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SEROLOGICAL MONITORING OF VESICULAR STOMATITIS NEW JERSEY VIRUS IN ENZOOTIC REGIONS OF COSTA RICA

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Abstract. The activity of vesicular stomatitis viruses was monitored on 3 dairy farms in Costa Rica. Antibody levels were measured and clinical disease monitored in 165 cattle during a 20 month period (1986–1988). Vesicular stomatitis New Jersey (VS NJ) virus was shown to be enzootic on these farms by a 94.2% prevalence of neutralizing antibody; this did not vary significantly between herds. The mean prevalence of antibody to vesicular stomatitis Indiana (VS IN) virus was 15.2%, but was significantly higher in 1 herd. A total of 25 cases (annual incidence rate of 9%) of clinical vesicular stomatitis (VS) was reported. VS NJ virus was identified as the causal agent by detection of VS NJ virus antigens by the complement fixation test. VS NJ virus was isolated in 11 cases. All episodes of disease occurred between November and January, the beginning of the dry season. Most animals maintained stable neutralizing antibody titers throughout the study, and all diseased animals were previously seropositive to VS NJ virus. A total of 31 animals with neutralizing antibodies to VS NJ virus had a VS NJ virus-specific IgM response, and 6 animals had IgM responses that persisted for as long as 6 months. There was no relation between IgM responses and clinical disease occurrence. VS NJ virus persisted predominately as a subclinical infection in cattle throughout the year in enzootic areas of Costa Rica. The humoral response did not prevent reinfection with VS NJ virus.

Domestic livestock of many Central and South American countries are frequently infected with vesicular stomatitis virus (VSV). This virus causes vesicular stomatitis (VS) characterized by vesicular lesions leading to loss of epithelium of the mouth, tongue, coronary band, and udder. These signs are clinically indistinguishable from those caused by foot and mouth disease virus (FMDV). In some cases, VS occurs as a subclinical (non-vesicular) debilitating disease.[1] There are at least 2 serotypes that cause VS, VS New Jersey (VS NJ) virus, and VS Indiana (VS IN) virus. Both are members of the genus Vesiculovirus within the family Rhabdoviridae.

In recent years, VS NJ virus has been the most frequently isolated virus in clinical cases of vesicular stomatitis in North America and Latin America.[2] In temperate regions of Mexico and the United States, VS causes epizootics at variable intervals, whereas from southern Mexico, throughout Central and South America, the disease is considered enzootic. In Costa Rica, VS appears regularly at the beginning of the dry season (November–December).[3–6] This disease is considered important because of the direct and indirect losses associated with clinical cases in epizootic and enzootic areas.[7,8] Because of its similarity to FMDV, it causes alarm and confusion when it appears in FMDV-free areas.

The natural cycle of VS NJ virus is not yet clear, particularly under enzootic conditions. Sequence analysis of viral isolates of epizootic and non-epizootic origin suggest that the virus may be maintained in a stable reservoir in nature and periodically escapes this niche to infect domestic animals.[9,10]

This study was a prospective serological survey in a VS enzootic region, to determine the level of viral activity during the presence and absence of disease in a group of adult dairy cattle. Neutralization surveys were used to show that VS NJ virus was enzootic in the 3 herds over the period. Virus isolation and complement fixation were used to document each occurrence of clinical VS. An IgM capture ELISA was used to follow VS NJ virus-specific IgM responses.

MATERIALS AND METHODS

Survey area

Three dairy farms (B, C, and G), comprising a total average population of 165 animals, participated from August 1986 to March 1988. These farms were located within a 20 mile radius of the town of Grecia in the province of Alajuela, Costa Rica. The altitudes were 1,600, 1,300 and 1,100 m above sea level for B, G, and C, respectively. The land is considered premontane wet forest, with an average annual rainfall >2,000 mm and a marked dry season from December to May.[11,12] The temperature range is 15–30°C (average 22.5°C) throughout the year.[11] This is an agricultural region where coffee and sugar cane are the major crops and dairy farms are small secondary operations.

Clinical cases

Farms were visited once every month. Cattle were observed during milking in order to detect active clinical cases or fresh scars. During the intervals between visits, farm personnel were asked to record and report any animals with vesicular disease. The farm personnel were given vials of transport medium (20% phosphate buffered glycerol, pH 7.2) and asked to collect epithelium and/or vesicular fluid from any vesicular lesion, write the cow number and the date on the label, and immediately inform the laboratory at the Veterinary School in Costa Rica. Samples collected during monthly visits or by farm personnel were delivered to the laboratory within 24 hr. Part of each sample was sent to the Vesicular Diseases Diagnostic Laboratory (LADIVES) in Panama for detection of VS viral antigen by the complement fixation test.[13] The remainder of each sample was frozen at −70°C. Once a case was identified as VS, virus was isolated on Vero cells and confirmed as VS NJ virus by a microtiter neutralization test with anti-VS NJ or anti-VS IN serum (kindly provided by Gene Erickson, National Veterinary Service Laboratory, U.S. Department of Agriculture, Ames, IA). The anti-VS NJ serum contained high titer antibody to 100 $TCID_{50}$ of VS NJ virus and did not neutralize 100 $TCID_{50}$ of VS IN virus at the lowest dilution (1:100) of antiserum. The reciprocal was also true for the anti-VS IN serum. The incidence rate of clinically observed VS was calculated as the number of new cases divided by the average population at risk during a given period of time[14] and may underestimate the real incidence rate because cases may have escaped observation.

Serological survey

Serum samples were collected from all adult cattle present on the farm on each sampling date. The mean number of animals sampled in each herd was 26, 42, and 20 for herds B, C, and G, respectively, for an average sample population of 88 animals (53% of the total population of 165 animals). All animals were sampled at least 5 times among 8 collection dates. Sera were collected approximately every 2 months, except from January to May 1987.

Serological assays

Sera were tested for neutralizing antibodies to VS NJ virus and VS IN virus by microtiter neutralization assay in 96-well plates.[15] The indicator cells were Madin Darby bovine kidney cells, purchased from American Type Tissue Culture (ATCC CCL22) and used at passage levels below 185. All sera were tested at 4-fold dilutions starting at 1:100 against 100 $TCID_{50}$ of VS NJ virus Greentree strain and VS IN virus Lab Strain (supplied by Thomas Monath, Centers for Disease Control, Fort Collins, CO). Both viruses were provided as lyophilized 10% suckling mouse brain. To control for interassay variation, serial serum samples from the same animal were titrated in the same assay. VS NJ and VS IN virus reference antisera and negative control serum were included in every neutralization assay.

An IgM antibody capture ELISA (MAC ELISA) was used for the detection of IgM antibodies to VS NJ virus. This assay was performed as described.[16] Affinity purified goat anti-bovine IgM (μ chain-specific) antibodies (Kirkegaard and Perry Laboratories, Inc., Gaithersburg, MD) were bound to wells of 96-well flat-bottomed polystyrene plates (Immulon II, Dynatech Laboratories, Inc., Alexandria, VA), followed by test serum diluted 1:100. Positive and negative control bovine anti-VS NJ sera from a calf (No. 001) experimentally infected at the University of Wisconsin, and a heifer (Juanita) experimentally infected at the Veterinary School in Costa Rica, were included in every plate. Positive serum was

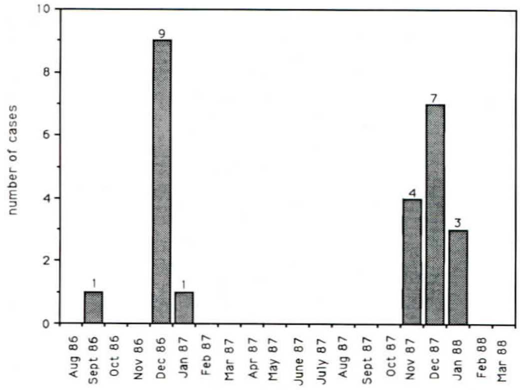

FIGURE 1. Total number and date of occurrence of clinical cases in 3 study herds.

the day 15 post-inoculation serum and the negative control was the pre-inoculation serum. Gradient purified VS NJ virus was then added and virus bound by VS NJ virus-specific IgM was detected using mouse IgG anti-VS NJ glycoprotein antisera conjugated to alkaline phosphatase and an appropriate chromogen. Gradient purified VS IN virus was used as the negative antigen control. Every serum sample was screened against both VS NJ and VS IN viral antigen. Antisera to the VS NJ virus glycoprotein was prepared by inoculation of mice with the glycoprotein that had been purified from the VS NJ (Greentree strain) virion by detergent disruption and centrifugation as described previously.[17] Optimal dilutions of the capture antibody, antigens, and conjugate were determined by checkerboard titrations. A serum was considered positive if it had a positive over negative ratio >2. This was determined by dividing the mean absorbance value of 1:100 diluted test serum by the mean absorbance of the negative control pre-inoculation serum. Positive samples were titrated at 4-fold dilutions starting at 1:100.

RESULTS

Clinical cases

A total of 25 clinical cases of vesicular disease were observed between August 1986 and March 1988. All cases were confirmed as VS NJ virus by detection of VS NJ viral antigen using routine diagnostic complement fixation tests at the LADIVES laboratory in Panama. In 11 cases, virus was isolated and identified as VS NJ virus by neutralization with reference serum. These 25 cases represented an annual incidence of 9% in the total average population of the 3 herds (165 animals). No differences were observed in case occurrence among herds ($P < 0.05$). Most cases occurred during the dry season, particularly during the months of November, December, and January (Fig. 1).

TABLE 1

Individual herd prevalence of neutralizing antibodies to VS NJ and VS IN viruses

Herd	Average population	Prevalence VS NJ virus	1986–1988 (SD)* VS IN virus
B	26	93.9 (3.58)	12.70 (4.5)
C	43	92.4 (6.13)	*23 (4.72)*†
G	19	96.3 (4.17)	10 (9.61)
Total	88	94.2 (4.78)	15 (8.6)

* Prevalence calculated as follows: (number of seropositive animals/population sampled) × 100. SD = standard deviation.
† Value was significantly different from the mean ($P < 0.05$) by analysis of variance.

Serological survey

The mean number of adult cattle tested during the 8 sampling dates for the 3 herds combined was 88. The overall average prevalence of neutralizing antibodies was 94.2% (4.8% SD) for VS NJ virus and 15.2% (8.6% SD) for VS IN virus (Table 1). No significant differences in the antibody prevalence to VS NJ virus were observed among herds. However, herd C had a statistically significant higher average prevalence of antibodies to VS IN virus (Table 1). No significant differences were observed in the prevalence of neutralizing antibodies to VS NJ virus nor VS IN virus among the 8 sampling dates (Fig. 2).

We attempted to reveal recent primary infections by measuring VS NJ virus-specific serum IgM with the MAC ELISA. The test proved to be specific for IgM. Positive control sera (day 15 post-inoculation) had absorptions >2 times (and >3 SD) above those of the negative control sera (Fig. 3). Most of the test sera failed to react in this assay, although many contained high titers of neutralizing antibodies directed against VS NJ virus. The MAC ELISA also proved specific for VS NJ virus. Sera positive for VS NJ IgM lacked reactivity with the VS IN viral antigen (Fig. 3). Thirty-one animals had at least one IgM response over the 20 month period of the study (Table 2). Sera from animals that experienced clinical disease and those that were not reported

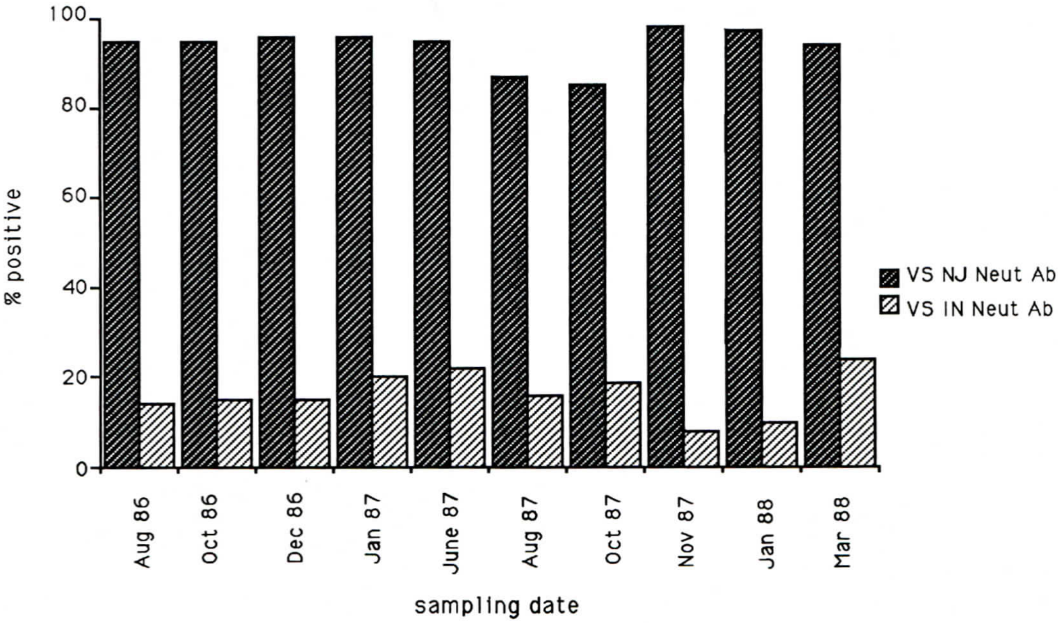

FIGURE 2. Seroprevalence of neutralizing antibodies in herds B, C, and G.

to have clinical disease were compared for the presence of specific IgM (Table 2). Of the 4 IgM positive animals with clinical disease, only 1 animal had an IgM response that corresponded with acute clinical symptoms (data not shown). The IgM responses were detected throughout the year and did not correspond with the seasonality of clinical disease (data not shown).

Many animals with and without clinical symptoms and all with previous neutralizing antibody titers to VS NJ virus had IgM titers that persisted for more than a month. Six of the 31 animals with persistent IgM responses had detectable IgM levels on 2 or more consecutive bleeding dates (Table 3).

Approximately 20 animals had drastic fluctuations in the neutralizing antibody titer to VS NJ virus from 1 bleeding to the next (2 month period) often with a change in titer >10-fold. Figure 4A demonstrates this type of fluctuating antibody response of a representative animal from each herd. All the sera of each animal from the consecutive bleedings were tested in duplicate in the same assay to preclude interassay variation. The fluctuations of antibody titers prevented us from making valid comparisons of average titers among herds or bleeding dates due to the high standard errors obtained (data not shown). Most animals maintained a relatively stable neutralizing antibody response throughout the 20 month test period. This stable neutralizing antibody response of 3 representative animals is shown in Figure 4B.

Unexpectedly, seropositive animals became clinically ill. Furthermore, all animals with clinical disease had neutralizing antibodies to VS NJ virus prior to the onset of illness. Table 4 shows the neutralizing antibody titers to VS NJ virus of animals from which at least 2 serum samples were available prior to the onset of clinical disease and the date that these animals were reported to have experienced clinical VS. In all cases, the causal agent was identified as VS NJ virus by the complement fixation test. In addition, in those animals marked by a dagger, virus was isolated and identified as VS NJ virus

TABLE 2

Number of animals with IgM responses in herds B, C, and G from August 1986 to March 1988

Herd	Clinical disease		No clinical disease	
	IgM	No IgM	IgM	No IgM
B	3	4	4	34
C	1	5	22	54
G	0	12	1	25
Total	4	21	27	113

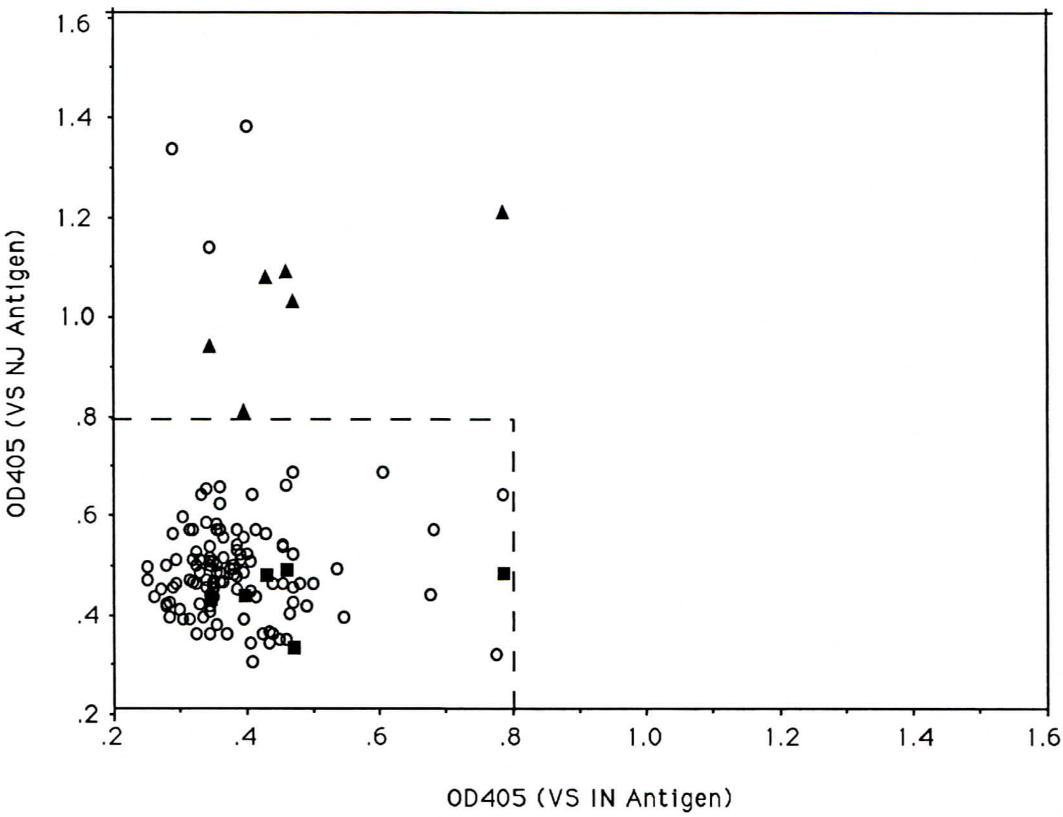

FIGURE 3. MAC ELISA optical densities (OD_{405}) of control sera and 125 test sera from herd B. Positive control sera (▲), negative control sera (■), and test sera (○) were tested against VS NJ virus and plotted against the OD_{405} of the same sera tested against VS IN virus. Dotted line delineates OD_{405} values accepted as positive or negative for VS NJ-specific IgM.

by a serum neutralization assay using anti-VS NJ virus reference serum. In the remaining animals, virus isolation attempts were unsuccessful but all were identified as VS NJ virus by the complement fixation test of epithelium samples.

TABLE 3

Animals with repeated IgM responses

Animal*	Neutalizing titer prior to first IgM	Date(s) of IgM response	Date of clinical disease
B647	1:400	08/87, 11/87, 01/88	12/87
B654	1:6400	12/86, 01/87	12/86
C27	1:1600	10/86, 3/88†	NA§
C30	1:400	08/87, 10/87	NA
C41	1:1600	06/87, 10/87‡	NA
C46	1:1600	06/87, 08/87	NA

* The letter indicates herd of origin.
† Animal was negative for IgM between 10/86 and 3/88.
‡ Not bled on 8/87.
§ Clinical disease not reported in these animals.

DISCUSSION

This study was initiated to examine some of the epidemiological aspects of VSV in an enzootic region. The serological response of the animals and the occurrence of clinical disease were used as indicators of viral activity in the cattle population throughout the study. The occurrence of clinical cases was seasonal, with the majority occurring during the beginning of the dry season (November–January). This agrees with the reported seasonal incidence of clinical cases in Costa Rica during the last 15 years.[5] We found that the seroprevalence to VS NJ virus in all 3 herds examined was very high throughout the 20 month period, whereas the seroprevalence to VS IN virus was relatively low. This was substantiated by the fact that only VS NJ virus was isolated from clinical cases. These findings also agree with reports that 82.3% of VS-related vesicular disease

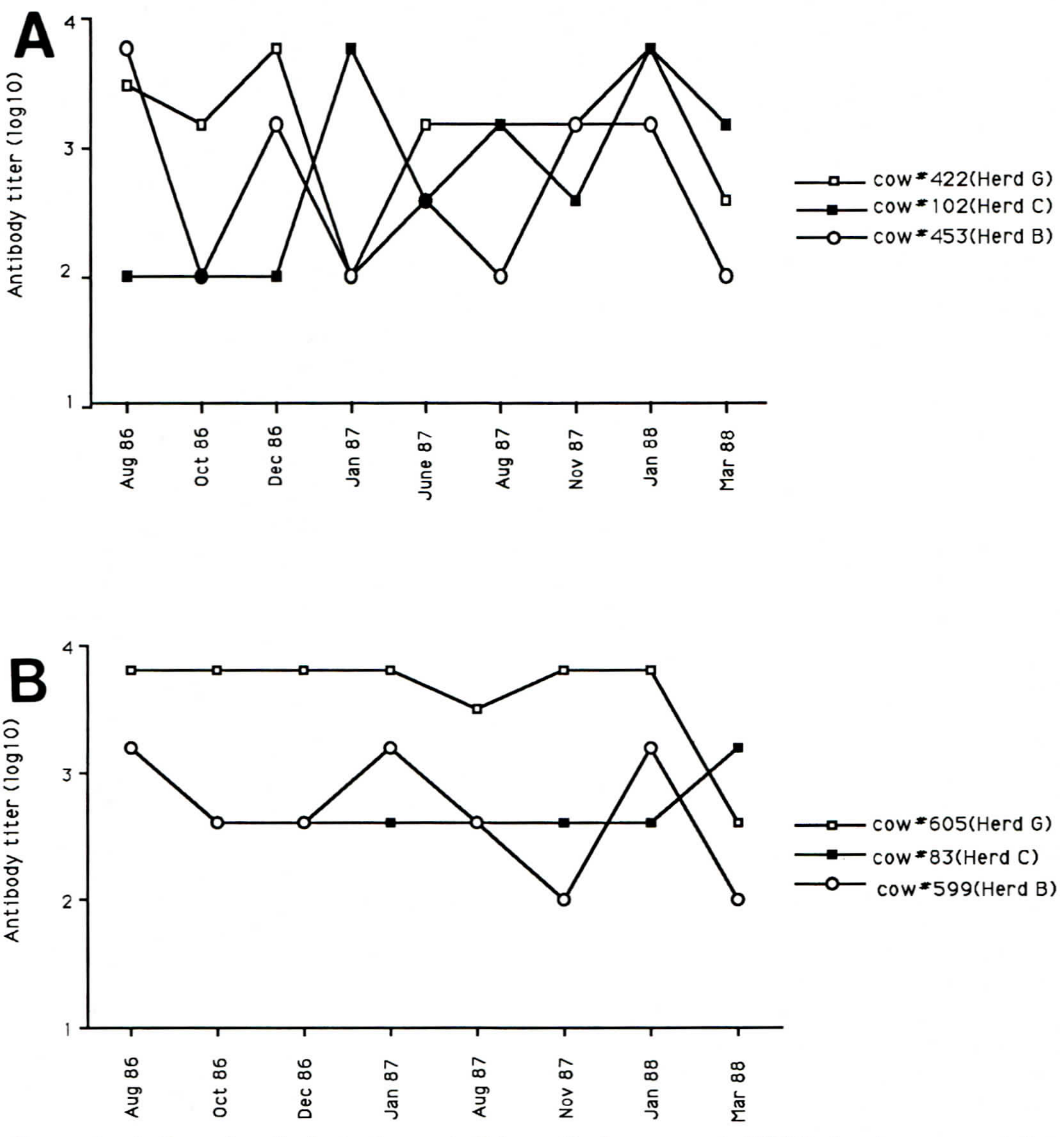

FIGURE 4. **A.** Examples of a fluctuating neutralizing antibody response to VS NJ virus in a representative animal from each herd. **B.** Maintenance of a stable neutralizing antibody response to VS NJ virus in a representative animal from each herd.

cases in Costa Rica are caused by VS NJ virus, whereas only 17.7% are caused by VS IN virus.[5,6] However, herd C, which had a higher prevalence of neutralizing antibodies to VS IN virus than the other 2 herds, had an outbreak caused by VS IN virus in January 1989 (data not shown), which demonstrated that VS IN virus was circulating, but not to the same extent as VS NJ virus.

One of the goals of this study was to determine if the antibody titers to VSV fluctuated according to the season. Since most clinical cases were observed from November to January, we might expect a drop in the number of seropositive animals in the months without clinical activity. However, this was not the case, and seroprevalences for VS NJ virus and VS IN virus did not fluctuate over the 20 month period.

Experimental infection with VS NJ virus results in seroconversion and a persistent fluctuating neutralizing antibody response for up to

TABLE 4
*Animals with neutralizing antibody titers to VS NJ virus susceptible to reinfection with the virus**

Animal	Aug 86	Oct 86	Dec 86	Jan 87	June 87	Aug 87	Nov 87	Dec 87	Jan 88	Mar 88
455†	1600	1600	400	1600	6400	400	400	—	—	—
478	—‡	1600	1600	—	1600	1600	—	—	1600	400
481	1600	1600	3200	—	—	—	6400	—	6400	—
580†	1600	6400	6400	—	1600	1600	—	—	—	—
594	1600	1600	>6400	—	—	—	>6400	—	>6400	>6400
604†	—	—	6400	—	400	—	1600	—	100	1600
646	—	—	1600	1600	100	400	>6400	—	>6400	100
4	>6400	1600	—	1600	1600	1600	—	—	—	—
24	1600	400	—	400	400	—	400	—	400	1600
114†	—	400	—	1600	400	400	400	—	1600	1600
485	400	400	1600	—	100	6400	3200	—	6400	400
540	<100	100	1600	—	100	400	1600	—	100	1600
545†	6400	100	400	1600	6400	1600	1600	—	—	—
581	1600	100	1600	—	100	<100	1600	—	1600	100

† VS NJ virus isolated from these animals at the time of clinical disease.
* Underlining indicates month when clinical disease was observed.
‡ Animals not bled on these dates.

several years.[18] The present survey demonstrated that a fluctuating antibody response was also common in naturally infected cattle in a VS NJ virus enzootic area. The fluctuations in neutralizing antibody titers were drastic and did not have a specific pattern that could be related to the appearance of clinical disease. There are several possible explanations for these antibody fluctuations. It may be possible that cattle are subjected to constant antigenic stimulation by virus circulating in nature. This possibility is supported by reports that both VS NJ virus and VS IN virus have been found in arthropods.[4,19–21] Another source of antigenic stimulation could be direct or indirect transmission from a mammalian reservoir. Neutralizing antibodies to VS NJ virus are found in wild animals.[22] Alternatively, VS NJ virus may persist in the cattle and occasionally be reactivated, causing clinical or subclinical disease. The possibility of persistence has been suggested by several authors.[23–25] Both mechanisms would provide means of intermittent antigenic stimulation to the animal, resulting in a persistent, fluctuating antibody response.

Isolation of VS NJ virus from previously seropositive cattle in the field showed that either reinfection or reactivation of VS NJ virus occurs under natural conditions. The serological history of animals with clinical disease did not show any specific serological trends, such as a drop in titer prior to the onset of disease, as has been suggested by other authors.[26] Experimental inoculation of animals with VS NJ virus has shown that reinfection with the same virus is possible.[1] Intramuscular vaccination with low titer VS NJ virus has been reported to protect animals from reinfection under field conditions; however, some vaccinated animals were still susceptible to experimental challenge.[27] This study documented the occurrence of clinical disease in animals with high neutralizing antibody titers under natural enzootic conditions.

Susceptibility of seropositive animals might be explained by 2 alternative mechanisms. First, it is possible that animals are being reinfected by viruses that are antigenically different from the virus that caused the initial infection, as has been shown to occur with influenza virus.[28] We are currently investigating the possibility of antigenic variation by monoclonal antibody analysis and sequencing of the glycoprotein gene of viruses isolated from these 3 herds. Second, it is possible that the immune response is simply not fully protective against disease, and that cattle remain susceptible despite a vigorous humoral response. This is consistent with the susceptibility of cattle vaccinated either with natural or recombinant VS viral proteins.[29]

The classical immune response to primary challenge with a virus involves the production of IgM followed by IgG. The IgM response is generally of low titer and short lived. Upon secondary infection, the predominant immunoglobulin detected is IgG.[30] The presence of IgM in serum detected by MAC ELISA is commonly used as an indicator of primary infection in many human and animal diseases, including experimental and natural infection with VS NJ virus.[16,22,31,32] We attempted to use the presence of VS NJ virus-specific IgM in serum samples to discriminate between primary and secondary VS NJ virus clinical or subclinical infections. All of the animals with IgM responses were previously seropositive with neutralizing antibodies to VS NJ virus. In most, the IgM response was not related to clinical disease, since most animals with IgM responses either did not have clinical disease or had clinical disease on a different date than the IgM response.

Therefore, in the case of VS NJ virus, an IgM response might not indicate primary infection but rather an acute episode of antigenic stimulation. If VS NJ virus is a persistent infection of cattle, this type of IgM expression would be similar to the IgM response seen in cytomegalovirus infection where detection of IgM is indicative of viral reactivation.[33] In the rhabdoviruses, a mechanism of viral persistence or latency has not been described. The fact that the IgM responses to VS NJ virus were not found more frequently in clinical cases might be because the date of bleeding often did not fall within 2 weeks of the onset of clinical symptoms, possibly precluding the detection of the short-lived IgM response. However, at least 6 of 31 animals had IgM responses on consecutive occasions for periods of up to 6 months. This suggested that the VS NJ virus IgM response in naturally infected cattle in enzootic regions was different from the IgM response in experimentally infected cattle, in which the IgM persisted for up to 30 days post-inoculation and then diminished.[16]

Another possible explanation for IgM responses detected in cattle is that VS NJ virus is antigenically changing in nature and IgM responses are mounted to the "new," or "different," epitopes on the viral glycoprotein. Sequence analysis of the glycoprotein gene should determine if nucleotide variation in the regions coding for the neutralizing epitopes is occurring in the field. It is also possible that the IgM response detected was due to cross-reactions with other serum proteins or immunoglobulin classes. We do not believe this to be the case. The MAC ELISA is a capture test that has been demonstrated to have a greater specificity than other non-capture IgM ELISA tests.[34] Its specificity for VS NJ virus-specific IgM is clearly shown in Figure 3. To preclude nonspecific binding of IgG, we used heavy chain-specific anti-IgM to capture IgM antibodies, a monospecific mouse anti-VS NJ virus glycoprotein antibody, and gradient purified virus, making it very unlikely that we were detecting cross-reactions. All dilutions of these reagents were optimized using sera from the experimentally inoculated calf (No. 001). With these sera, we showed that VS NJ-specific IgM was first detected 4–5 days after inoculation, peaked at 15–20 days, and then diminished to undetectable levels in 30–50 days (data not shown). The neutralizing antibody titers and IgG levels detected by ELISA on these same sera were detectable 4–5 days post-inoculation and persisted until the animal was killed at day 60 post-inoculation (data not shown). This further substantiated the specificity of the MAC ELISA for detecting VS NJ virus-specific IgM.

This study is one of the few prospective serological studies carried out in an enzootic region. The most prevalent virus is VS NJ virus, which was also the major cause of clinical disease. In most cases, the neutralizing antibody response persisted at high titers throughout the 20 month survey period and was not protective against clinical VS NJ virus. VS NJ virus-specific IgM responses occurred in previously seropositive animals, and could not be used as indicators of primary infection in enzootic regions.

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REFERENCES

1. Hanson RP, 1981. Vesicular stomatitis in horses, cattle and swine, New Jersey Indiana viruses. Gibbs EP, ed. *Virus diseases of food animals, vol. II.* New York: Academic Press, 517–539. UI: 8209177
2. *Foot and mouth disease and vesicular stomatitis—epidemiological report,* 1988. Rio de Janeiro: Pan American Foot and mouth disease center.
3. Barreto CH, 1979. *Diagnostico particular de estomatitis vesicular en bovinos de la Republica de Costa Rica. Informe preliminar,* 2nd ed. San Salvador: Organismo International Regional de Sanidad Agropecuaria.
4. Francy DB, Moore CG, Smith GC, Jakob WL, Taylor SA, Calisher CH, 1988. Epizootic vesicular stomatitis in Colorado, 1982: isolation of virus from insects collected along the Northern Rocky Mountain Front Range. *J Med Entomol 25:* 343–347. UI:89055739
5. Perez E, Cornelissen B, 1988. Retrospective study of temporal distribution of vesicular stomatitis in cattle in Costa Rica, 1972–1986. *Prev Vet Med 6:* 1–8.
6. Smith S, 1986. *Annual report of vesicular diseases in Costa Rica.* APHIS, USDA.
7. Goodger WJ, Thurmond M, Nehay J, Mitchell J, Smith P, 1985. Economic impact of an epizootic of bovine vesicular stomatitis in California. *J Am Vet Med Assoc 186:* 370–373. UI:85130513
8. Leder RR, Maas J, Lane VM, Evermann JF, 1983. Epidemiologic investigation of vesicular stomatitis its economic impact. *Bovine Practitioner 18:* 45–49.
9. Nichol ST, 1988. Genetic diversity of enzootic isolates of vesicular stomatitis virus New Jersey. *J Virol 62:* 572–579. UI:88091069
10. Nichol ST, Rowe JE, Fitch WM, 1989. Glycoprotein evolution of vesicular stomatitis virus New Jersey. *Virology 168:* 281–291. UI: 89130940
11. Barrantes JA, Liao A, Rosales A, 1988. *Atlas climatologico de Costa Rica. Ministerio de Agricultura y Ganaderia.* San Jose, Costa Rica: Instituto Meteorologico Nacional.
12. Holdridge L, 1978. *Ecologia basada en zonas de vida.* San Jose, Costa Rica: Instituto Interamericano de Ciencias Agricolas (IICA).
13. Test results, 1984. Laboratorio de Diagnostico de Enfermedades Vesiculares, COPFA. Luis C. Roquebert, Director, Apartado 327, Panama 1, Panama.
14. Schwabe CW, Riemann HP, Franti CE, 1977. *Epidemiology in veterinary practice.* Philadelphia: Lea and Febiger, 14. UI:7703002
15. Jenney EW, Snyder ML, 1981. *Serologic microtitration techniques.* Ames, IA: USDA-NVSL, 38–39.
16. Vernon S, Webb PA, 1985. Recent vesicular stomatitis virus infection detected by immunoglobulin M antibody capture enzyme-linked immunosorbent assay. *J Clin Microbiol 22:* 582–586. UI:86086281
17. Kelley JM, Emerson SU, Wagner RR, 1972. The glycoprotein of vesicular stomatitis virus is the antigen that gives rise to and reacts with neutralizing antibodies. *J Virol 10:* 1231–1235. UI: 7301233
18. Geleta JN, Holbrook AA, 1961. Vesicular stomatitis—patterns of complement-fixing serum-neutralizing antibody in serum of convalescent cattle horses. *Am J Vet Res 22:* 713–719
19. Sudia WD, Fields BN, Calisher CH, 1967. The isolation of vesicular stomatitis virus (Indiana strain) and other viruses from mosquitoes in New Mexico, 1965. *J Epidemiol 86:* 598–602. UI:68130477
20. Tesh RB, BN Chaniotis, KM Johnson, 1971. Vesicular stomatitis virus, Indiana serotype: multiplication transmission by experimentally infected Phlebotomine sandflies (Lutzomyia trapidoi). *Am J Epidemiol 93:* 491–495. UI: 71262946
21. Tesh RB, Chaniotis BN, Peralta PH, Johnson KM, 1974. Ecology of viruses isolated from Panamanian plebotomine sandflies. *Am J Trop Med Hyg 23:* 258–269. UI:74126690
22. Webb PA, McLean RG, Smith GC, Ellenberger JH, Francy DB, Walton TE, Monath TP, 1987. Epizootic vesicular stomatitis in Colorado, 1982: some observations on the possible role of wildlife populations in an enzootic maintenance cycle. *J Wildl Dis 23:* 192–198. UI:87226513
23. Fultz PN, Shadduck JA, Kang CY, Streilein JW, 1982. Vesicular stomatitis virus can establish persistent infections in Syrian hamsters. *J Gen Virol 63:*493–497. UI:83110935
24. Hughes JV, Doll SC, Johnson TC, 1985. Hypothermia-inducing peptide promotes recovery of vesicular stomatitis virus from persistent animal infections. *J Virol 53:* 781–785. UI: 85135019
25. Rosenthal KL, Zinkernagel RM, Hengartner H, Groscurth P, Dennert G, Takayesu D, Prevec L, 1986. Persistence of vesicular stomatitis virus in cloned interleukin-2-dependent natural killer cell lines. *J Virol 60:* 539–547. UI:87036915
26. Orrego AU, Arbelaez G, Rocha J, 1987. *Avances en las investigaciones sobre la estomatitis vesicular en Colombia,* 1st ed. Bogota, Colombia: Instituto Colombiano Agropecuario.
27. Lauerman LH, Hanson RP,1963. Field trial of live virus vaccination procedure for prevention of vesicular stomatitis in dairy cattle. III. Evaluation of emergency vaccination in Georgia. *Proc US Livestock Sanitary Assoc 63:* 473–482.
28. Webster RB, Laver WB, Air GM, Schild GC, 1982. Molecular mechanisms of variation in influenza viruses. *Nature 296:* 115–112. UI:82148582
29. Mackett M, Yilma T, Rose JK, Moss G, 1985. Vaccinia virus recombinants: expression of VSV genes protective immunity of mice cattle. *Science 227:* 433–435. UI:85090448

30. Roitt IM, ed., 1980. *Essential immunology.* St. Louis: Blackwell Scientific Publications. UI: 8100533
31. Gadkari DA, Shaikh BH, 1984. IgM antibody capture ELISA in the diagnosis of Japanese encephalitis, West Nile and dengue virus infections. *Indian J Med Res 80:* 613–619. UI: 85181398
32. Innis BL, Nisalak S, Nimmannitya S, Kusalerdchariya S, Chongswasdi V, Suntayakorn S, Puttisri P, Hoke CH, 1989. An enzyme linked immunosorbent assay to characterize Dengue infections where dengue and Japanese encephalitis co-circulate. *Am J Trop Med Hyg 40:* 418–427. UI:89226115
33. Buimovici-Klein E, Tinker, MK, O'Beirne, AJ, Lange M, Cooper Z, 1983. IgM detection by ELISA in the diagnosis of cytomegalovirus infections in homosexual and heterosexual immunosuppressed patients. *Arch Virol 78:* 203–212. UI:84103664
34. Briantais MJ, Grangeot-Keros L, Pillot J, 1984. Specificity and sensitivity of the IgM capture immunoassay: studies of possible factors inducing false positive or false negative results. *J Virol Methods 9:* 15–26. UI:85055360