CHAPTER 2.5.14.

VENEZUELAN EQUINE ENCEPHALOMYELITIS

SUMMARY

Venezuelan equine encephalomyelitis (VEE) viruses, of the genus Alphavirus of the family Togaviridae, cause disease ranging from mild febrile reactions to fatal encephalitic zoonoses in Equidae and humans. They are transmitted by haematophagous insects, primarily mammalophilic mosquitoes.

The VEE complex of viruses includes six antigenic subtypes (I–VI). Within subtype I there are five antigenic variants (variants AB–F). Originally, subtypes I-A and I-B were considered to be distinct variants, but they are now considered to be identical (I-AB). Antigenic variants I-AB and I-C are associated with epizootic activity in equids and human epidemics. Historically, severe outbreaks have involved many thousands of human and equine cases. The other three variants of subtype I (I-D, I-E, I-F) and the other five subtypes of VEE (II–VI) circulate in natural enzootic cycles. Equidae serve as amplifying hosts for epizootic VEE strains while enzootic VEE viruses cycle primarily between sylvatic rodents and mosquitoes. Enzootic variants and subtypes have been considered to be nonpathogenic for equids, but can cause clinical disease in humans. During 1993 and 1996 however, limited outbreaks of encephalitis in horses in Mexico were shown to be caused by enzootic VEE viruses of subtype I-E.

Identification of the agent: Diagnosis of VEE virus infection can be confirmed by the isolation, identification, and antigenic classification of the isolated virus.

A presumptive diagnosis of equine encephalomyelitis can be made when susceptible animals in tropical or subtropical areas display clinical signs of encephalomyelitis where haematophagous insects are active. VEE virus can be isolated in cell cultures or in laboratory animals using the blood or serum of febrile animals in an early stage of infection. It is recovered less frequently from the blood or brain tissue of encephalitic animals.

VEE virus can be identified by complement fixation, haemagglutination inhibition, plaque reduction neutralisation (PRN), or immunofluorescence tests using VEE-specific antibodies. Specific identification of epizootic VEE variants can be made by the indirect fluorescent antibody test, or a differential PRN test using subtype- or variant-specific monoclonal antibody, or by nucleic acid sequencing.

Serological tests: Specific antibodies may be demonstrated by PRN tests against epizootic VEE virus variants or by IgM capture enzyme-linked immnosorbent assay. Antibody can also be demonstrated by the haemagglutination inhibition or the complement fixation tests.

Any diagnosis of VEE in an individual that is based on seroconversion in the absence of an epizootic should be made with care. Infections of equids with enzootic VEE viruses produce a low level viraemia accompanied by antibody development, but without clinical disease in most cases. Antibody induced by such subclinical infections may be reactive to epizootic VEE virus variants.

Requirements for vaccines and diagnostic biologicals: The only acceptable vaccines against VEE are an attenuated virus vaccine, made with strain TC-83, or inactivated virus preparations also made from this strain. Attenuated virus is immunogenic when given by intramuscular injection, but sometimes causes adverse reactions in the recipient.

Formalin-inactivated virulent VEE virus preparations should never be used in equids, as residual virulent virus can remain after formalin treatment, and thereby cause severe illness in both animals and humans. Epizootics of VEE have occurred from the use of such formalin-treated viruses.
A. INTRODUCTION

Venezuelan equine encephalomyelitis (VEE) is an arthropod-borne inflammatory viral infection of equines and humans, resulting in mild to severe febrile and, occasionally fatal, encephalitic disease.

VEE viruses form a complex within the genus Alphavirus, family Togaviridae. The VEE virus complex is composed of six subtypes (I–VI). Subtype I includes five antigenic variants (AB–F), of which variants 1-AB and 1-C are associated with epizootic VEE in equids and concurrent epidemics in humans (2–4, 8–10). The epizootic variants 1-AB and 1-C are thought to originate from mutations of the enzootic 1-D serotype (11); 1-AB and 1-C isolates have only been obtained during equine epizootics. The enzootic strains include variants 1-D, 1-E and 1-F of subtype I, subtype II, four antigenic variants (A–D) of subtype III, and subtypes IV–VI. Normally, enzootic VEE viruses do not produce clinical encephalomyelitis in the equine species (9), but in 1993 and 1996 in Mexico, the 1-E enzootic subtype caused limited epizootics in horses. The enzootic variants and subtypes can produce clinical disease in humans (3, 4, 5, 8, 10).

Historically, epizootic VEE was limited to northern and western South America (Venezuela, Colombia, Ecuador, Peru and Trinidad) (4). From 1969 to 1972, however, epizootic activity (variant 1-AB) occurred in Guatemala, El Salvador, Nicaragua, Honduras, Costa Rica, Belize, Mexico, and the United States of America (USA) (Texas). Epizootics of VEE caused by I-AB or I-C virus have not occurred in North America and Mexico since 1972. Recent equine and human isolations of epizootic VEE virus were subtype 1-C strains from Venezuela in 1993, 1995 and 1996 and Colombia in 1995.

The foci of enzootic variants and subtypes are found in areas classified as tropical wet forest, i.e. those areas with a high water table or open swampland areas with meandering sunlit streams. These are the areas of the Americas where rainfall is distributed throughout the year or areas permanently supplied with water. Enzootic viruses cycle among rodents, and perhaps birds, by the feeding of mosquitoes (3, 4, 8, 10). Enzootic VEE strains have been identified in the Florida Everglades (subtype II), Mexico (variant I-E), Central American countries (variant I-E), Panama (variants I-D and I-E), Venezuela (variant I-D), Colombia (variant I-D), Peru (variants I-D, III-C, and III-D), French Guiana (variant III-B and subtype V), Ecuador (variant I-D), Suriname (variant II-A), Trinidad (variant II-A), Brazil (variants I-F and III-A and subtype IV), and Argentina (subtype VI). In an atypical ecological niche, variant III-B has been isolated in the USA (Colorado and South Dakota) in an unusual association with birds (3, 4, 8, 10).

A tentative diagnosis of viral encephalomyelitis in equids can be based on the occurrence of acute neurological disease during the summer in temperate climates or in the wet season in tropical or subtropical climates. These are the seasons of haematophagous insect activity. Virus infection will result in clinical disease in many equids concurrently rather than in isolated cases. Epizootic activity can move vast distances through susceptible populations in a short time (3, 4, 8, 10). Differential diagnoses include eastern or western equine encephalomyelitis (Chapter 2.5.5), Japanese encephalitis (Chapter 2.1.7.), West Nile fever (Chapter 2.1.20), rabies (Chapter 2.1.13) and other infectious, parasitic, or non-infectious agents producing similar signs.

Human VEE virus infections have originated by aerosol transmission from the cage debris of infected laboratory rodents and from laboratory accidents. Infections with both epizootic and enzootic variants and subtypes have been acquired by laboratory workers (6). Severe clinical disease or death can occur in humans. Those who handle infectious VEE viruses or their antigens prepared from infected tissues or cell cultures should be vaccinated and shown to have demonstrable immunity in the form of VEE virus-specific neutralising antibody (1, 4). All procedures producing aerosols from VEE virus materials should be conducted in biosafety cabinets at containment level 3 (see Chapter 1.1.2 Biosafety and biosecurity in the veterinary microbiological laboratory and animal facilities) (6, 7).

B. DIAGNOSTIC TECHNIQUES

1. Identification of the agent

A confirmatory diagnosis of VEE is based on the isolation and identification of the virus or on the demonstration of seroconversion. The period of virae mia coincides with the onset of pyrexia within 12–24 hours of infection. Virae mia terminates 5–6 days after infection, and coincides with the production of neutralising antibodies and the appearance of clinical neurological signs. Frequently, VEE viruses cannot be isolated from the brains of infected equids. Blood samples for virus isolation should be collected from febrile animals that are closely associated with clinical encephalitic cases.

Virus may be isolated from the blood or sera of infected animals by inoculating 1–4-day-old mice or hamsters intracerebrally or by the inoculation of other laboratory animals, such as guinea-pigs and weaned mice. It may
also be isolated by the inoculation of various cell cultures including African green monkey kidney (Vero), rabbit kidney (RK-13), baby hamster kidney (BHK-21), or duck or chicken embryo fibroblasts, or by inoculation of embryonated chicken eggs. Details of virus identification techniques are described in Chapter 2.5.5.

Isolates can be identified as VEE virus by complement fixation (CF), haemagglutination inhibition (HI), or plaque reduction neutralisation (PRN) tests, or by immunofluorescence as described in Chapter 2.5.5. The VEE virus isolates can be characterised by the indirect fluorescent antibody or PRN tests using monoclonal antibody or by nucleic acid sequencing. The VEE virus characterisation should be carried out in a reference laboratory (see Table given in Part 3 of this Terrestrial Manual).

2. Serological tests

Diagnosis of VEE virus infection in equids requires the demonstration of specific antibodies in paired serum samples collected in the acute and convalescent phases. After infection, PRN antibodies appear within 5–7 days, CF antibodies within 6–9 days, and HI antibodies within 6–7 days. The second convalescent phase serum sample should be collected 4–7 days after the collection of the first acute phase sample or at the time of death. The serological procedures are described in detail in Chapter 2.5.5. Vaccination history must be taken into account when interpreting any of the VEE serological test results. In horses not recently vaccinated with an attenuated live virus strain, demonstration of VEE-specific serum IgM antibodies in a single serum sample supports recent virus exposure.

Any diagnosis of VEE in an individual that is based on seroconversion in the absence of an epizootic should be made with care. Although enzootic subtypes and variants are nonpathogenic for equids, infection will stimulate antibody production to epizootic VEE virus variants.

C. REQUIREMENTS FOR VACCINES AND DIAGNOSTIC BIOLOGICALS

The acceptable vaccines against VEE infection are an attenuated virus vaccine, strain TC-83, and an inactivated virus preparation made from that strain (3, 4, 8, 10). The inactivated vaccine is now the most widely used, and is marketed in EEE/VEE, EEE/VEE/VEE, EEE/VEE/WEE/tetanus toxoid, and EEE/VEE/WEE/VEE/West Nile virus/tetanus toxoid combinations.

Inactivated vaccine should be administered in two doses with an interval of 2–4 weeks between doses. Annual revaccination is recommended.

Attenuated vaccine should be reconstituted with physiological saline and used immediately. Multidose vials are kept on ice while the vaccine is being used. Any vaccine not used within 4 hours of reconstitution should be safely discarded. Foals under 2 weeks of age and pregnant mares should not be vaccinated. Animals are vaccinated subcutaneously in the cervical region with a single dose. Revaccination is not recommended.

NOTE: Formalin-treated preparations of virulent epizootic VEE virus should never be used in equids. Residual virulent virus can remain after formalin treatment, and result in severe illness. Epizootics of VEE have occurred in Central and Southern America from the use of such preparations (8, 12).

1. Seed management

a) Characteristics of the seed

Attenuated VEE virus vaccine strain TC-83 originated from the Trinidad donkey strain (a variant of I-AB) of epizootic VEE virus isolated in 1944. This strain was derived by serial passage of the Trinidad donkey strain in fetal guinea pig heart cells. It is safe and immunogenic at the established passage levels, and induces protective immunity in vaccinated equids, although adverse reactions can sometimes occur. The vaccine was originally developed for use in personnel involved in high-risk VEE virus research. Suitable seed lots should be maintained at −70°C in a lyophilised state.

b) Method of culture

The virus is grown in fetal guinea pig heart cell cultures in a suitable medium.
c) Validation as a vaccine
The cells used for vaccine production must be free from bacterial, fungal, mycoplasmal, and viral contamination. VEE virus is identified in batches of vaccine by PRN tests against hyperimmune serum. For inactivated vaccines of cell culture origin, strain TC-83 virus is treated with formaldehyde.

2. Method of manufacture (see footnote 1)
Vaccine is produced by harvesting supernatant fluid from fetal guinea pig heart monolayers in which the replication of attenuated VEE virus has occurred. The monolayers are maintained at approximately 37°C. The time of harvesting is determined by the occurrence of characteristic cytopathic changes when approximately 70–100% of the cell sheet is affected, typically 1–3 days after infection. The supernatant fluid is clarified by low speed centrifugation and suitable stabilisers are added to protect the virus during freezing and lyophilisation.

3. In-process control (see footnote 1)
Cultures should be examined daily for cytopathic changes. After harvesting, the virus suspension should be tested for the presence of microbial contaminants.

Inactivated vaccines derived from attenuated strain TC-83 virus should be checked to exclude the presence of viable virus after formalin treatment.

4. Batch control (see footnote 1)
a) Sterility
Tests for sterility and freedom from contamination of biological materials may be found in Chapter 1.1.9.

b) Safety
Safety tests of the inactivated vaccine are described in Chapter 2.5.5.

Safety tests of the attenuated vaccine are conducted in mice. A 0.5 ml dose is injected intraperitoneally or subcutaneously into each of eight mice, and the animals are kept under observation for 7 days. If adverse reactions attributable to the product occur during this period, the product is considered to be unsatisfactory.

c) Potency
Potency tests of the inactivated vaccine are described in Chapter 2.5.5 except that antibody titre in inoculated guinea-pigs will be ≥1/4.

Potency of the attenuated vaccine can be determined by testing in horses. Each of 20 susceptible horses is inoculated subcutaneously with 1 ml of lyophilised vaccine that has a reconstituted virus titre of at least 2.5 log_{10} TCID_{50} (50% tissue culture infective dose) per ml. For a valid test, at least 19 of 20 vaccinated horses must have HI antibody titres of at least 1/20 or serum neutralising antibody titres of at least 1/40 within 21–28 days of vaccination.

When tested at any time within the expiration period following lyophilisation, the product must have a virus titre of 0.7 log_{10} greater than that used to test horses as described above, but no less than 2.5 log_{10} TCID_{50}/dose.

The final product must be free from bacterial, fungal, mycoplasmal, or extraneous viral contaminants.

d) Duration of immunity
Comprehensive studies on duration of immunity are not available. An annual revaccination is recommended for the inactivated vaccine. Foals that are vaccinated at under 1 year of age should be revaccinated before the next vector season. Revaccination with the attenuated vaccine is not recommended.

e) Stability
The lyophilised vaccine is stable and immunogenic for 3 years if kept refrigerated at 2–7°C. After 3 years, vaccine should be discarded. The vaccines should be used immediately after reconstitution. Multidose vials of the attenuated vaccine should be kept on ice while being used. All unused vaccine should be safely discarded 4 hours after reconstitution.

f) Preservatives
The preservatives used are thimerosal at a 1/1000 dilution and antibiotics (neomycin, polymyxin, amphotericin B, and gentamicin).
g) Precautions (hazards)

Pregnant mares and foals under 2 weeks of age should not be vaccinated.

All personnel handling infectious VEE viruses or their antigens prepared from infected tissues or cell cultures should be vaccinated and shown to have demonstrable immunity in the form of VEE virus-specific neutralising antibody. All procedures producing aerosols from VEE virus materials should be conducted in biosafety cabinets with biocontainment and efficient filtration of the exhaust air from the laboratory (6, 7).

5. Tests on the final product

a) Safety and potency

Safety and potency tests are as outlined above under Batch control (Sections C.4.b and C.4.c). The attenuated vaccine must have a virus titre of no less than 2.5 log_{10} TCID_{50}/dose.

REFERENCES


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NB: There is an OIE Reference Laboratory for Venezuelan equine encephalomyelitis (see Table in Part 3 of this Terrestrial Manual or consult the OIE Web site for the most up-to-date list: www.oie.int).