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Field Studies of an Attenuated Venezuelan Equine Encephalomyelitis Vaccine (Strain TC-83)

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A series of field studies using strain TC-83 attenuated Venezuelan equine encephalomyelitis vaccine in horses was made to determine the rate of seroconversions, the postvaccination viremia, and the possibility of adverse reactions to the vaccine. The rate of seroconversions varied from 50% in one study to 91 and 100% in two others. The highest level of viremia measured was 7×10^3 to 8×10^3 plaque-forming units per ml. No adverse reactions to the vaccine were observed in any horses, including 42 pregnant mares and their resulting foals.

During 1969 and 1970, an epizootic of Venezuelan equine encephalomyelitis (VEE) occurred in Central America (3, 6; T. E. Walton et al., Amer. J. Epidemiol, *in press*) and subsequently spread through Mexico and into the United States (5). Control of the epizootic was attempted primarily through vaccination with the strain TC-83 attenuated VEE vaccine developed by the U.S. Army Medical Research and Development Command (USAMRDC) (1). This report describes field studies of the vaccine in equines to assay immune responses, resulting viremia levels, and the occurrence of adverse reactions to the vaccine.

MATERIALS AND METHODS

Vaccine. The strain TC-83 vaccine was supplied by USAMRDC in 100-dose vials of lyophilized virus. The diluent consisted of 10% human serum albumin in Hanks balanced salt solution in 50-ml vials. The dose of vaccine used was 0.5 ml of the reconstituted product given subcutaneously or intramuscularly within 4 hr of reconstitution. The vaccine used in study 1 was from lot 5B and that in studies 2 and 3 was from lot 5.

Antibody titration. Serum was assayed for virus neutralizing antibody by measuring plaque reduction by the method of Earley et al. (2). The titrations were made in serial fourfold dilutions from 1:8 through 1:8,192; titers were recorded as the reciprocal of the highest dilution giving an 80% or greater reduction in plaque counts. In some cases, the sera were tested only at a 1:8 dilution for a reduction in plaque count of 80% or more. One or two virus strains were included in each test at a level of 50 to 150 plaque-forming units (PFU) per well.

Virus strains. In addition to the TC-83 strain of VEE, an epizootic isolate from the Central American outbreak, designated MF-8 (antigenic subtype I-B; references 3, 7) was used in the plaque reduction test to measure virus neutralizing antibody.

Virus assay of serum. Horse blood which was to be assayed for virus was collected in sterile Vacutainer tubes. The serum was separated by centrifugation within 3 to 5 hr, sealed in 1-dram vials, and promptly frozen at −70 C or lower. The quantity of virus was assayed later by plaque counts in 35-mm dishes of Vero cell monolayer cultures (a continuous line of African green monkey kidney cells). Sera were routinely tested at 10^{-1} and 10^{-2} dilutions; the quantity of virus then was calculated in terms of PFU/ml. Sera with virus titers too high to be measured at 10^{-2} were retitrated by using a portion from a previously unthawed vial of serum and extending the dilutions to 10^{-4}. Comparisons of serum virus assays of strain TC-83 virus in suckling mice with the above plaque method showed no significant differences in sensitivity.

Virus isolations. Sera from horses with a measurable viremia and selected sera from horses which showed encephalitic signs were inoculated into Vero cell monolayers or suckling mice for virus isolation and subsequent identification. From the known viremic sera, a previously unthawed portion was used to inoculate 0.2 ml of serum into each of four tubes of Vero cell culture. The resulting culture fluid was used as the virus pool. Selected sera from encephalitic horses were inoculated intracerebrally into suckling mice by using 0.02 ml of serum inoculum for each of eight mice. From the dead or moribund mice, a 10% brain suspension was prepared in phosphate-buffered saline solution (*p*H 7.3) with 0.5% gelatin.

Classification of viral isolates. Two methods were used to determine whether viruses isolated from the sera of vaccinated horses were vaccine virus or the epizootic agent. The serum isolates made in Vero cell culture or suckling mouse brain were each inoculated subcutaneously into five weanling mice by using 0.2 ml of a 10^{-1} dilution of the virus pool. Our unpublished

studies clearly indicated that vaccine virus has little apparent effect on these animals by this route, whereas subcutaneous inoculation of 0.2 ml of a 10^{-1} dilution of Vero cell cultivated epizootic virus or infected mouse brain suspensions of the epizootic virus invariably killed all mice inoculated in this manner. The mouse-virulent isolates and selected nonvirulent isolates then were typed by the kinetic hemagglutination-inhibition (KHI) test as described by Young and Johnson (7).

Study 1. A group of 36 horses on a large ranch in Costa Rica was used to investigate the extent of viremia resulting from vaccination. They were vaccinated during the 1970 epizootic. The animals were bled before vaccination and again at 39, 63, 87, and 111 hr thereafter for serum virus assays. Additional sera were collected at 40 days postvaccination to determine the antibody responses.

Study 2. From a group of approximately 150 horses at a thoroughbred breeding farm in western Panama which were bled and vaccinated at day 0, we selected 100 animals which were free of pre-existing neutralizing antibody and were readily identifiable when bled on day 30 postvaccination. The resulting sera were used to determine the percentage of animals that converted serologically after vaccination. All of the horses, including 42 pregnant mares, were observed daily by the farm manager, Angel Rodriguez-Martin, an experienced veterinarian and equine obstetrician, for adverse reactions to the vaccine.

Study 3. From a riding club in the Panama Canal Zone, 20 horses seronegative for VEE virus antibody were selected for study of virus levels, leukocyte responses, body temperature fluctuations, and antibody responses after vaccination. The horses were bled immediately prior to vaccination and at daily intervals thereafter for 8 days. Complete blood counts were made, and body temperatures were taken daily. Sera were collected for antibody assay at 50 days postvaccination.

RESULTS

Postvaccination seroconversions of the horses in studies 1, 2, and 3 are shown in Table 1. Ten of the original 36 horses in study 1 are not included. Five of these had VEE-neutralizing antibody at the time of vaccination, and another five were excluded for reasons described below. The data in Table 1 show that 100, 91, and 50% of the horses in the three respective groups converted to a seropositive status against the epizootic strain after vaccination with strain TC-83. They also show that the antibody titer was higher against the vaccine virus than against the epizootic strain. The variation in responses in groups of horses was quite marked with respect to study 3, where only 50% of the animals developed detectable antibody titers against the epizootic virus. This variation was consistent with that shown by Walton et al. (Amer. J. Epidemiol., *in press*) in Nicaraguan horses which received this vaccine.

The leukocyte counts and body temperatures of the 20 horses in study 3 showed little variation. During the 8 days of the study, mean leukocyte counts varied from 8,250 to 8,800 per mm^3 and mean daily body temperatures varied from 99.3 to 100.1 F. These small fluctuations did not follow a pattern that could be interpreted as a response to vaccination.

Viremic responses after vaccination were measured in two groups of horses, studies 1 and 3. The horses in the latter group showed no measurable virus in any of the daily sera taken during the 8 days after administration of vaccine. This is o interest in connection with the low level and low incidence of seroconversion and the general absence of fever and leukopenia in this group of animals.

Sera showing peak viremia levels from 14 horses in study 1 were inoculated into Vero cell cultures for virus isolation as described above. All produced a distinct cytopathic effect typical for VEE virus, and the cell culture fluids were harvested for virus pools. Upon inoculation into adult mice, four cell culture isolates and one suckling mouse brain isolate from five different horses produced death in all of the mice. The four mouse-virulent cell culture isolates and three nonlethal isolates

TABLE 1. *Neutralizing antibody responses of horses vaccinated with attenuated TC-83 VEE vaccine, Costa Rica and Panama, 1970*

Study	TC-83 (vaccine virus)		MF-8 (epizootic virus)	
	Seroconversions/total	Geometric mean titer[a]	Seroconversions/total	Geometric mean titer[a]
1	26/26 (100%)	2,048	26/26 (100%)	196
2	Not done	Not done	91/100 (91%)	Not done
3	12/20 (60%)	161[b]	10/20 (50%)	84[b]
Total	38/46 (83%)	916	127/146 (87%)	155

[a] Geometric mean of reciprocal of highest titers giving 80% plaque reduction.
[b] Calculated only for those animals showing an antibody titer of 1:8 or higher.

TABLE 2. *Viremia[a] of horses during a VEE epizootic, Costa Rica, 1970*

Horse	PFU of virus/ml of blood at daily intervals postvaccination					
	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5
1	0[b]	Not done	0 Encephalitic	0 Died with severe encephalitis		
2	1.6×10^5	Not done	0 Encephalitic	0 Died with severe encephalitis		
3	0	Not done	1.2×10^3	6×10^4	8×10^2	0 No clinical illness
4	0	Not done	3×10^2	0	0	0 No clinical illness
5	0	Not done	0	8×10^1	0	0 Encephalitic and survived

[a] Isolates found to be epizootic virus (see text).
[b] No PFU were detected, but virus was isolated by suckling mouse inoculation.

TABLE 3. *Viremia in horses after vaccination with strain TC-83 vaccine, Costa Rica, September 1970*

Time (hr) postvaccination	No. with viremia/total	Serum virus titers (PFU/ml)
39	3/26	7×10^3–8×10^3
		4×10^2
		5×10^2–6×10^{2a}
63	2/26	2×10^3–3×10^3
		2×10^{2a}
87	4/26	1×10^2
		1×10^2
		1×10^2–2×10^2
		1×10^{2a}
111	3/26	5×10^2
		3×10^2
		4×10^3

[a] Denotes viremia in the same horse on three different bleedings.

were typed by the KHI test. The mouse-virulent isolates developed a pattern similar to the epizootic I-B subtype. The avirulent isolates were distinct from the epizootic strain and similar to the strain TC-83 virus. The viremia patterns of horses that had mouse-virulent virus are shown in Table 2. With one exception, the viremias attributable to epizootic virus commenced before the third day postvaccination.

The 26 horses retained in study 1 exhibited varying levels of viremia as shown in Table 3. Measurable virus was detected in 10 horses. One of these horses was viremic for 3 consecutive days, whereas virus was detected in the sera of the nine others only once. The maximum titer was 7×10^3 to 8×10^3 PFU/ml at 39 hr postvaccination, and the second highest titer of 4×10^3 PFU/ml was found in a 111-hr serum. The virus content in the sera of the other horses ranged down to undetectable levels.

All of approximately 900 horses at the ranch in study 1 were vaccinated during a 2-day period in September 1970 in the midst of an epizootic. Approximately 30 deaths with encephalitis had occurred at the time of vaccination, and an additional 12 deaths occurred during the following 8 to 9 days. No horse deaths attributable to encephalitis occurred subsequent to 9 days postvaccination despite the persistence of the rainy season through October 1970.

None of the horses at the farm described in study 2 presented evidence of adverse reactions attributable to the vaccine. Most of the horses were valuable thoroughbred stock and were observed daily by Rodriguez-Martin. He found that approximately 10% of the horses exhibited a mild lassitude within 2 to 3 days postvaccination; fevers, when present, were in the range of 102 to 103 F. The farm also maintains thorough breeding records, and the mares are examined periodically for pregnancy. At the time of vaccination, there were 42 pregnant mares in various stages of gestation as follows: 3 at 1 to 2 months, 13 at 3 to 4 months, 23 at 5 to 6 months, and 3 at 7 months or more. In the period immediately after the vaccination, no abortions occured. However, one mare in the fifth month of pregnancy at the time of vaccination aborted two months later, and a second mare aborted an 8-month fetus 6 months after vaccination. None of the other 40 mares aborted, nor were the resulting foals abnormal in

any way. The farm has experienced a 3 to 4% abortion rate per year. Twelve of the 42 mares, including the two that aborted, were subsequently diagnosed as positive for equine infectious anemia by Leroy Coggins, Cornell University, on the basis of an immunodiffusion test for antibodies associated with that disease. Rodriguez-Martin has observed that abortions are not uncommon in mares afflicted with infectious anemia.

DISCUSSION

Because of the possibility that the vaccine virus may revert to virulence through successive horse-mosquito-horse passages, the magnitude of the viremia resulting from vaccination is of great interest. Our data show that low levels of virus circulating in the blood of horses may be expected after vaccination. The question of whether an occasional horse may show higher titers, sufficient to infect mosquitoes, can be resolved only by further study.

The antibody response to vaccination was clearly substantial in two of the groups of horses, but the development of relatively low levels of VEE subtype I-B neutralizing antibody in only half of the horses in a third group and similar results in Nicaraguan horses (Walton et al., Amer. J. Epidemiol., *in press*) indicates that some variation within a lot of vaccine may be expected. The variation may reflect poor handling or faulty reconstitution of the vaccine, but care was taken to maintain the vaccine in accordance with the recommendations of the USAMRDC. The possibility that an individual bottle of vaccine was defective or improperly handled is supported by the failure of horses in study 3 to produce measurable viremia and by the absence of leukocyte and temperature responses. Walton et al. (Infect. Immunity, *in press*) found distinct leukopenia and body temperature elevations in experimentally vaccinated horses. We therefore submit that this variation must be considered in planning extensive programs of vaccination.

It should be emphasized, however, that the abrupt cessation of horse deaths 8 to 9 days after vaccination of the 900 horses at the farm in study 1 was an indication of a high degree of vaccine efficacy. This leads us to infer that the strain TC-83 vaccine prevents VEE virus-induced disease within 3 days postvaccination. Our contention is based in part on experimental evidence gathered by Thomas Walton in our laboratory and by Henderson et al. (4) that no horses died of VEE prior to 5 days postinfection, and on the failure to observe the initiation of epizootic virus-induced viremias after 3 days postvaccination (Table 2). Therefore, the cessation of horse deaths 8 to 9 days postvaccination in this and other studies (D. H. Martin et al., Amer. J. Epidemiol., *in press*) provided evidence for early protection by TC-83 vaccine.

Although adverse reactions, including abortions, have been reported to MARU staff members during the Central American vaccination programs, we saw no evidence of this. Fevers, when observed, did not exceed 102 to 103 F. The 42 pregnant mares showed no indication of vaccine-induced abortion, and the foals were normal. We have observed numerous large-scale horse vaccination programs in Central America where the animals have experienced rough handling. It is our opinion that the reported abortions, if true, are more likely to have been caused by the trauma received than by the vaccine.

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