CHAPTER 2.5.13.

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 limited outbreaks of encephalitis in horses in Mexico were shown to be caused by enzootic VEE viruses of subtype I-E. More recently, sporadic outbreaks have occurred in Mexico, Central America, and northern and western parts of South America. Human enzootic subtypes reach more broadly into northern Central America and South America* (*Weaver, 2012*).

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 polymerase chain reaction, 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 immunosorbent 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: *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 (Calisher *et al*., 1980; Monath & Trent, 1981; Pan-American Health Organization, 1972; Walton, 1981; Walton *et al*., 1973; Walton & Grayson, 1989). The epizootic variants 1-AB and 1-C are thought to originate from mutations of the enzootic 1-D serotype (Weaver *et al*., 2004); 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 (Walton *et al*., 1973), 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 (Monath & Trent, 1981; Pan-American Health Organization, 1972; Powers *et al*., 1997; Walton, 1981; Walton & Grayson, 1989).

Historically, epizootic VEE was limited to northern and western South America (Venezuela, Colombia, Ecuador, Peru and Trinidad) (Pan-American Health Organization, 1972). 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 swampy 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 (Monath & Trent, 1981; Pan-American Health Organization, 1972; Walton, 1981; Walton & Grayson, 1989). 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 1-D, III-C, and III-D), French Guiana (variant III-B and subtype V), Ecuador (variant I-D), Suriname (variant III-A), Trinidad (variant III-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 (Monath & Trent, 1981; Pan-American Health Organization, 1972; Walton, 1981; Walton & Grayson, 1989). Everglades virus is a subtype II VEE virus that infects rodents and dogs in Florida.

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 (Monath & Trent, 1981; Pan-American Health Organization, 1972; Walton, 1981; Walton & Grayson, 1989). 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 (American Committee on Arthropod-Borne Viruses [ACAV], 1980). 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 (Berge *et al*., 1961; Pan-American Health Organization, 1972). If vaccination is not a viable option, additional personal protective equipment to include respiratory protection is recommended for all procedures. Laboratory manipulations should be carried out at an appropriate biosafety and containment level determined by biorisk analysis (see Chapter 1.1.3 *Biosafety and biosecurity in the veterinary microbiology laboratory and animal facilities*).

B. DIAGNOSTIC TECHNIQUES

Table 1. *Test methods available for the diagnosis of Venezuelan equine encephalomyelitis and their purpose*

Method	Purpose				
	Population freedom from infection	Individual animal freedom from infection	Confirmation of clinical cases	Prevalence of infection – surveillance	Immune status in individual animals or populations post-vaccination
Agent Identification	–	+	+++	–	–
IgM Capture ELISA	–	–	++	–	–
Plaque reduction neutralisation (paired samples)	+++	+	++	++	+++
Hemagglutination inhibition (paired samples)	+	++	++	++	++
Complement fixation (paired samples)	–	+	++	–	–

Key: +++ = recommended method; ++ = suitable method; + = may be used in some situations, but cost, reliability, or other factors severely limits its application; – = not appropriate for this purpose. although not all of the tests listed as category +++ or ++ have undergone formal standardisation and validation, their routine nature and the fact that they have been used widely without dubious results, makes them acceptable.
ELISA = enzyme-linked immunosorbent assay.

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 viraemia coincides with the onset of pyrexia within 12–24 hours of infection. Viraemia 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 reverse transcriptase-polymerase chain reaction (RT-PCR), 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 4 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

1. Background

The acceptable vaccines against VEE infection are an attenuated virus vaccine, strain TC-83, and an inactivated virus preparation made from that strain (Monath & Trent, 1981; Pan-American Health Organization, 1972; Walton, 1981; Walton & Grayson, 1989). Directions for use provided with commercial products should be followed; examples of typical guidelines are below.

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. Animals over 3 months of age are vaccinated subcutaneously in the cervical region with a single dose. Annual revaccination is recommended.

Guidelines for the production of veterinary vaccines are given in Chapter 1.1.6 on *Principles of veterinary vaccine production*. The guidelines given below and in chapter 1.1.6 are intended to be general in nature and may be supplemented by national and regional requirements.

2. Outline of production and minimum requirements for vaccines

a) Characteristics of the seed

See chapter 1.1.6 for general requirements for master seeds and allowable passages for vaccine production. Suitable seed lots should be maintained at –70°C in a lyophilised state.

i) *Biological characteristics of the master seed*

The 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.

ii) *Quality criteria* (*sterility, purity, freedom from extraneous agents*)

The MSV must be tested for purity, identity, and freedom from extraneous agents at the time before it is used in the manufacture of vaccine. The MSV must be free from bacteria, fungi and mycoplasma. The MSV is cultured on a Vero cell line and an embryonic equine cell type with confirmation by the fluorescent antibody technique to demonstrate freedom from equine herpesvirus, equine adenovirus, equine arteritis virus, bovine viral diarrhoea virus, reovirus, and rabies virus extraneous agents The MSV must also be free from extraneous virus by cytopathic effect (CPE) and haemadsorption on cell culture.

iii) *Validation as a vaccine strain*

In an immunogenicity trial, the MSV at the highest passage level intended for production must prove its efficacy (protection) in the guinea-pig vaccination/serology potency test.

iv) *Emergency procedure for provisional acceptance of new master seed virus* (*MSV*) *in the case of an epizootic* (*with pathogens with many serotypes, e.g. bluetongue virus, highly pathogenic avian influenza, FMD, etc*.)

In emergency epizootic situations, provisional acceptance of a new strain could be based on a risk analysis of the possibility of contamination of the antigen produced from the new MSV with extraneous agents. This risk assessment should take into account the characteristics of the process, including the nature and concentration of the inactivant for inactivated vaccines, before allowing or not the early release of the new product However, 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 (Walton, 1981; Weaver *et al.,* 1999).

b) Method of manufacture

i) *Procedure*

The MSV should be propagated in cell lines known to support the growth of VEE. See chapter 1.1.6 for additional guidance on the preparation and testing of master cell stocks. Cell lines should be free from extraneous viruses, bacteria, fungi, and mycoplasma. Viral propagation should not exceed five passages from the MSV, unless further passages prove to provide protection in the host animal.

The susceptible cell line is seeded into suitable vessels. Minimal essential medium, supplemented with fetal bovine serum (FBS), may be used as the medium for production. Incubation is at 37°C.

Cell cultures are inoculated directly with VEE working virus stock, which is generally 1 to 4 passages from the MSV. Inoculated cultures are incubated for 1–3 days before harvesting the culture medium. During incubation, the cultures are observed daily for CPE and bacterial contamination.

The TC-83 VEE vaccine strain may be chemically inactivated with formalin and mixed with a suitable adjuvant. The duration of the inactivation period is based on demonstrated inactivation kinetics.

The preservatives used are thimerosal at a 1/1000 dilution and antibiotics (neomycin, polymyxin, amphotercin B, and gentamicin).

ii) *Requirements for ingredients*

All ingredients used in the manufacture of VEE vaccine should be defined in approved manufacturing protocols and consistent from batch to batch. See chapter 1.1.6 for general guidance on ingredients of animal origin. Ingredients of animal origin should be sourced from a country with negligible risk for transmissible spongiform encephalopathies (TSEs).

iii) *In-process controls*

Production lots should be examined daily for cytopathic changes. After harvesting, the virus suspension should be tested for the presence of microbial contaminants. Production lots of VEE must be titrated in tissue culture before inactivation to standardise the product. Low-titred lots may be concentrated or blended with higher-titred lots to achieve the correct titre.

Inactivated VEE lots must be tested for completeness of inactivation in 6- to 12-hour old chicks.

iv) *Final product batch tests*

Sterility

Inactivated and live vaccine samples are examined for bacterial and fungal contamination. The volume of medium used in these tests should be enough to nullify any bacteriostatic or fungistatic effects of the preservatives in the product. To test for bacteria, ten vessels, each containing a minimum of 120 ml of soybean casein digest medium, are inoculated with 0.2 ml from ten final-container samples. The ten vessels are incubated at 30–35°C for 14 days and observed for bacterial growth. To test for fungi, ten vessels, each containing a minimum of 40 ml of soybean casein digest medium, are inoculated with 0.2 ml from ten final-container samples. The vessels are incubated at 20–25°C for 14 days and observed for fungal growth. Individual countries may have other requirements.

Identity

Separate batch tests for identity should be conducted if the batch potency test, such as tissue-culture titrations of live virus vaccines, does not sufficiently verify the identity of the agent in the vaccine. Identity tests may include fluorescent antibody or serum neutralisation assays.

Safety

Batch safety testing for VEE inactivation is conducted in 6- to 12-hour old chicks. Ten chicks are inoculated subcutaneously with 0.5 ml of product and observed daily for 10 days. If unfavourable reactions occur, the batch is unacceptable.

Batch potency

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

c) Requirements for authorisation/registration/licensing

i) *Manufacturing process*

For registration of vaccine, all relevant details concerning manufacture of the vaccine and quality control testing (see Section C.2.a and b) should be submitted to the authorities. This information shall be provided from three consecutive vaccine batches with a volume not less than 1/3 of the typical industrial batch volume.

In-process controls are part of the manufacturing process.

ii) *Safety requirements*

The final inactivated vaccine formulation should be tested in a limited number of target animals prior to a larger-scale field study. The final vaccine formulation should not cause adverse reactions.

Field safety studies should be conducted before any vaccine receives final approval. Generally, two serials should be used, in three different geographical locations under typical animal husbandry conditions, and in a minimum of 600 animals. The vaccine should be administered according to label recommendations (including booster doses) and should contain the maximum permissible amount of VEE antigen. (If no maximum antigen content is specified, serials should be of anticipated typical post-marketing potency.) About one-third of the animals should be at the minimum age recommended for vaccination.

- *Precautions* (*hazards*)

Vaccine should be identified as harmless or pathogenic for vaccinators. Manufacturers should provide adequate warnings that medical advice should be sought in the case of self-injection (including for adjuvants, oil-emulsion vaccine, preservatives, etc.) with warnings included on the product label/leaflet so that the vaccinator is aware of any danger.

iii) *Efficacy requirements*

To register a commercial vaccine, a batch or batches produced according to the standard method and containing the minimum amount of antigen or potency value shall prove its efficacy (protection) in the guinea-pig vaccination/serology potency test; each future commercial batch shall be tested before release to ensure it has the same potency value demonstrated by the batch(es) used for the efficacy test(s).

iv) *Vaccines permitting a DIVA strategy* (*detection of infection in vaccinated animals*)

Vaccination is only recommended for horses in VEE-positive areas. Vaccinated horses may develop a serological titre, which may interfere with the ability to export the horse.

v) *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.

v) *Stability*

The lyophilised vaccine is stable and immunogenic for 2 years if kept refrigerated at 2–7°C. After 2 years, vaccine should be discarded.

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NB: There is an OIE Reference Laboratory for Venezuelan equine encephalomyelitis
(see Table in Part 4 of this *Terrestrial Manual* or consult the OIE web site for the most up-to-date list:
http://www.oie.int/en/our-scientific-expertise/reference-laboratories/list-of-laboratories/).
Please contact the OIE Reference Laboratories for any further information on
diagnostic tests, reagents and vaccines for Venezuelan equine encephalomyelitis