Laboratory Studies of Venezuelan Equine Encephalitis Virus in Equines, Texas, 1971

CHARLES H. CALISHER,¹* AND KATHRYN S. C. MANESS

Center for Disease Control, Atlanta, Georgia 30333

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During the summer and fall of 1971, epizootic and epidemic Venezuelan equine encephalitis was detected in Texas. Isolates of epizootic (IB) and vaccine (TC-83) strains were distinguished by virulence of the former for guinea pigs. Vaccine virus was isolated from 1 to 14 days after vaccination and neutralization tests demonstrated the appearance of antibody about a week after vaccination. Viremia titers of subtype IB in horses ranged from 2.2 to 8.3 log₁₀ suckling mouse intracranial 50% lethal doses per ml. Of 101 equines from which Venezuelan equine encephalitis virus (IB or TC-83) strains were isolated, 87 had no neutralizing antibody against Venezuelan, eastern or western equine encephalitis viruses.

The strain of epidemic Venezuelan equine encephalitis (VEE) virus, now known as type I, subtype B, was first isolated in Peru in 1942 (11). This same variety was again recognized in the winter and spring of 1969 when Ecuador suffered an epizootic of VEE (7); and in June through September 1969, the same virus subtype was active in Central America, notably Guatemala, El Salvador, and Costa Rica (13). Its means of transport from the infected areas of Ecuador into Central America remains unexplained. However, the fact that it spanned such a considerable distance in a very short time suggested that other areas still further north could also be at risk. The epizootic did indeed persist and extended south into Nicaragua and north into Mexico. Thousands of equines died on the Isthmus of Tehuantepec, Mexico, in 1970, and a strain of type IB was isolated from a vampire bat (*Desmodus rotundus*) collected in the state of Chiapas that same year (5). In April 1971, scientists from Mexico and the United States confirmed the presence of VEE in Tampico; and by late June it was active in the vicinity of Matamoros on the Mexico-Texas border (3). Sudia et al. have summarized the virus-vector-host relationships of this epizootic in an excellent series of publications (14-16).

This report presents laboratory data on equine specimens accumulated in the surveillance and control operations during the epizootic in the southcentral United States, primarily Texas.

MATERIALS AND METHODS

Sampling. At the time of the epizootic, prospective experimental protocols had not been developed. Specimens were obtained primarily by personnel concerned with monitoring the spatial and temporal spread of epizootic VEE virus in the United States. Consequently, serial sampling was virtually nonexistent. Sampling was performed when an illness compatible with that of VEE was detected in horses or to affirm virus and seronegativity at the time of vaccination. In addition, specimens were frequently obtained at intervals after vaccination to determine the efficacy of the vaccine in individual horses. Specimens collected for autopsy in the field and blood or sera collected for virus isolation or serology were shipped on dry ice (-60 C) to Atlanta. Approximately 1,585 of the specimens were sera; 99% of the animals from which specimens were taken were horses, whereas the remainder were donkeys or mules.

Virus isolation. All sera, whole blood specimens, and exudate from clots were inoculated into litters of six 2- to 4-day-old Ha/ICR mice (SM) by the intracranial (i.c.) route (0.02 ml/mouse). Other tissues were macerated by grinding with sterile alundum in a mortar, and 10% suspensions in 0.75% bovine albumin-phosphate-buffered saline, pH 7.2 to 7.4, were centrifuged at 1,600 × g for 30 min at 4 C. The supernatant fluids were then inoculated i.c. into SM as above. All animals were inoculated in a special security facility to assure virus containment. Mice were observed 7 to 14 days for signs of illness; those developing such signs were euthanized and brains were aspirated by a 19-gauge needle on a 1-ml disposable syringe. Samples of the brain harvest were suspended at 1:10 dilution in bovine albumin-phosphate-buffered saline for inoculation of guinea pigs and complement fixation (CF) tests; the remainder was stored at -60 C for later reference. Specimens

¹ Present address: Vector-Borne Diseases Division, Center for Disease Control, Fort Collins, Colo. 80522.

not tested the day of harvest were also stored at -60°C . Approximately 10% of the primary material and isolates required a second inoculation in mice because of cannibalism, nonspecified deaths, and/or toxic properties of the inocula. Airborne cross-contamination between cages of inoculated animals was minimized by use of commercially available filter covers (Iso-Cap Filter, Carworth Farms Co., New City, N. Y.) on the mouse cages and hand-fitted fiberglass filters (filter media no. FM-004, Standard Safety Equipment Co., Palatine, Ill.) on the guinea pig cages.

Virus identification. CF tests. Due to the suspected concurrent presence of Eastern equine encephalitis (EEE) and Western equine encephalitis (WEE) in the VEE epizootic area, a presumptive identification of isolates from field submissions was rapidly provided by a CF test using crude SM brain suspension. Infected SM brains of littermates were pooled, prepared as 10 or 20% suspensions in veronal buffer, and centrifuged at $7,500 \times g$ for 30 min, and the supernatant fluids were diluted 1:4 with veronal buffer. These clarified supernatants served as crude CF antigens and were tested without further dilution against twofold dilutions of inactivated (56 $^{\circ}\text{C}$, 30 min) hyperimmune mouse ascitic fluids prepared against VEE, EEE, and WEE. The CF test was performed by the Laboratory Branch complement-fixation technique adapted for use in microtiter plates (1) by using seven 50% units of complement to compensate for any anti-complementary properties of crude SM brain suspensions. The CF test was considered confirmatory for EEE and WEE without further testing.

Neutralization tests and guinea pig virulence tests. Isolates identified in CF tests as VEE virus strains were confirmed by neutralization (N) tests in 3-week-old mice, inoculated i.c., by using a constant serum-virus dilution procedure and hyperimmune mouse ascitic fluids prepared against VEE, EEE, and WEE viruses. Differentiation of the epidemic VEE subtype IB strains from TC-83 vaccine strains was accomplished by testing the virulence of isolates for 150- to 350-g (1- to 4-month-old) guinea pigs inoculated subcutaneously (2). Two to five guinea pigs were inoculated with 0.1 ml of each isolate previously identified as VEE by CF tests. The IB subtype killed the guinea pigs, whereas the vaccine strain did not.

VEE isolates representative of different geographic areas or time periods were selected for further testing by a short incubation hemagglutination-inhibition (HI) test, as modified by Young and Johnson, as the method of choice for accurate determination of VEE virus subtypes (17). The modified technique is as follows: an incubation period of 22 $^{\circ}\text{C}$ instead of 4 $^{\circ}\text{C}$, an incubation time of 2 h instead of 18 h, and specific antisera production in spiny rats (*Proechemys semispinosus*) rather than laboratory mice (sera kindly supplied to us by Middle America Research Unit).

Avidity for specific antibody is manifested by an increased number of antigen (hemagglutinin) units inhibited by a given serum. Hemagglutinins for

each strain were produced by inoculation of three 32-ounce (ca. 960 ml) prescription bottles of Vero cell cultures grown and maintained with Medium 199 containing antibiotics. Cultures were harvested when cytopathic effect was extensive (3+ to 4+) and most cells had detached from the glass; storage was at -60°C without added serum. For seed virus stocks, 3% inactivated fetal bovine serum was added to the unclarified supernatant fluid, and the suspensions were stored at -60°C .

Antibody studies. HI tests. Because a very large number of specimens was to be received for serological testing, the rapid, inexpensive HI technique was considered the test of choice. Standard HI tests were performed by a microtiter modification of the method of Clarke and Casals (4), in disposable "U" plates. Sera were adsorbed with kaolin and goose cells before testing. During the peak of the epidemic, however, confirming HI results by serum-dilution N tests doubled the workload. Therefore, later in the investigation, the HI tests were seldom done and reliance was placed on the N tests alone.

N tests. The presence of antibody to VEE, EEE, and WEE viruses was determined by serum dilution N tests in primary Pekin duck embryo cell cultures. Approximately 100 plaque-forming units of each of the three viruses were tested against 1:5 and 1:50 dilutions of inactivated test sera. Serum-virus mixtures were incubated for 1 h, and then 0.1 ml was dropped onto a monolayer of Pekin duck embryo cells. After an additional 1-h adsorption period, the cells were overlaid with a medium containing 1% Noble agar and 1:60,000 neutral red. Cultures were examined daily for up to 3 days, and the number of plaques was recorded. A serum was considered positive if the number of plaques did not exceed 10% of the number observed with known negative control sera.

Initially, N tests of sera were carried out using only a single dilution, 1:2, but later, to obtain a more quantitative result, two serum dilutions were used, 1:5 and 1:50. Each serum was thus eventually N tested at two dilutions against VEE, EEE, and WEE viruses; a total of six bottles of duck embryo cell monolayers were required for each serum tested.

RESULTS AND DISCUSSION

Isolation results. One or more tissues from each of the only 10 suspect horse cases tested yielded VEE virus. Onset of illness in these horses was 1 to 2 days before death. The results of virus isolation attempts with various autopsy tissues, other than serum or blood, from those 10 horses are shown in Table 1. Various unspecified portions of brain tissue had been obtained from nine of the ten horses; VEE virus was isolated from all nine samples. The next most common source of virus was spleen, with isolations from seven of eight such specimens tested. Gochenour (6) has shown that target organs of VEE virus strains often are those composed

TABLE 1. Isolations of VEE virus, epizootic subtype IB, from tissues of Texas equines 30 June to 31 August 1971

Horse no.	Tissue ^a					
	Brain	Spleen	Heart	Lung	Liver	Kidney
1	+	NT	NT	NT	NT	NT
2	+	+	0	0	0	0
3	NT	+	0	0	0	0
4	+	+	0	0	0	0
5	+	0	0	0	NT	NT
6	+	+	NT	0	0	NT
7	+	+	+	NT	+	+
8	+	+	NT	NT	0	NT
9	+	+	NT	+	NT	NT
10	+	NT	NT	NT	NT	NT
Fraction positive	9/9	7/8	1/5	1/6	1/6	1/4

^a +, VEE subtype IB was isolated from this tissue; 0, no virus isolated from this tissue; NT, not tested.

primarily of nervous or reticuloendothelial tissues, so that the brain and spleen results in Table 1 were not unexpected. The virus isolations obtained from heart, lung, liver, and kidney tissues shown in Table 1 might be due to contamination of these organ samples with infective serum or blood since, in all of these cases, the spleen was infected as well. Neither blood nor sera from these horses was received for testing.

Virus isolation attempts were performed on 1,654 blood or serum specimens (Table 2). VEE virus was obtained from 6 of 37 whole blood specimens and 11 of 32 clots, but from only 103 of 1,585 sera tested. The sampling technique and timing of the collections were probably responsible for the differences in success of isolation between whole blood, clots, and sera. That is, under the emergency conditions prevailing early in the outbreak, specimens were more frequently sent to Atlanta without complete field processing, and this was a time when equines were more likely to be found infected; the specimens tended to be from horses with highly suspect illness in areas most heavily affected by the epizootic. Somewhat later, specimen selection was improved in that specimens were obtained from a wider geographic range and included a wider range of clinical illnesses designed to minimize chances for missing atypical cases. Most sera were separated before shipping to the Center for Disease Control. Many of these later specimens were from horses that had already been vaccinated, which reduced the likelihood of active infection.

Epizootic versus vaccine strains. Since vaccination with TC-83 was being carried out on a wide scale, the vaccine virus could also be isolated on occasion. Therefore, TC-83 and subtype IB VEE virus isolates had to be distinguished. Workers in Panama had reported differences among certain VEE virus strains both by short-incubation HI tests and by plaque morphology and pH optimum for hemagglutination (17). We were generally able to confirm their short-incubation HI findings. However, plaque morphology and pH optima were not useful in our hands, and we were unable to distinguish the TC-83 vaccine strain from either the prototype VEE IA, from which it had been derived, or from the present epidemic subtype IB; this is as Young and Johnson have reported (17). All three produced small plaques in Vero cells and had low pH (6.0 to 6.2) optima. These characteristics are useful and distinctive in separating the so-called endemic strains of VEE (subtypes ID and IE and types II, III, and IV) from epidemic subtypes IA, IB, and IC, since the endemic strains produce much larger plaques in Vero cells and give maximum hemagglutinin titers over a wider pH range (6.2 to 6.6).

The virulence test for guinea pigs by the peripheral route, a method used by the developers of the TC-83 vaccine to distinguish it from its parent IA strain, was a practical solution. All virus isolates identified as VEE by CF tests were tested for guinea pig virulence. The results reported elsewhere (2) can be summarized by stating that all of the VEE virus isolates from unvaccinated equines killed guinea pigs within 18 to 72 h after inoculation, depending upon the age and weight of the guinea pig. All of the guinea pig virulent isolates of VEE virus from unvaccinated equines were from Texas or Mexico. Twenty-five of 114 VEE virus isolates from vaccinated equines also killed guinea pigs and all were from known epizootic areas of Texas.

Of 114 isolations of VEE virus from vaccinated equines, dates of vaccination and diagnostic sampling were available for 86 (Table 3). Vaccine virus (TC-83) was isolated from 63, and the epizootic IB strain was isolated from 23,

TABLE 2. Isolations of VEE virus from equine blood or serum—Texas, 30 June–31 August 1971

Fraction	No. with virus/no. tested	% Positive
Whole blood	6/37	16
Clot	11/32	34
Serum	103/1585	6

TABLE 3. Numbers of VEE virus isolations from equine sera by day after vaccination

Strain ^a	Day after vaccination												
	0	1	2	3	4	5	6	7	9	10	11	14	25-61
Vaccine	1	4	5	16	13	4	9	4	4	1	1	1	0
Epizootic	1	2	1	2	1	1	6	0	2	0	0	0	7

^a Determined by guinea pig inoculation (2).

based on virulence for guinea pigs. Sixty of the 63 isolations of TC-83 were obtained within the first 9 days after vaccination and more than half of these were obtained within the first 4 days. However, isolations were made as long as 14 days after vaccination.

Most (16 of 23) of the epizootic strain isolates from vaccinated equines were obtained from animals bled within 9 days of vaccination. In these cases, infection with epizootic virus presumably occurred before vaccine-induced immunity developed. However, seven isolates were made from equines bled at long intervals (25 to 61 days) after vaccination. A number of possible explanations for the latter isolates exist: (i) the field records may have been incorrect, (ii) the equines may not have been vaccinated, or (iii) vaccination may not have failed to prevent a subsequent infection or to suppress infection from a prevaccination exposure.

Clinical. The date of onset of typical signs of encephalitis was known for 17 equines from which epizootic VEE virus was isolated. Table 4 shows the levels of viremia by day after onset in these animals. On the first and second days after onset, titers ranged from 2.2 to 8.3 log₁₀ SM i.c. LD₅₀/ml. Since a titer of 5.0 log₁₀ SM i.c. LD₅₀/ml has been suggested (12) as the approximate threshold for infection of reasonably efficient mosquito vectors, viremia titers of 8.3 log₁₀ SM i.c. LD₅₀/ml would probably be sufficient to infect practically any *Aedes* or *Psorophora* mosquito which might take a blood meal from such a horse. Such high viremias, which are known to occur in many infected equines, undoubtedly account for the rapid spread of VEE virus during epizootics.

In contrast, TC-83 virus titers in 15 virus-positive sera, from equines bled 1 to 3 days after vaccination in states other than Texas, ranged from 3.2 to 5.5 log₁₀ SM i.c. LD₅₀/ml; generally, the viremia was in the lower range. These data suggest that a small proportion of vaccinated equines might serve to infect some individuals of the efficient vector species with the vaccine virus, but this would probably not be a usual circumstance. Findings in experimental studies with TC-83 virus support the field viremia results (10). Isolations of strains of VEE viruses

TABLE 4. VEE subtype IB viremias in horses by day after onset

Day after onset	No. of horses	Titer (SM i.c. LD ₅₀ /ml) ^a	
		Range	Geometric mean
0	7	2.2-8.2	5.6
1	5	5.2-8.0	6.5
2	4	2.2-8.3	6.3
3	1	0.5	0.5
Unspecified	23	2.2-6.8	4.2

^a Log₁₀.

with some of the characteristics of TC-83 from mosquitoes collected in Louisiana (12) and Mexico (T. H. Work, personal communication) during vaccination campaigns further indicate the possibility of mosquito infection and virus transmission in the field, but the isolates were few in comparison with the several millions of equines vaccinated.

Central nervous system disorders were noted in 16 of 19 equines from which epizootic VEE virus was isolated. Rectal temperatures in these equines ranged from 38.6 to 41.4 C (mean = 40.4 C). There was no apparent relationship between viremia titer and temperature or central nervous system disorders.

Serological findings. The coexistence in Texas of epizootic VEE virus, TC-83 vaccine virus, and EEE and WEE viruses (suspected in nature and known in the form of bivalent EEE-WEE vaccine) confounded interpretation of serological results on equine sera. Most of the specimens submitted were single sera which prevented confirmation by comparison of serial antibody determinations. In addition, the specificity of the HI test is complicated by cross-reactions within the North American group A arboviruses. Since N tests were necessary to confirm the HI test results, HI and N test results of 103 single sera were compared to determine whether the HI test could be eliminated and only N tests performed. HI and N test results for these sera are presented in Table 5. These comparative data demonstrated that the

TABLE 5. *HI and N antibody titers in 103 equine sera, Texas, 1971*

Specimen no.	Vacc. ^c status	Antigen (HI) ^a				Virus (N) ^b			VEE virus isolation ^d
		(GJ9-IBJ) VEE	(FE3-7C) VEE	(NJO) EEE	(Fleming) WEE	(TC-83) VEE	(NJO) EEE	(Fleming) WEE	
1									
2	>6	320	160	20	160	≥50		5	TC-83
3				10	40		≥50	≥50	
4					10		5	5	
5					40		≥50	≥50	
6		10		10	20		5	5	
7		40	20	40	80		≥50	≥50	
8									IB
9	<7				40			≥50	
10		80	40	20	20	5	5	5	
11				10	10		5	5	
12		10	10	80	80		≥50	≥50	
13	<7	40	40		10	5			
14		40	20	10	80	5		≥50	
15	<7	10			20	5			
16	>6	≥1,280	≥1,280	80	160	≥50	5	5	
17	>6	160	80	40	80	5		5	
18	>6	80	160	20	20	≥50	5	5	
19		10	10	10	10	5	5	5	
20	>6	320	160	40	40	≥50	5	5	
21		80	160	<10	<10	≥50	<5	<5	
22		320	640	10	10	≥50	<5	<5	
23		<10	<10	<10	<10	≥50	<5	<5	
24		320	320	20	40	≥50	<5	≥50	
25	<7	640	≥1,280	160	≥1,280	≥50	5	5	
26		≥1,280	≥1,280	160	640	≥50	5	5	
27	<7	160	320	20	40	≥50	≥50	<5	
28	<7	<10	<10	<10	<10	<5	<5	<5	
29	<7	40	40	<10	<10	5	<5	<5	TC-83
30		20	20	<10	<10	≥50	<5	<5	
31		20	20	<10	<10	5	<5	<5	
32	<7	<10	<10	<10	<10	<5	<5	<5	TC-83
33		<10	<10	<10	<10	<5	<5	<5	IB
34		<10	<10	<10	<10	<5	<5	<5	IB
35	<7	<10	<10	<10	<10	<5	<5	<5	TC-83
36	<7	40	20	<10	<10	≥50	<5	<5	
37		160	160	10	20	≥50	<5	<5	
38		80	80	40	160	≥50	5	5	
39	<7	10	10	<10	20	<5	<5	≥50	
40		160	160	<10	<10	≥50	<5	<5	
41		80	80	10	40	≥50	<5	≥50	
42	<7	160	160	<10	10	≥50	<5	<5	
43	>6	160	160	40	320	≥50	<5	5	
44	>6	≥1,280	≥1,280	160	≥1,280	≥50	<5	≥50	
45		≥1,280	≥1,280	160	640	≥50	5	5	
46		80	160	20	20	≥50	5	5	
47		<10	<10	<10	<10	<5	<5	<5	IB
48		<10	<10	<10	<10	<5	<5	<5	
49	<7	10	80	40	160	≥50	<5	5	
50	<7	<10	<10	<10	<10	<5	<5	<5	IB
51	<7	20	20	<10	<10	≥50	<5	<5	
52	>6	40	40	10	320	5	<5	≥50	
53		≥1,280	≥1,280	160	320	≥50	5	5	
54	<7	20	10	<10	10	5	<5	5	
55	<7	160	80	20	640	≥50	<5	≥50	
56		<10	<10	<10	20	<5	<5	≥50	
57		20	20	<10	20	5	<5	5	
58		<10	<10	<10	160	<5	<5	≥50	
59	<7	80	80	<10	20	≥50	<5	<5	
60	>6	80	160	10	40	≥50	<5	5	

TABLE 5—Continued

Specimen no.	Vacc. ^c status	Antigen (HI) ^a				Virus (N) ^b			VEE virus isolation ^d
		(GJ9-IBJ) VEE	(FE3-7C) VEE	(NJO) EEE	(Fleming) WEE	(TC-83) VEE	(NJO) EEE	(Fleming) WEE	
61		40	40	<10	20	50	<5	≥50	
62	<7	160	40	20	80	≥50	<5	5	
63		<10	<10	<10	20	<5	<5	≥50	IB
64		<10	<10	<10	<10	<5	<5	<5	IB
65		320	160	20	80	≥50	<5	5	
66		<10	<10	<10	<10	<5	<5	<5	IB
67		<10	<10	<10	<10	<5	<5	<5	
68	>6	80	80	20	40	≥50	5	5	
69	>6	10	10	80	80	<5	5	≥50	
70	>6	40	40	20	20	≥50	5	5	
71		10	10	<10	<10	≥50	<5	<5	IB
72		<10	<10	<10	<10	<5	5	5	
73		10	10	<10	<10	5	<5	<5	
74		320	320	20	10	≥50	<5	<5	
75		160	160	<10	<10	≥50	<5	<5	
76	<7	<10	<10	<10	<10	<5	<5	<5	TC-83
77	<7	10	10	10	40	5	5	≥50	
78		<10	<10	<10	<10	<5	5	<5	
79		<10	<10	<10	<10	<5	<5	<5	
80		<10	<10	<10	<10	<5	<5	<5	
81		<10	<10	<10	<10	<5	<5	<5	
82		<10	<10	<10	<10	<5	<5	<5	
83		<10	<10	<10	40	<5	<5	≥50	
84		<10	<10	<10	<10	<5	<5	<5	
85		<10	<10	<10	<10	<5	<5	<5	
86		80	40	10	40	≥50	<5	5	
87	<7	320	320	10	20	≥50	<5	5	
88		80	80	<10	40	≥50	<5	≥50	
89		80	40	10	160	≥50	<5	5	
90	<7	320	160	<10	10	5	<5	<5	
91		40	<10	<10	40	≥50	<5	5	
92		80	10	<10	<10	≥50	<5	<5	
93		≥1,280	320	160	≥1,280	≥50	≥50	≥50	
94		40	20	<10	<10	≥50	<5	<5	
95		20	10	10	320	<5	≥50	≥50	
96	>6	≥1,280	640	80	≥1,280	≥50	5	≥50	
97	>6	40	20	20	20	<5	≥50	≥50	
98		640	160	20	40	≥50	5	<5	
99		160	160	10	<10	≥50	<5	<5	
100	<7	<10	<10	10	10	≥50	<5	<5	IB
101		80	40	20	10	≥50	<5	<5	
102	<7	40	20	20	20	<5	<5	<5	
103		160	160	10	20	≥50	<5	<5	

^a A blank indicates <10.^b A blank indicates <5.^c A blank indicates no history of recent vaccination with TC-83 vaccine; <7 indicates vaccination with TC-83 less than 7 days previously; >6 indicates vaccination with TC-83 more than 6 days previously.^d Strain of VEE virus isolated: a blank indicates no virus isolated; TC-83 indicates vaccine virus isolated; IB indicates epizootic subtype IB isolated.

N test was specific and capable of providing more useful epizootological information than the HI test. High (≥1:50) and low (1:5) N titers were generally comparable to high (≥1:40) and low (<1:20) HI titers. In other tests we found that N antibody at 1:5 was roughly equivalent to a long neutralization index of 1.0 to 2.5, a titer of

1:50 to a long neutralization index of 2.0 to 4.0 and a titer of ≥1:500 to a long neutralization index of 3.0 to 6.0. Therefore, all further testing of equine sera was carried out by the N test.

Impact of vaccination. Reliable date records were available on sera from 708 equines vaccinated with TC-83 in Texas and neighboring

states (Table 6). Of these animals, 294 were bled 0 to 6 days after vaccination and the remainder were bled a week or more after vaccination. Of the 0- to 6-day bleeding group, 78.2% (230/294) had no detectable N antibody titer (<1:5) to VEE; on the other hand, 18.6% (77/414) of those bled 7 days or more after vaccination lacked detectable N antibody. When one excludes equines from Texas, some of which may have had field-acquired VEE antibody, the results are even more striking: 90.4% (94/104) of those bled <7 days after vaccination lacked N antibody to VEE virus as compared to 18.7% (38/203) of those bled 1 or more weeks after vaccination.

These findings indicate that some Texas horses were infected before vaccination and support the results shown in Table 3; active infection with epizootic VEE virus in some horses apparently occurred even when they were given vaccine as many as 9 days before detectable signs appeared. Thus, administration of TC-83 vaccine closely after or concurrently with exposure to field VEE virus may not interfere with the usual course of development of the field virus in the few horses which produce antibody unusually late or do not produce antibody after TC-83 vaccination. Clearly, how-

ever, vaccination was generally highly effective, as shown by 78 to 90% of the horses eventually exhibiting N antibody.

Whether or not prior existence of EEE and/or WEE antibody in horses protects them against infection with epizootic VEE is still moot. However, the results shown in Table 7 suggest the possibility of some such cross-protection. Thirty-seven of 47 (78.7%) of the epizootic VEE isolates and 50 of 54 (92.6%) of the TC-83 isolates were from equines with no detectable N antibody against VEE, EEE, or WEE viruses. Cross-protection, if it does exist, may have contributed to the slowing of the epizootic in south Texas. In one major ranch in Kleburg County, for example, where vaccination with bivalent EEE-WEE vaccine has been an annual occurrence, no equine deaths were reported. Although this was one of the first ranches to effect complete vaccination of horses with the TC-83 vaccine, epizootic VEE was identified north and west of that ranch within too short a time for full immunological response to have been attained.

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TABLE 6. Numbers of horses tested listed by N antibody titers to VEE virus and by day after vaccination, U.S., July-September 1971

Titer	Days after vaccination							
	0-6(%)		7-14(%)		15-21(%)		>21(%)	
	Texas	Other	Texas	Other	Texas	Other	Texas	Other
<1:5	136 (72)	94 (90)	19 (18)	28 (22)	6 (14)	5 (22)	14 (21)	5 (10)
1:5	13 (7)	9 (9)	20 (19)	23 (18)	10 (24)	4 (17)	9 (14)	8 (16)
≥1:50	41 (22)	1 (1)	64 (62)	78 (60)	26 (62)	14 (61)	43 (65)	38 (75)

TABLE 7. Numbers of horses from which VEE virus was isolated (U.S., 1971), listed by VEE strain isolated and VEE, EEE, and WEE N antibody titers

VEE Ab titer	EEE Ab titer	VEE virus strain isolated					
		Epizootic strain (IB) WEE Ab ^a titer:			VEE vaccine virus WEE Ab titer:		
		<5	5	>50	<5	5	>50
<5	<5	37	1	1	50	3	0
	5	0	1	0	0	1	0
	≥50	0	0	0	0	0	0
5	<5	3	0	0	0	0	0
	5	0	0	1	0	0	0
	≥50	0	0	0	0	0	0
≥50	<5	3	0	0	0	0	0
	5	0	0	0	0	0	0
	≥50	0	0	0	0	0	0

^a Ab, N antibody.

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