Estimation of the time of seroconversion to the New Jersey serotype of vesicular stomatitis virus in sentinel cattle of dairy herds located at high and low elevations in southern Mexico

Aiko D. Adell, DVM, MPVM; Andres M. Perez, DVM, PhD; Roberto Navarro Lopez, DVM, MSc; Irene Lopez Gonzalez, BS; Pedro Paz Ramirez, DVM; Luis L. Rodriguez, DVM, PhD

Objective—To estimate the time of seroconversion to the New Jersey serotype of vesicular stomatitis virus (VSNJV) in sentinel cattle of dairy herds located at high and low elevations in southern Mexico and to determine the factors associated with an increase in VSNJV transmission.

Animals—471 dairy cattle in 4 free-ranging dairy herds located at high and low elevations in southern Mexico.

Procedures—Serum samples from all cattle were screened by use of serum neutralization (SN) tests for antibodies against VSNJV. Cattle with SN titers < 1:20 were designated as sentinel cattle and tested every 10 weeks for seroconversion to VSNJV (SN titer ≥ 1:80). A Cox proportional hazards regression model was used to compare the hazard for seroconversion between sentinel cattle located at high and low elevations and kept under similar management and nutritional conditions.

Results—Hazard of VSNJV seroconversion was significantly higher for sentinel cattle located at high elevations, compared with the hazard for sentinel cattle located at low elevations. Dairy cattle located at high elevations seroconverted to VSNJV more frequently during the rainy season and the beginning of the dry season.

Conclusions and Clinical Relevance—Seroconversion to VSNJV was more likely in dairy cattle in southern Mexico located at high elevations than in dairy cattle located at low elevations. These findings should contribute to understanding the dynamics of VSNJV infection in endemic areas and should be useful in the design of effective preventive and control strategies to decrease the impact of future VSV incursions.(Am J Vet Res 2010;71:1451–1456)
South America, Central America, and southern Mexico, with outbreaks being reported every year throughout these regions. In contrast, VSV is not endemic in the United States. In addition, outbreaks of VSV typically occur in the United States only every 5 to 10 years. It is believed that the risk for an outbreak of VSV in an endemic region is associated with environmental conditions that promote or prevent VSV transmission. Typically, the highest incidence of VSV coincides with years that are warmer and wetter, compared with the incidence in cooler and drier years. Risk for VSV transmission also appears to be associated with the elevation at which a susceptible population of animals is located. In study conducted in Costa Rica, the odds of being seropositive for antibodies against VSNJV in cattle herds located at elevations of 500 to 1,500 m above sea level were 3.6 times as high as those for cattle herds located at elevations < 500 m above sea level. However, because of the cross-sectional nature of the design of that study, it is possible that unmeasured variables may have affected the results. Prospective studies conducted in the United States and El Salvador in which investigators used sentinel animals did not explicitly explore the association between elevation and seroconversion rates. The purpose of the study reported here was to estimate the time of seroconversion to VSNJV in sentinel cattle of dairy herds located at high and low elevations in southern Mexico and to determine the factors associated with an increase in VSV transmission.

**Materials and Methods**

**Sample population**—Dairy cattle (n = 471) of 4 free-range dairy herds located in Tocotalpa, State of Tabasco, and in Ocozocautitlan, Villaflorres, and Arriaga, State of Chiapas, were enrolled in the study between January 2000 and November 2002. Herds were included on the basis of the willingness of herd owners to participate. Management practices of these 4 herds were similar, and number of cattle in each herd (range, 72 to 136 cows; mean, 188 cows) was similar and typical of dairy herds located in southern Mexico and northern Central America. Cattle in these herds ranged from 2 to 8 years of age (mean, 2.3 years). Two herds were located at high elevations (altitude, 600 or 820 m above sea level [designated as herds 1 and 2, respectively]) in the State of Chiapas. The remaining 2 herds were located at low elevations (altitude, 200 or 100 m above sea level [designated as herds 3 and 4, respectively]) near the Pacific coast and the Gulf of Mexico. The area in which these 4 herds were located was characterized by a tropical climate with mean temperatures ranging from 22°C to 28°C. Annual rainfall ranged from 1,227 to 1,441 mm and 1,102 to 3,724 mm for the high- and low-elevation herds, respectively. Demographic conditions and management practices, such as breed and age of the animals, nutrition system, and sanitary management, were similar for the 4 herds.

**Sample collection and SN tests**—Blood samples were collected from the jugular or coccygeal vein from every cow in each herd at the beginning of the study by use of a needle and transferred into a glass tube with a clot activator gel by official veterinarians employed by the Mexican Animal Health Service. Samples were stored briefly in the shade at ambient temperature (not recorded) for 15 minutes, centrifuged at 1,431 X g for 5 minutes, and transferred into 1.8-mL sterile vials. Vials were stored in containers filled with coolant gel at 20°C for ≤ 6 hours while in transit to the headquarters and were then stored at –70°C until processing. Samples were analyzed by use of microtiter SN tests at a reference laboratory as previously described. Cattle with SN antibody titers < 1:20 (the lowest dilution tested) to VSNJV were selected as sentinel cattle. Follow-up SN testing was performed on samples obtained from sentinel cattle approximately every 10 weeks throughout the 34-month duration of the study to evaluate seroconversion to VSNJV infection. Sentinel cattle with SN titers ≥ 1:80 were considered to have seroconverted to VS

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positive cattle. However, the use of an SN titer $\geq 1:160$ to define seroconversion may increase the specificity and decrease the sensitivity of the SN test, compared with changes in sensitivity and specificity when an SN titer $\geq 1:80$ is used. Computations for statistical analyses were made by use of a commercial software program. A value of $P < 0.05$ was used to indicate significance for all analyses.

Results

Populations of sentinel cattle were homogenous for breed and sex. Seroconversion to the Indiana serotype of VSV was observed in only 1 cow; therefore, results and analyses reported here refer to cattle that seroconverted to VSNJV. Age was not significantly ($P = 0.82$) associated with VSNJV seroconversion in sentinel cattle; therefore, the matched control design used here resulted in a population of sentinel cattle in each of the 4 herds that was homogeneous for every factor and condition recorded, except for elevation. At the initiation of the study (0 months), VSNJV seroprevalence ranged from 38% to 58% (mean, 50%) but did not differ significantly ($P = 0.25$) among the 4 herds.

The proportion of clinical disease attributable to VSNJV was greater in high-elevation herds, compared with this proportion in low-elevation herds (Table 1).

The proportion of sentinel cattle that seroconverted to VSNJV in these 4 herds was 50% when an SN titer $\geq 1:80$ was used to define seroconversion and 43% when an SN titer $\geq 1:160$ was used to define seroconversion. In addition, when an SN titer $\geq 1:80$ was used to define seroconversion, sentinel cattle in high-elevation herds were at a significantly higher risk for seroconversion to VSNJV than were sentinel cattle in low-elevation herds. The pattern of seroconversion did not vary when an SN titer $\geq 1:160$ was used to indicate seroconversion.

Table 1—Comparison of the results of SN testing for VSNJV seroconversion in 4 herds of dairy cattle located at high and low elevations in southern Mexico.

<table>
<thead>
<tr>
<th>Variable</th>
<th>High elevation</th>
<th>Low elevation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Herd 1</td>
<td>Herd 2</td>
</tr>
<tr>
<td>Elevation (m above sea level)</td>
<td>600</td>
<td>630</td>
</tr>
<tr>
<td>No. of cattle from which samples were obtained at the initiation of the study</td>
<td>130</td>
<td>113</td>
</tr>
<tr>
<td>Proportion of cattle with SN titers $\geq 1:80$ for VSNJV at the initiation of the study*</td>
<td>0.48</td>
<td>0.55</td>
</tr>
<tr>
<td>No. of cattle with clinical signs of VSNJV</td>
<td>26</td>
<td>17</td>
</tr>
<tr>
<td>No. of sentinel cattle†</td>
<td>14</td>
<td>29</td>
</tr>
<tr>
<td>No. of sentinel cattle that seroconverted to VSNJV during the study*</td>
<td>11</td>
<td>28</td>
</tr>
<tr>
<td>Proportion of sentinel cattle that seroconverted to VSNJV during the study*</td>
<td>0.79</td>
<td>0.97</td>
</tr>
<tr>
<td>Median time to VSNJV seroconversion in sentinel cattle (mo)</td>
<td>17‡</td>
<td>19‡</td>
</tr>
</tbody>
</table>

*An SN titer $\geq 1:80$ represents a 4-fold increase over the lowest serum dilution (1:20) tested and is recommended for indication of infection (seroconversion) with VSNJV in endemic areas. †Cattle with SN titers $\geq 1:20$ were selected for use as sentinel cattle. ‡Median time to seroconversion was 25 months when an SN titer $\geq 1:160$ was used to define seroconversion.

— = Not determined because the proportion of sentinel cattle with an SN titer $\geq 1:80$ for VSNJV was $< 0.5$.

Figure 1.—Percentages of sentinel dairy cattle with an SN titer $\geq 1:80$ used to indicate seroconversion to VSNJV in high-elevation herds (herds 1 [600 m above sea level; A] and 2 [1,000 m above sea level; B]) and low-elevation herds (herds 3 [200 m above sea level; C] and 4 [100 m above sea level; D]). Seroconversion was detected during 2000 (white bars), 2001 (gray bars), and 2002 (black bars). The pattern of seroconversion did not vary when an SN titer $\geq 1:160$ was used to indicate seroconversion.
Discussion

In the study reported here, results of a prospective cohort study conducted in an area endemic for VSNJV are described. Analysis of these results suggests that time to seroconversion was significantly shorter for sentinel cattle in high-elevation herds, compared with that for sentinel cattle in low-elevation herds. These results will help characterize the nature and extent of the association between epidemiological factors and the risk for VSNJV seroconversion in areas endemic for VSNJV. Furthermore, these results will be useful for the design of disease control programs in areas endemic for VSNJV, and ultimately, they might help veterinarians to anticipate epidemics of VSNJV in areas nonendemic for VSNJV.

Sentinel cattle in southern Mexico in high-elevation herds seroconverted to VSNJV more frequently and rapidly than did sentinel cattle in low-elevation herds. Seroconversion was not a random event throughout the year; rather, VSNJV seroconversion in sentinel cattle in high-elevation herds peaked during the rainy season and at the beginning of the dry season, which is consistent with information known regarding seasonal dynamics for the transmission and spread of VSV.

The findings of the study reported here are consistent with the findings of earlier studies in which investigators reported that elevation, rainfall, season,19 and presence of running water (eg, rivers, irrigation channels, and drainage ditches) are factors that influence transmission of VSV.4,12 Investigators in other studies4,6 have reported a higher incidence of VS in cattle during the summer and spring, compared with the incidence of VS in cattle during the winter and fall. Analysis of the results of another study17 and the results of the present study suggest that the number of VSNJV epemics peaks during the rainy season. Some investigators believe that the incidence of VS is highest during the summer in places with a temperate climate12 but that disease incidence is highest after the rainy season in regions with a tropical climate.18

The association between rate of seroconversion and season and between seroconversion and elevation suggests that VS transmission may be influenced by the nature and abundance of the population of hematophagous insects, which are recognized vectors for the spread of VSV. It has been reported in earlier studies19 that VSV is able to replicate in certain species of sand flies (Lutzomyia spp),19 mosquitoes (Aedes spp),20,21 biting gnats (Culicoides spp),22 and black flies (Simulium spp).15,23,24 In addition, sand flies and black flies are capable of transmitting VSNJV both transovarially and horizontally to susceptible vertebrate hosts.4,19,25 Furthermore, transmission of VSNJV from black flies to mice has been confirmed.23 Investigators of another study24 reported that swine bitten by black flies developed lesions at the site of the bites, and this indicates that VSNJV–infected black flies can biologically transmit the virus to susceptible swine. Similarly, allowing experimentally infected black flies to feed at the coronary band of the hoof as well as on the lips of cattle resulted in clinical signs of VS.15 Investigators of another study12 reported the transmission of VSV to cattle by VSV-infected biting gnats (Culicoides spp), and other investigators reported26,27 that sand flies can transmit the virus to pigs, which suggests that both biting gnats and sand flies may also play a role as biological vectors for VSNJV. However, despite experimental evidence that suggests that VS is a vector-borne disease, it is still unclear which insect species are the most essential vectors for the transmission and spread of VSV under natural conditions. For that reason, it is also unknown whether the association between the rate of VSV seroconversion and season and between seroconversion and elevation in the study reported here can be explained by a difference in the abundance of insect species that are proven
 vectors for the transmission of VSV in high-elevation herds, compared with results for low-elevation herds.

Notably, if transmission of VSV is associated with a species-specific insect vector,12 the differences in the species, habitats, and size of the arthropod populations in high-elevation herds and low-elevation herds may explain the variations in the frequency and speed of seroconversion reported in the present study. Some investigators have reported13,19 that vectors of VSV, such as black flies, are more prevalent in forests and adjacent to streams, which are ecological conditions that are encountered more commonly by cattle located at high elevations than by cattle located at low elevations. Investigators of another study10 reported a lower risk high elevations than by cattle located at low elevations. are encountered more commonly by cattle located at to streams, which are ecological conditions that are prevalent in forests and adjacent to streams, which are ecological conditions that are encountered more commonly by cattle located at high elevations than by cattle located at low elevations. Investigators of another study10 reported a lower risk high elevations than by cattle located at low elevations.

In conclusion, the rate of VSNJV seroconversion and time to VSNJV seroconversion were associated with elevation and season in regions of Mexico endemic for VS. Consideration of ecological factors in the design and implementation of VS control and prevention strategies may be an effective method for reducing the prevalence of VS caused by VSV in endemic regions and, ultimately, for preventing or minimizing the impact of VSV epidemics in nonendemic areas.

References


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