

## EQUINE HERDS AS SENTINELS FOR VENEZUELAN EQUINE ENCEPHALITIS VIRUS ACTIVITY, NICARAGUA 1977<sup>1,2</sup>

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*Collection of sera from 93 horses on Nicaraguan ranches and subsequent serologic testing for VEE virus antibody show that such horses can be used as cheap and effective sentinels for detecting VEE virus activity. The results also draw attention to the need for periodic monitoring of equine herds' VEE antibody levels in parts of Central America susceptible to the disease.*

### Introduction

The Central American equine epizootic and epidemic of Venezuelan equine encephalitis (VEE) that began in June 1969 near the Guatemala-El Salvador border has been well-documented (1-6). That outbreak spread southeast, reaching Guanacaste, Costa Rica, by 1970, and north, reaching southern Texas in 1971.

The last known cases of equine infection by the exotic equine-virulent subtype I-AB strain of VEE virus in Central America occurred in Nicaragua's Chinandega Department during April-July 1972 (7). Whether that activity was caused by virus remaining from the 1969 outbreak in Nicaragua or whether it resulted from reintroduced virus is unknown (7). Extensive searches for persistent activity by epizootic strains of VEE virus in Guatemala, El Salvador, and Nicaragua during 1970-1975—employing sentinel horses and sentinel hamsters, serologic surveys of humans and wild rodents, and attempts to isolate the virus

from mosquitoes—all yielded negative results (7,8).

Because of the importance of VEE virus to the health of humans and equine animals, it is important to continue monitoring VEE virus activity in tropical America. For that reason, the search for VEE virus activity in the Pacific lowlands of Nicaragua was continued in 1977 by testing sera from readily available herds of equine animals in natural habitats. These sera were tested for VEE virus antibodies engendered by epizootic strains (rather than enzootic or vaccine strains) of the virus. This article reports the results of that investigation.

### Materials and Methods

The target population consisted of young equine animals not previously inoculated with VEE virus vaccine. Ninety-three horses at two ranches in Chinandega Department and two others in Rivas Department were bled by jugular venipuncture into 50 ml disposable plastic tubes. (These two departments are on the Pacific Coast of Nicaragua and border Honduras and Costa Rica, respectively.) The blood was allowed to clot on "wet" ice (H<sub>2</sub>O). The clot was then freed from the sides of the tube so that it could settle and retract, and 5-6 ml of serum were subsequently decanted into two 15 x 48 mm screw-capped vials. These sera were stored on dry ice until their arrival in New York, where they were kept at -20°C.

Plaque-reduction neutralization tests were performed in chicken embryonic cell cultures

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as previously described, using cells in three wells of plastic plates per serum with combined bottom surfaces of 6 cm (8). The virus strains used were: the TC83 vaccine strain of VEE virus, the 69Z1 subtype I-AB epizootic VEE strain isolated from a human in Guatemala, and the 68U201 Central American enzootic VEE subtype I-E strain isolated from a sentinel hamster in Guatemala (8).

The sera, heated to 60°C for 20 minutes, were first screened at a 1:4 dilution in a virus-dilution neutralization test. Selected positive sera were then tested in serum-dilution neutralization tests that employed three-fold dilutions of serum, starting at 1:4, and about 180 plaque-forming units of TC83, 250 of 69Z1, or 800 of 68U201. A log<sub>10</sub> neutralization in-

dex greater than 1.6 (98 per cent plaque reduction or greater) was considered positive.

## Results

Neutralizing antibodies to the epizootic strain (69Z1) and the vaccine strain (TC83) were found in sera from 30 and 34 of the 93 horses tested, respectively (Table 1). Most of the positive sera were obtained from animals five to 10 years of age—that is, from animals that were alive in 1972 during the last known period of epizootic VEE virus activity in Nicaragua. The antibodies were found in sera from horses living in both the northern and southern parts of Nicaragua's Pacific lowlands.

**Table 1. Prevalences of plaque-reduction neutralization antibodies to epizootic and vaccine strains of VEE virus found in sera from 93 "young unvaccinated" horses bled in Nicaragua during August 1977.**

Location	Age in years	Approximate time of birth	Fractions of horses with detectable N antibody in serum to VEE HI subtype I	
			Epizootic strain 69Z1	Vaccine strain TC83
Chinandega Department, about 15 km southwest of the Honduran border	6-7	1970-71	2/2	2/2
	5	1972	1/2	1/2
	4	1973	1/4	2/4
	3	1974	1/7	1/7
	2	1975	0/1	0/1
	1	1976	0/7	2/7
Subtotal			5/23	8/23
Chinandega Department, about 20 km southwest of the Honduran border	6-10	1967-71	10/14	11/14
	5	1972	1/7	1/7
	4	1973	0/6	0/6
	3	1974	1/2	1/2
	2	1975	0/3	0/3
Subtotal			12/32	13/32
Rivas Department, about 25 km north of the Costa Rican border	6	1971	2/6	2/6
	5	1972	0/1	0/1
	4	1973	1/3	1/3
	2	1975	0/2	0/2
	1.5	1976	0/4	0/4
Subtotal			3/16	3/16
Rivas Department, about 30 km north of the Costa Rican border	6-7	1970-71	2/6	2/6
	5	1972	5/6	4/6
	4	1973	1/1	1/1
	3	1974	1/2	1/2
	2-2.5	1975	1/7	2/7
Subtotal			10/22	10/22
Total			30/93(32%)	34/93(37%)

Serum-dilution plaque-reduction neutralization tests were carried out on 20 sera from "young unvaccinated" horses that were positive to the epizootic and vaccine VEE strains (Table 2). Five of seven sera from horses born after the epizootic (1973-1974) had antibodies that were induced by vaccine virus; two horses yielded uninterpretable results. Three of the 13 horses born during the outbreak (1971-1972) had antibodies probably induced by epizootic virus, and five had antibodies probably induced by vaccine virus; the remaining five sera yielded uninterpretable antibody patterns. Thus, these sentinel horses yielded no unequivocal evidence of epizootic VEE virus infections between 1972 and August 1977.

## Discussion

This serologic survey of 93 horses bled at four ranches in Nicaragua's Chinandega and Rivas Departments in 1977 revealed no evidence of natural VEE virus activity since the end of the outbreak in 1972. These results were in accord with those of the last preceding survey of Nicaraguan equine animals in 1975 (7). The results also showed that over 65 per cent of the horses sampled lacked VEE virus-specific neutralization antibodies detectable at a 1:4 dilution of serum and thus were probably susceptible to infection.

This survey again demonstrated the feasibility of utilizing herds of equine animals on

**Table 2. Results of plaque-reduction serum-dilution neutralization tests using epizootic, enzootic, and vaccine strains of VEE virus to test sera from 20 "young unvaccinated" horses bled in Nicaragua during August 1977.**

Time of birth of horses	Reciprocal of serum dilution N antibody titer against VEE HI subtype I			Probable source of VEE viral antigenic stimulus of antibody production		
	Epizootic strain 69Z1	Vaccine strain TC83	Enzootic strain 68U201	Epizootic virus	Vaccine virus	Enzootic virus
<i>After epizootic:</i>						
1973	12	108	<4		+	
1973	12	108	12		+	
1974	12	972	<4		+	
1974	36	>2,196	<4		+	
1974	12	36	4		+	
1973	4	4	4	?	?	?
1974	12	12	4	?	?	
<i>During epizootic:</i>						
1971	324	108	36	+		
1971	108	36	36	+		
1971	324	108	108	+		
1971	108	324	36		+	
1972	4	12	<4		+	
1972	36	108	12		+	
1972	36	108	4		+	
1972	4	36	12		+	
1971	36	36	12	?	?	
1972	12	12	4	?	?	
1972	36	36	4	?	?	
1972	4	4	<4	?	?	
1970	12	36	36		?	?

ranches for inexpensive monitoring of VEE virus activity. Such animals are easily bled with a sterile needle that allows blood to flow directly into a sterile test tube. Centrifugation in the field is not necessary because the volume of blood is sufficient to allow for decanting of serum after the clot has retracted.

At present, in 1982, ten years have passed since the last equine fatalities from VEE virus occurred in Chinandega Department during 1972. Thus, an even larger proportion of the equine population than that found in this study may be susceptible to VEE virus infection unless vaccination programs have been unusually thorough. Accordingly, a periodic program for monitoring VEE antibody in

equine herds should be instituted in this and other Central American regions susceptible to VEE virus activity.

The presence of VEE neutralizing antibodies induced by vaccine virus in horses said by ranch personnel to be "unvaccinated" merely emphasizes the unreliability of such statements obtained in the field without further documentation. Ideally, therefore, VEE vaccination and antibody monitoring programs for equine animals should be done with marked equines—so that besides monitoring antibody levels in the general population, workers can follow the serologic histories of individual animals.

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Chinandega Department) and Hacienda Palermo and La Estancia (in Rivas Department) and their field hands were most generous with their time and effort in helping us to obtain equine blood samples.

### SUMMARY

Plaque-reduction neutralization tests were performed with 93 horse sera collected in the Pacific coastal lowlands of Nicaragua during August 1977. The results reveal that about 65 per cent of the animals were without neutralizing antibodies to epizootic, enzootic, or vaccine strains of Venezuelan equine encephalitis (VEE) virus detectable at a 1:4 serum dilution, and thus were probably susceptible to VEE virus infection. Five of seven horses born in 1973 or 1974 had neutralizing antibody patterns indicative of infection by the vaccine strain of VEE virus, while the two others had unin-

terpretable antibody patterns. Antibody prevalences were similar whether measured with the VEE vaccine strain or an epizootic VEE virus strain.

This survey demonstrated that naturally occurring herds of equine animals can be effectively used as sentinels for monitoring the activity of VEE virus and the effectiveness of VEE equine vaccination programs. It also indicated the need for a program providing periodic monitoring of VEE antibody in equine herds within portions of Central America susceptible to VEE virus activity.

### REFERENCES

- (1) Frank, P. T., and K. M. Johnson. An outbreak of Venezuelan equine encephalomyelitis in Central America: Evidence for exogenous source of a virulent subtype. *Am J Epidemiol* 94:487-495, 1971.
- (2) Sudia, W. D., R. D. Lord, V. F. Newhouse,

D. L. Miller, and R. E. Kissling. Vector-host studies of an epizootic of Venezuelan equine encephalomyelitis in Guatemala, 1969. *Am J Epidemiol* 93:137-143, 1971.

(3) Scherer, W. F., J. V. Ordoñez, P. B. Jahrling, B. A. Pancake, and R. W. Dickerman. Observations of equines, humans, and domestic and wild vertebrates during the 1969 equine epizootic and epidemic of Venezuelan encephalitis in Guatemala. *Am J Epidemiol* 95:255-266, 1972.

(4) Hinman, A. R., J. E. McGowan, Jr., and B. E. Henderson. Venezuelan equine encephalomyelitis: Surveys of human illness during an epizootic in Guatemala and El Salvador. *Am J Epidemiol* 93:130-136, 1971.

(5) Walton, T. E., F. E. Brautigam, J. A. Ferrer, and K. M. Johnson. Epizootic Venezuelan equine encephalomyelitis in Central America. Dis-

ease pattern and vaccine evaluation in Nicaragua, 1969-1970. *Am J Epidemiol* 95:247-254, 1972.

(6) Martin, D. H., G. A. Eddy, W. D. Sudia, W. C. Reeves, V. F. Newhouse, and K. M. Johnson. An epidemiologic study of Venezuelan equine encephalomyelitis in Costa Rica, 1970. *Am J Epidemiol* 95:565-578, 1972.

(7) Scherer, W. F., J. V. Ordoñez, R. W. Dickerman, and J. E. Navarro. Search for persistent epizootic Venezuelan encephalitis virus in Guatemala, El Salvador, and Nicaragua during 1970-1975. *Am J Epidemiol* 104:60-73, 1976.

(8) Scherer, W. F., K. Anderson, B. A. Pancake, R. W. Dickerman, and J. V. Ordoñez. Search for epizootic-like Venezuelan encephalitis virus at enzootic habitats in Guatemala during 1969-1971. *Am J Epidemiol* 103:576-588, 1976.

### RUBELLA IN THE UNITED STATES

A record low number of 2,077 rubella cases was reported in the United States for 1981. This represented a 47 per cent decline from the 1980 total of 3,904 cases (the previous record low) and an 82 per cent decline from the 1979 total of 11,795 cases. However, during the first 38 weeks of 1982 (ending 25 September), 2,018 cases were reported—a 13 per cent increase over the number of cases reported during the same period of 1981. This increase was due to a three-fold increase in the reported cases from California, where the respective totals were 445 cases during the first 38 weeks of 1981 and 1,319 cases during the same period of 1982. Reported cases of rubella from all other states declined by 52 per cent during the first 38 weeks of 1982 as compared with the first 38 weeks of 1981.

Since 1979, the annual provisional total of both laboratory-confirmed and unconfirmed congenital rubella syndrome cases has declined sharply—from 53 among children born in 1979 to 17 among those born in 1980, and to five among those born in 1981. This decline correlates with the decline in the reported incidence of rubella among women of childbearing age.

*Note:* The initial recommendation of the U.S. Public Health Service Immunization Practices Advisory Committee for rubella control was to vaccinate pre-school and elementary school children of both sexes; vaccination of older individuals received only secondary emphasis. This approach caused a dramatic decline in rubella incidence and eliminated the characteristic six to nine year cycle of epidemics.

Sources: World Health Organization, *WHO Epidemiological Record* 58(7):48-49, 1983; and US Centers for Disease Control, *Morbidity and Mortality* 31(42), 1982.