Possible Evidence for Interference with Venezuelan Equine Encephalitis Virus Vaccination of Equines by Pre-Existing Antibody to Eastern or Western Equine Encephalitis Virus, or Both

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During 1971, an epizootic of Venezuelan equine encephalitis (VEE) reached the United States. Laboratory tests were performed on a large number of sick, healthy, unvaccinated, and vaccinated horses. Neutralization (N) tests in cell cultures revealed that 153 of 193 (79.3%) equines outside the state of Texas and 175 of 204 (85.8%) within Texas (82.6% overall) had detectable N antibody to VEE virus a week or more after vaccination. Twenty-six of 40 (65%) non-Texas equines and 18 of 29 (62%) Texas equines which had no detectable antibody against VEE virus a week or more after vaccination had N antibody against Eastern equine encephalitis (EEE) or Western equine encephalitis (WEE) virus or both, whereas only 50 of 153 (32.7%) non-Texas equines and 82 of 175 (46.9%) Texas equines with demonstrable N antibody against VEE also had N antibody against EEE and/or WEE virus. In vaccinated equines, significant negative correlations were found between the occurrence of antibody to VEE and antibody to EEE and/or WEE virus. These findings support the hypothesis that pre-existing antibody to EEE and/or WEE virus may modify or interfere with infection by VEE virus. The epizooologic significance of this possibility is discussed briefly.

Venezuelan equine encephalitis (VEE) is an acute febrile disease which may affect the central nervous system of man, equines, and other mammalian hosts (5). The etiologic agent, VEE virus, is a group A arbovirus which, by hemagglutination inhibition (HI), complement fixation, neutralization (N), and other tests, is serologically related to Eastern equine encephalitis (EEE) and Western equine encephalitis (WEE) virus (2). Recently, Young and Johnson (7) distinguished serologically between four major types of VEE virus and between those subtypes responsible for epizootics and those which have been found only enzootically. Subtype IB, which in 1969 caused an epizootic in Ecuador (4) and then alternately moved or “jumped” northward, reached northern Mexico in the late spring of 1971 (3). Because extension of the disease into Texas was considered likely, a cooperative program of surveillance was established by the United States Department of Agriculture, Texas Animal Health Commission, United States Public Health Service, and other governmental agencies. In late June of 1971, the presence of the virus in Texas was established by virus isolation from a horse in Three Rivers, Live Oak County. Where this horse acquired its infection is not known. The epizootic spread toward the west and the north from the Brownsville area, and the infection was eventually detected in 30 counties bordering Mexico and the Gulf Coast. Over 1,500 equines died of VEE in Texas, and 110 humans contracted the infection. Because of the importance of this disease, an extensive program of mosquito control, equine vaccination, quarantine (premise, county, and state), and disease and serological surveillance was undertaken (6; R. B. Zehmer, P. B. Dean, W. D. Sudia, C. H. Calisher, G. E. Sather, and R. L. Parker, to be published).

The serological surveillance consisted of sam-
pling suspect, prevaccinated, vaccinated, and asymptomatic equines in the epizootic zone and other areas of Texas, in the states which border Texas, and eventually in most other states. Since nearly 3,000 equines were sampled, the results of serological testing of a large proportion of these could reflect the level of immunity to VEE virus in vaccinated and unvaccinated equines and also permit estimation of antibody levels to EEE and WEE viruses.

The results of the serological tests are presented in this report. These results suggest that pre-existing antibody to EEE or WEE virus or both, interferes with subsequent VEE virus infection.

MATERIALS AND METHODS

At the outset of the studies, HI tests were used to detect antibody. However, it rapidly became clear that for a number of reasons this test alone would not provide interpretable results. First, EEE and/or WEE virus probably was, or had been, active in most of the areas under surveillance. Thus, naturally acquired infections evoked detectable levels of HI antibody to these viruses in many equines; and because of the broadness of the immune response to group A arboviruses as measured by HI tests, confusing cross-reactivity with VEE virus occurred. Furthermore, many equines not naturally infected had been vaccinated with commercially available EEE-WEE bivalent or WEE univalent vaccine. Second, most of the serological sampling was done during an ongoing VEE vaccination campaign, which naturally led to complications in interpreting laboratory results. Third, epizoodemic VEE virus (subtype IB) was actively infecting horses in the areas of south Texas during the time of the most extensive surveillance efforts. Fourth, for the vast majority of equines, only single serum would be available. Fifth, neutralization is considered the most reliable and specific test. Therefore, it appeared to be more economically and scientifically sound to test all equine sera by N tests only and thus dispense with the HI tests altogether.

For the N tests, each serum was inactivated at 56°C for 30 min and diluted 1:5 and 1:50; 0.1-ml samples of each dilution were incubated for 1 h at 37°C with an equal volume of diluent containing approximately 100 plaque-forming units of VEE virus (TC-83 vaccine strain), EEE virus (New Jersey strain), or WEE virus (Fleming strain). The serum-virus mixtures were then inoculated onto monolayer cultures of primary Pekin duck embryo cells, allowed to adsorb for 1 h at 37°C, and overlaid with a medium composed of Earles balanced salt solution (10%), yeast extract (0.033%), lactalbumin hydrolysate (0.165%), inactivated newborn calf serum (2%), NaHCO₃ (0.224%), antibiotics (penicillin, 200 U/ml; streptomycin, 100 μg/ml; mycostatin, 100 μg/ml), neutral red (1:80,000), Noble agar (1%), and distilled water (80%). Cultures were observed daily for 2 to 3 days for the appearance of plaques. Serum dilutions were recorded as positive for antibody if 90% or greater plaque reduction was observed.

All results presented in this paper were from tests performed on specimens collected by the veterinary staffs of the U.S. Department of Agriculture and the U.S. Public Health Service (Center for Disease Control). Despite various conditions during the epizootic, the field workers made every effort to collect precise and pertinent information on each specimen. The filed data probably contain some errors, but these errors probably are of minor importance. For the purposes of this paper, field-collected data obtained during the peak epizootic period in the United States were included.

In these studies we observed that equines rarely produce detectable antibody before the seventh day after vaccination with TC-83 strain (R. B. Zehmer et al., to be published). Consequently, we included only those results which were determined for sera collected 7 or more days after vaccination with TC-83. No attempt was made to certify the history of vaccination with EEE-WEE vaccines, since we felt that such determinations would be unreliable.

RESULTS AND DISCUSSION

Table 1 presents the N test results with sera from equines in the United States which had not received the attenuated (TC-83) VEE virus vaccine. Only 3 out of 373 (0.8%) of the unvaccinated equines from outside the state of Texas had detectable antibody to VEE virus. This is not unexpected, since clinical evidence and isolation of VEE virus in the United States outside of Texas were also lacking. The three sera with N antibody to VEE had low titers (1:5) but high (≥1:50) N antibody titer to either EEE or WEE virus. The VEE antibody can thus be explained on the basis of cross-reaction. There was a significant positive correlation (P < 0.001) between the presence of N antibody to EEE and WEE viruses. This was expected since (i) the two viruses are closely related serologically, (ii) many of the equines may have been naturally infected with either or both viruses, and (iii) many had been vaccinated with commercially available bivalent EEE-WEE or monovalent WEE virus vaccines.

In contrast, 103 (49.5%) of 208 nonvaccinated Texas equines had detectable N antibody against VEE virus. Eighteen (8.6%) had a titer of 1:5 and 85 (40.9%) had a titer of ≥1:50. These results reflect the activity of epizootic VEE virus in Texas. In addition, of the 103 with VEE N antibody, 41 (39.8%) had antibody to EEE and/or WEE virus, whereas 48 (45.7%) of 105 without VEE N antibody had EEE and/or WEE antibody.

Serological results for vaccinated equines are shown in Table 2. Despite a history of vaccina-
### Table 1. Numbers of unvaccinated equines, 1971, classified by reciprocal VEE, EEE, and WEE neutralizing antibody titers

| Cross-reaction | WEE (non-Texas) | Cross-reaction | WEE (Texas) |
|----------------|-----------------|----------------|-------------|
| VEE            | EEE             | <5            | 5           | ≥5          | VEE            | EEE             | <5          | 5           | ≥5          |
| <5             | <5              | 112 (30.2)    | 20 (5.4)    | 98 (26.5)   | <5             | 56 (27.7)       | 22 (10.8)   | 13 (6.3)    |
| 5              | 5               | 20 (5.4)      | 6 (1.6)     | 16 (5.7)    | 5              | 2 (1.0)        | 3 (1.5)     | 3 (1.5)     |
| ≥5             | 66 (17.8)       | 11 (3.0)      | 21 (4.3)    |             | ≥5             | 1 (0.5)       | 1 (0.5)     | 3 (1.5)     |
| 5              | <5              | 0             | 0           | 1 (0.3)     | 5              | 12 (5.8)       | 5 (2.5)     | 1 (0.5)     |
| ≥5             | 2 (0.6)         | 0             | 0           | ≥5          | 0              | 0             | 0           |             |
| ≥5             | <5              | 0             | 0           | 0           | ≥5             | 50 (24.2)      | 8 (3.9)     | 8 (3.9)     |
| 5              | 0               | 0             | 0           |              | 5              | 4 (2.0)       | 10 (4.8)    | 2 (1.0)     |
| ≥5             | 0               | 0             | 0           | ≥5          | 1 (0.5)        | 0             | 2 (1.0)     |             |

* Percent of total is given in parentheses.
* Numbers (<5, 5, and ≥5) represent reciprocal neutralizing antibody titers.

### Table 2. Numbers of vaccinated equines classified by reciprocal VEE, EEE, and WEE neutralizing antibody titers

| Cross-reaction | WEE (non-Texas) | Cross-reaction | WEE (Texas) |
|----------------|-----------------|----------------|-------------|
| VEE            | EEE             | <5            | 5           | ≥5          | VEE            | EEE             | <5          | 5           | ≥5          |
| <5             | <5              | 14 (72)       | 1 (0.5)     | 5 (2.6)     | <5             | 11 (5.4)        | 8 (3.9)     | 1 (0.5)     |
| 5              | 2 (1.0)         | 0             | 2 (1.0)     |             | 5              | 5              | 2 (1.0)     | 5 (2.5)     |
| ≥5             | 7 (3.6)         | 2 (1.0)       | 7 (3.6)     | ≥5          | 0              | 0              | 1 (0.5)     |             |
| 5              | <5              | 18 (9.3)      | 0           | 2 (1.0)     | 5              | 14 (6.8)       | 2 (1.0)     | 2 (1.0)     |
| ≥5             | 1 (0.5)         | 0             | 2 (1.0)     | ≥5          | 0              | 2 (1.0)       | 5 (2.5)     |             |
| ≥5             | <5              | 85 (44.0)     | 10 (5.2)    | 5 (2.6)     | ≥5             | 79 (38.7)      | 13 (6.4)    | 9 (4.4)     |
| 5              | 7 (3.6)         | 2 (1.0)       | 5 (2.6)     |              | 5              | 6 (2.9)       | 17 (8.3)    | 8 (3.9)     |
| ≥5             | 2 (1.0)         | 0             | 4 (2.0)     | ≥5          | 1 (0.5)        | 1 (0.5)       | 3 (1.5)     |             |

* Percent of total is given in parentheses.
* Numbers (<5, 5, and ≥5) represent reciprocal neutralizing antibody titers.

Interference with VEE virus infection, no detectable N antibody to VEE virus was found in 40 (20.7%) of 193 equines outside Texas and in 29 (14.2%) of 204 within Texas. The prevalence of EEE and WEE N antibody in equines lacking a detectable immunological response to VEE vaccination was higher than in those with detectable VEE antibody. Of 69 vaccinated equines without detectable VEE antibody, 44 (63.8%) had antibody to EEE and/or WEE virus. In contrast, of 328 horses showing VEE antibody responses, only 132 (40.2%) had EEE and/or WEE antibody.

Statistically, there is a moderate but significant negative correlation (P < 0.01) between the occurrence of VEE antibody and/or WEE antibody in VEE-vaccinated equines and a highly significant negative correlation (P < 0.001) between the occurrence of VEE antibody and either EEE or WEE antibody or with both EEE and WEE antibody. Interference between pre-existing antibody to EEE and/or WEE virus and VEE vaccination is therefore indicated. Casals and Francy (personal communications) have made similar observations by HI tests with VEE-vaccinated horses. Further, Byrne et al. (1) showed that EEE-immune burros were partially resistant to VEE virus challenge. If pre-existing antibody to EEE or WEE virus, or both, can prevent or alter the course of infection with VEE virus, this effect could conceivably reduce the efficacy of VEE vaccine or might have slowed the spread of VEE in the United States in 1971, irrespective of vaccination.

Further studies are now in progress to determine viremia and N antibody levels and resistance to VEE vaccination and epizootic subtype
IB in equines with pre-existing or no antibody to EEE or WEE virus, or both. Preliminary results indicate that, under experimental conditions, such an interference occurs.

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