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

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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)

Vesicular stomatitis is a disease of cattle, horses, pigs, and small ruminants caused by a vesiculovirus of the family Rhabdoviridae that is commonly referred to as VSV.[1–6] In addition, VSV infects a variety of wildlife species. This virus is transmitted by insects such as sand flies (*Lutzomyia* spp), black flies (*Simulium* spp), and biting gnats (*Culicoides* spp).[7] It is a negative-sense, single-stranded RNA virus with the capacity to replicate in high numbers.[1–6] Strains of VSV are grouped into 2 serotypes (New Jersey and Indiana).[5,6]

Abbreviations

CI	Confidence interval
SN	Serum neutralization
VS	Vesicular stomatitis
VSNJV	New Jersey serotype of vesicular stomatitis virus
VSV	Vesicular stomatitis virus

Economic losses resulting from infection with VSV infection are associated with direct impacts on milk production and weight loss in infected animals and indirect costs derived from the treatment of infected animals, quarantine of infected premises, and cancellation of public events such as horse shows and cattle auctions.[3–6] For example, the economic impact of an outbreak of VS in the United States has been estimated at $100 to $200/cow and a mean loss of $15,565/ranch infected with VSV.[8,9] This disease, which is considered one of the most important diseases included in a list of differential diagnoses when examining animals with disease of the feet and oral cavity, is endemic in northern

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South America, Central America, and southern Mexico, with outbreaks being reported every year throughout these regions.[1,3,6] In contrast, VSV is not endemic in the United States. In addition, outbreaks of VS typically occur in the United States only every 5 to 10 years.[1,6]

It is believed that the risk for an outbreak of VS in an endemic region is associated with environmental conditions that promote or prevent VSV transmission. Typically, the highest incidence of VS coincides with years that are warmer and wetter, compared with the incidence in cooler and drier years.[4,6] Risk for VSV transmission also appears to be associated with the elevation at which a susceptible population of animals is located. In a study[10] 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,[10] it is possible that unmeasured variables may have affected the results. Prospective studies conducted in the United States[11] and El Salvador[12] 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 Ocozocoautla, Villaflores, 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 156 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° to 28.2°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 $\times$ 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[a] as previously described.[13] 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 VSNJV and were subsequently classified as VSNJV positive because a 4-fold increase over the first serum dilution tested is recommended for diagnosing infection (seroconversion) with VSNJV in endemic areas.[14–16] Sentinel cattle with SN titers < 1:80 were still considered VSNJV negative. Investigators in another study[10] reported an association between the risk for VS and altitudes ranging from 500 to 1,500 m above sea level.

Statistical analysis—Samples were obtained from cattle of low- and high-elevation herds at approximately the same times, which meant that we did not have to make adjustments for the effect of nominal variables (eg, season or month of sample collection) during statistical analyses. Seroprevalence at the initiation of the study was computed as the proportion of VSNJV–positive cattle in each herd and was compared among herds by use of a χ^2 test. In addition, a χ^2 test was used to determine whether an association existed between age and seroconversion to VSNJV in sentinel cattle. Incidence was calculated as the proportion of seronegative cattle that became VSNJV positive as described. When cattle herds were homogenous for factors such as breed and sex, and when age was not associated with seroconversion to VSNJV, risk of seroconversion was calculated as the proportion of sentinel cattle that seroconverted in each herd.

A Cox proportional hazards regression model was used to assess the hypothesis that the hazard for VSNJV seroconversion was significantly higher for cattle exposed to VSNJV (ie, sentinel cattle in high-elevation herds [herds 1 and 2]), compared with VSNJV seroconversion in cattle not exposed to VSNJV (ie, sentinel cattle in low-elevation herds [herds 3 and 4]). The event of interest was the time to VSNJV seroconversion. Sentinel cattle removed (eg, died or culled) from any herd before seroconversion were considered censored data. The model was adjusted by the herd of origin from which the blood samples were collected to account for lack of independence among subsequent observations. Time to seroconversion of 50% of the sentinel cattle (median survival time) was calculated for each herd.

An SN titer ≥ 1:80 could be considered a nonspecific titer for the identification of VSNJV–positive cattle because this study was conducted in an area endemic for VSNJV. Therefore, analyses were repeated by use of an SN titer ≥ 1:160 for the identification of VSNJV–

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.

Variable	High elevation		Low elevation	
	Herd 1	Herd 2	Herd 3	Herd 4
Elevation (m above sea level)	600	820	200	100
No. of cattle from which samples were obtained at the initiation of the study	130	113	72	156
Proportion of cattle with SN titers ≥ 1:80 for VSNJV at the initiation of the study*	0.48	0.55	0.38	0.58
No. of cattle with clinical signs of VSNJV	26	17	0	9
No. of sentinel cattle†	14	29	21	28
No. of sentinel cattle that seroconverted to VSNJV during the study*	11	28	4	3
Proportion of sentinel cattle that seroconverted to VSNJV during the study*	0.79	0.97	0.19	0.11
Median time to VSNJV seroconversion in sentinel cattle (mo)	17‡	19‡	—	—

*An SN titer ≥ 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.[14–16] †Cattle with SN titers < 1:20 were selected for use as sentinel cattle. ‡Median time to seroconversion was 25 months when an SN titer ≥ 1:160 was used to define seroconversion.

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

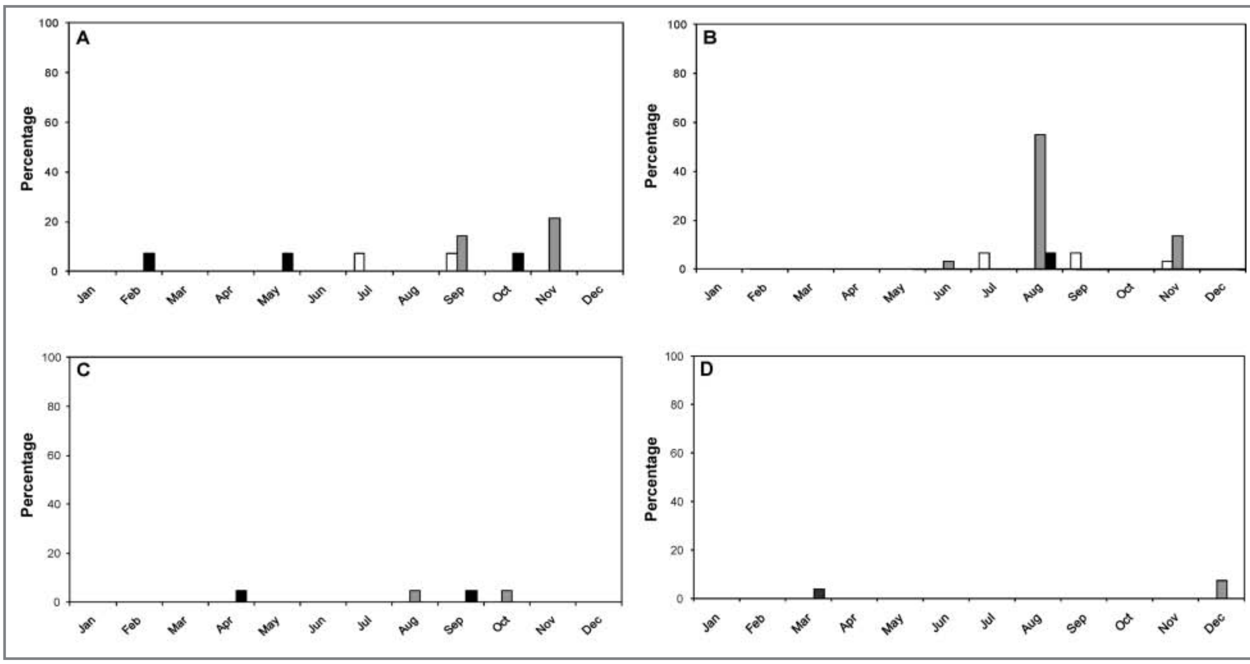

Figure 1—Percentages of sentinel dairy cattle with an SN titer ≥ 1:80 used to indicate seroconversion to VSNJV in high-elevation herds (herds 1 [600 m above sea level; A] and 2 [1,000 m above sea level; B]) and low-elevation herds (herds 3 [200 m above sea level; C] and 4 [100 m above sea level; D]). 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 ≥ 1:160 was used to indicate seroconversion.

positive cattle. However, the use of an SN titer ≥ 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 ≥ 1:80 is used. Computations for statistical analyses were made by use of a commercial software program.[b,c] 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 ≥ 1:80 was used to define seroconversion and 43% when an SN titer ≥ 1:160 was used to define seroconversion. In addition, when an SN titer ≥ 1:80 was used to define seroconversion, sentinel cattle in high-elevation herds were at a significantly higher risk for seroconversion to

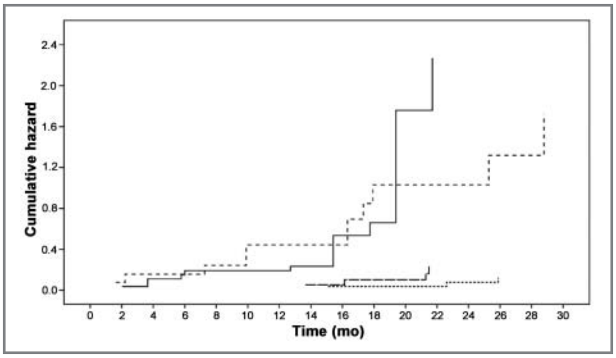

Figure 2—Graph depicting results of a Cox proportional hazards regression model for analysis of the time to seroconversion to VSNJV in sentinel cattle located in high-elevation herds (herd 1, 600 m above sea level [short-dashed line]; herd 2, 1,000 m above sea level [solid line]) and low-elevation herds (herd 3, 200 m above sea level [long-dashed bold line]; herd 4, 100 m above sea level [dotted line]). Seroconversion to VSNJV was defined as an SN titer ≥ 1:80. Outcome did not vary when an SN titer ≥ 1:160 was used to indicate seroconversion.

VSNJV (relative risk, 6.35; 95% CI, 3.18 to 12.69; *P* = 0.01), compared with the risk for sentinel cattle in low-elevation herds. Similarly, when an SN titer ≥ 1:160 was used to define seroconversion, sentinel cattle in high-elevation herds were at a significantly higher risk for seroconversion to VSNJV (relative risk, 7.98; 95% CI, 3.43 to 18.53; *P* = 0.01), compared with the risk for sentinel cattle in low-elevation herds. During the rainy season (from May through October) and the beginning of the dry season (in November), incidence of VSNJV seroconversion in sentinel cattle in the high-elevation herds (79% and 97%, respectively) was higher, compared with the incidence of VSNJV seroconversion in sentinel cattle in the low-elevation herds (19% and 11%, respectively; **Figure 1**).

Hazard of VSNJV seroconversion was higher for sentinel cattle in high-elevation herds, compared with that for sentinel cattle in low-elevation herds, regardless of whether an SN titer ≥ 1:80 (hazard ratio, 3.56; 95% CI, 2.43 to 5.58; *P* = 0.01) or ≥ 1:160 (hazard ratio, 3.63; 95% CI, 2.37 to 6.21; *P* = 0.01) was used to define seroconversion (**Figure 2**). Median survival time ranged from 17 to 25 months for sentinel cattle in high-elevation herds, whereas the median survival time could not be determined for sentinel cattle in low-elevation herds because of the low proportion that seroconverted to VSNJV. Clinical signs of VS were observed during the study period in 3 of the herds (Table 1).

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,[10] and presence of running water (eg, rivers, irrigation channels, and drainage ditches) are factors that influence transmission of VSV.[4,12] Investigators in other studies[4,6] 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 study[17] and the results of the present study suggest that the number of VSNJV epidemics peaks during the rainy season. Some investigators believe that the incidence of VS is highest during the summer in places with a temperate climate[12] 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 studies[19] 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 study[24] 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 study[22] reported the transmission of VSV to cattle by VSV-infected biting gnats (*Culicoides* spp), and other investigators reported[24,26] 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,[1,27] 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 reported[13,19] 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 study[10] reported a lower risk for VSNJV seroconversion in low-elevation herds, compared with the risk of VSNJV seroconversion in high-elevation herds, which is consistent with the results of the present study. Furthermore, other investigators of VS have suggested that VSV is enzootic in low-elevation herds that are located close to a seacoast and that VSV behaves epizootically in high-elevation herds,[17] which may explain the faster seroconversion rate observed in the study reported here. Investigators in 1 study[27] determined that the distribution of sand flies varied with the level of elevation in areas endemic for VSNJV. Sand flies, including *Lutzomyia shannoni*, are more frequently encountered at lower elevations in areas endemic for VS, whereas black flies are more frequently encountered at higher elevations.[1] Therefore, genus- or species-specific differences in the ability of insects to maintain infections or to serve as vectors for VSV may explain the association between elevation and disease prevalence and between elevation and rate of seroconversion in the study reported here.

Results of the present study provide support for our hypothesis that ecological factors play a role in the epidemiology of VS in endemic regions. Furthermore, these results may have an impact on the design of VS control strategies. Control of VS epidemics in the United States and Mexico is based on the quarantine of infected cattle and premises suspected of being infected and, sometimes, on the restriction of animal movement in VSV-affected regions.[24] However, if VSV transmission is associated with ecological conditions, such as elevation, weather, and insect vectors, these conditions should be considered to more effectively prevent or mitigate the spread of VSV. Selective application of control and prevention strategies in regions and during periods in which conditions for disease spread are more favorable, such as those reported in the present study, may help reduce the incidence of VS in areas endemic for VSV and, ultimately, reduce the risk for epidemics of VSV in nonendemic regions of Mexico and in the United States.

Alternatively, it is possible that the association between VSNJV seroconversion and high-elevation herds in the study reported here may have been caused by the presence or absence of epidemiological factors other than elevation or by epidemiological factors associated with elevation that were not investigated. For example, differences in the rate of seroconversion may be explained, in part, by the presence of different isolates of VSNJV at different elevations. Other investigators of VSNJV have reported[1] that VSNJV isolates found in bovids located in low-elevation herds (eg, tropical dry forest of Costa Rica) belonged to a genetic lineage that was different than that of an isolate found in bovids located in high-elevation herds (eg, humid forest). Therefore, it also is possible that VSV strains that caused disease in the study reported here were isolates of different genetic lineages, which may have resulted in variations of the virulence of VSNJV and the differences in the frequency and speed of VSNJV seroconversion between high- and low-elevation herds. Moreover, differences in the rate of VSNJV seroconversion also may have been affected by differences in the humidity or the relative abundance of standing water in the proximity of the herds included in the present study. Unfortunately, such hypotheses could not be investigated because no other VSNJV isolates were collected during the present study and variations in humidity were not recorded.

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.

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b. SPSS, SPSS Inc, Chicago, Ill.
c. JMP software, SAS Institute Inc, Cary, NC.

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