

TRANSPLACENTAL TRANSMISSION OF VENEZUELAN EQUINE ENCEPHALITIS VIRUS IN HORSES*

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Abstract. Transplacental passage of Venezuelan equine encephalitis virus, epizootic strain P-676, occurred in four of nine fetuses studied. The mares were infected near term. Virus was recovered in high titer from fetal blood and organs, while no virus was detected in maternal blood but neutralizing antibodies were present. No evidence of in utero infection was found in two fetuses from mares infected with MF-8, another epizootic strain of Venezuelan equine encephalitis virus.

Transplacental infection due to togaviruses has been described in various animals. Transplacental transmission of Japanese B encephalitis virus was demonstrated in swine which were infected during different stages of gestation.¹ Placental passage and infection of mouse fetuses occurred when pregnant mice were inoculated with St. Louis encephalitis virus.² Venezuelan equine encephalitis (VEE) virus, vaccine strain (TC-83), when inoculated into pregnant mice passed through the placenta and caused fetal infection and reduction in litter size.³

Venezuelan equine encephalitis virus comprises two major categories. One is composed of strains known as enzootic in which the virus cycles in rodents from the tropical forest. These strains have been shown not to be lethal for horses. The other category comprises those strains known as epizootic which produce high equine mortality. No epizootic strain has been shown to cycle enzootically in rodents. To date, no mechanism has been found for the maintenance of the equine-virulent strains of VEE virus during interepizootic periods; it has been suggested, however, that epizootic VEE virus may remain dormant or latent for prolonged periods in mammalian hosts, with activation later.⁴

Preliminary observation, made at the Middle America Research Unit, suggested fetal infection had occurred following inoculation of a mare with

MF-8 strain during the late stage of gestation. Because the observation was casual, a more detailed study was undertaken to evaluate the possibility of fetal infection in horses by epizootic VEE virus represented by the MF-8 and P-676 strains which belong to the 1-ABC subtype of the VEE virus grouping scheme.⁵

MATERIALS AND METHODS

Horses

Local crossbred pregnant mares (*Equus caballus*) 4-10 years old were used. Pregnancy was determined by rectal palpation. All mares used were free of antibodies to equine infectious anemia and to Venezuelan and eastern equine encephalitis viruses. Animals received treatment for external and intestinal parasites and were considered healthy.

Viruses

The VEE viral strain MF-8 was isolated from a human in Honduras during the 1969 epizootic,⁶ the virus pool employed had had one passage in mice, one in Vero cells and one in primary fetal equine kidney cells. Virus inoculum was prepared in phosphate-buffered saline, pH 7.2, containing 0.5% gelatin (PBS-G).

Strain P-676 was isolated from mosquitoes in Venezuela during 1963. The P-676 pool used by us had had one passage in mice, two in Vero and one in primary fetal equine kidney cells; virus inoculum was prepared as described for strain MF-8. Because it had been reported that P-676 had a lower pathogenicity than MF-8 for horses,⁷ this strain replaced MF-8 in the study after the second experiment.

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TABLE 1

Reactions among pregnant mares infected with epizootic Venezuelan equine encephalitis virus, strains MF-8 and P-676

| Mare no. | Months of gestation | Peak viremia | Day of death, abortion, or delivery* | Mare | | Fetus or foal | | |
|---------------------|---------------------|--------------|--------------------------------------|----------|-----------------|---------------|--------|----------|
| | | | | Antibody | Virus in organs | Virus in | | Antibody |
| | | | | | | Blood | Organs | |
| Strain MF-8 | | | | | | | | |
| 230 | 4 | 8.2† | 6D | ≥64‡ | -§ | - | - | <4 |
| 231 | 7 | 5.7 | 7D | ≥64 | - | - | - | <4 |
| Strain P-676 | | | | | | | | |
| 233 | 5 | 6.4 | 2D | <4 | ND | - | - | <4 |
| 242 | 10 | 8.5 | 5D | 4 | + | + | + | <4 |
| 239 | 11 | 5.3 | 5DL | 8 | ND | + | + | <4 |
| 232 | 10 | 8.2 | 5D | 16 | + | + | + | <4 |
| 244 | 11 | 7.1 | 5D | 32 | ND | - | - | <4 |
| 243 | 9 | 6.5 | 7A | ≥64 | ND | + | + | <4 |
| 241 | -10 | 7.2 | 7A | ≥64 | ND | - | - | <4 |
| 246 | 10 | 7.6 | 7D | ≥64 | - | - | - | <4 |
| 234 | 10 | 6.5 | 9D | ≥64 | - | - | - | <4 |

* D, died; DL, delivered; A, aborted.

† Maximum titer in dex per milliliter (SMICLD₅₀).

‡ Reciprocal serum titer neutralizing 90% of the test dose.

§ +, positive results; -, negative results; ND, not done.

Inoculation, observation and collection of specimens

Horses were inoculated subcutaneously with 1.0 ml inoculum containing 10,000–100,000 suckling mouse intracerebral LD₅₀ units of virus (SMICLD₅₀). Rectal temperatures were taken once daily during the period of observation from 1 day preinoculation to 12 days postinoculation (PI), unless the animal died. Heparinized blood was collected daily from each horse from day 0 through day 8 and on days 10 and 12.

Specimens were taken aseptically during necropsy of horses or fetuses; these included brain, spinal cord, cerebellum, lymph nodes (mesenteric, inguinal, prescapular), heart, lungs, spleen, kidneys, adrenal glands, ovary or testes, thymus, bone marrow, uterus, placenta, amniotic fluid and blood.

Serological tests

Tests for plaque neutralization were carried out in Vero cells as reported elsewhere,⁸ with overnight incubation of serum-virus mixtures. The overlay medium consisted of Eagle's minimum essential medium with hepes and tricine, incubation was carried out at 34°C without CO₂, and neutral red was added on the 2nd day after inoculation.

Serum titers were recorded as the highest dilution of serum/plasma giving 90% reduction of 60–80 plaque-forming units.

Titration of crude suckling mouse brain CF antigen were performed using a microtechnic,⁹ with 4–8 antibody units of VEE immune mouse ascitic fluid. The immunodiffusion test,¹⁰ to determine the presence of antibodies for equine infectious anemia, was performed at the Panama Viejo veterinary laboratory.

Virus isolation and titration

Attempts to isolate VEE virus from samples of plasma and tissue specimens were made by intracerebral inoculation (0.02 ml) of 2-3-day-old mice. Tissue specimens were washed in medium 199 to remove excess blood and were then macerated in a tissue grinder using PBS-G as diluent to produce a 20% suspension of tissue. This material was centrifuged at 1,000 × g for 15 min and the supernatant was stored at -70°C until assayed. Titrations of plasma and tissue specimens were performed by inoculation of 10-fold serial dilutions of the suspension, and only mice which became sick or died within the first 3 days were considered in the study. Isolates were identified as VEE virus by the complement-fixation micro-test.

RESULTS

Eleven mares and their fetuses were studied, and results are summarized in Table 1. The gestational stages of the mares infected with strain MF-8 were 4 and 7 months. The peak of viremia occurred on days 2-3 PI, and virus titers were 8.2 and 5.7 dex/ml, respectively. Mare 230 died on day 6 and 231 on day 7. At this time both mares had neutralizing antibodies, while virus was absent from the plasma and organs. Their fetuses were alive when autopsy was performed on them; no virus was found in fetal organs or plasma, and no antibodies were present.

The stages of gestation of the nine mares infected with P-676 varied from 5-11 months; the peak of viremia appeared between days 2 and 3 PI, and titers ranged from 5.3 to 8.5 dex/ml. Mare 239 delivered a live foal on day 5, mares 242 and 232 died on day 5 PI, and mare 243 aborted on day 7. The foal and the fetuses of these four mares were viremic and virus was detected in the organs, while antibodies were absent from the sera. These mares, at the time of autopsy, abortion or delivery had neutralizing antibodies in their sera and virus was no longer found in the plasma. Virus was recovered from the uterus of the two mares, 232 and 242.

Neither virus nor antibodies were detected in fetuses of the remaining five mares (233, 234, 241, 244, and 246). Virus was absent from the plasma of all these mares at the time of autopsy or abortion, and antibodies were present in their sera with the exception of mare 233, which died on day 2 PI. No virus was found in organs of the mares 234 and 246.

As may be observed in Table 2, virus was widespread in the organs of the four fetuses infected transplacentally. Titers in some of their organs were equal to or greater than virus titers in the blood, indicating virus replication in fetal tissues.

DISCUSSION

The transplacental passage of epizootic VEE virus, strain P-676, observed in four out of nine mares examined, indicates this is a common phenomenon at least in those mares infected during the last trimester of gestation. Fetuses from mares infected with MF-8, another VEE epizootic strain, were virus-negative, but the number examined was small. Both VEE strains had the ability to induce high viremias in pregnant mares. It

TABLE 2

Virus titers in blood and organs from fetuses or foals of mares infected with Venezuelan equine encephalitis virus, strain P-676

| Organ | Fetus or foal | | | |
|------------|---------------|-----|-----|-----|
| | 232 | 239 | 242 | 243 |
| Blood | 8.4* | 8.3 | 3.5 | 6.2 |
| Brain | 7.3 | 7.2 | 4.2 | 5.2 |
| Kidney | 8.2 | 7.7 | 5.2 | 5.3 |
| Liver | 7.5 | 8.0 | 4.2 | 5.3 |
| Lung | 7.7 | 7.3 | 7.2 | 5.2 |
| Lymph node | 6.6 | 8.2 | 7.2 | 5.2 |
| Spleen | 7.3 | 8.2 | 8.3 | 5.3 |
| Thymus | 8.5 | 8.3 | 6.3 | 6.3 |

* Titers in dex per ml or per gram of tissue (SMDCI)₅₀

is not known when the virus crosses the placental barrier and reaches the fetal tissues, but we suggest it could be during the period of maximum viremia.

The infected fetuses examined on day 5 PI probably had an infection of 2-3 days' duration, while the one aborted on day 7 PI probably had an infection of 4-5 days. This latter fetus had high virus titer in blood and organs and no detectable antibodies, a finding different from what has been observed in horses in which, by that time of infection, virus is absent from the blood and antibodies are present. These results showed that VEE infection may last longer in the fetus than in the adult horse. Two cases of abortion were observed, but more information will be needed in order to determine whether epizootic VEE virus induces abortion.

The finding that VEE virus in pregnant mares crosses the placental barrier and infects the fetus raises the possibility that such fetal infection may become latent, with the foal being born without signs of VEE infection. If this were to occur, reactivation of the VEE latent infection might happen later in life under certain conditions of stress. Such a mechanism could contribute to VEE virus survival during the interepizootic periods.

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