 16,000 leukocytes/ml (reference range: 13,000–24,000) with neutrophilia (83%; reference range: 53–74%). The anti-inflammatory and antibiotic treatment was continued but the calf died and was submitted to the Department of Veterinary Diagnostic Medicine, University of Minnesota, St. Paul, Minnesota, for postmortem examination. Tissue samples, including small and large intestine, lung, brain, liver, kidney, spleen, heart, intestinal lymph node, and adrenal gland, were fixed in 10% buffered formalin and embedded in paraffin. Sections cut at 4 μm were stained with hematoxylin and eosin (HE). Additional sections of the intestine were stained with a modified Gram stain. Fresh samples of lung and liver were submitted for aerobic culture. The intestine was cultured under aerobic and anaerobic conditions by routine laboratory procedures. Feces were submitted for routine parasitologic examination by flotation techniques. Fecal samples were examined for viruses by using direct negative-contrast transmission electron microscopy as previously described.8

Avidin–biotin–peroxidase complex method was used for immunohistochemical demonstration of coronaviral and rotaviral antigens as previously described.4,19 Two monoclonal antibodies against ruminant coronavirus (bovine and elk coronavirus) spike protein4 (clone Z3A5) and nucleocapsid protein4 (clone 8F2), 1 monoclonal antibody against porcine transmissible gastroenteritis virus8 (TGE; clone 14-E3), and 1 monoclonal antibody against bovine rotavirusb (clone 9-10) served as primary antibodies.3,16,19

At necropsy, the calf was in a good nutritional state. The colon and cecum were moderately distended and filled with yellow–brown watery fluid. The mucosa of the proximal colon and cecum had multiple, slightly raised, well-demarcated, red–white mottled areas, which were up to 10 cm in length by 5 cm in width (Fig. 1). The mesentery was edem-