VENEZUELAN EQUINE ENCEPHALITIS

AETIOLOGY

Classification of the causative agent

Venezuelan equine encephalomyelitis (VEE) viruses are taxonomically classified within the genus *Alphavirus* of the family Togaviridae (formerly the Group A arboviruses). The VEE complex of viruses includes six antigenic subtypes (I–VI) divided by antigenic variants. 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/epidemic activity in equids and humans. 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.

Resistance to physical and chemical action

Temperature:	Thermal inactivation point (TIP) for Alphaviruses is 58°C and virion half-life is 7 hours at 37°C
pH:	Alphavirus virions are stable in alkaline environment of pH 7–8 but inactivated quickly at acidic pH
Chemicals/Disinfectants:	Inactivated by various common disinfectants; sensitive to organic solvents and detergents 1% sodium hypochlorite, 70% ethanol, 2% glutaraldehyde and formaldehyde
Survival:	The agent is susceptible to radiant sunlight, moist or dry heat and drying thus cool, moist, dark conditions favour survival

EPIDEMIOLOGY

Hosts

- Enzootic VEE viruses (I-D, I-E, I-F and subtypes II–VI) are primarily found cycling between sylvatic rodents and Culex mosquitoes (*Melanoconion* subgenus)
    - birds may also be involved in enzootic virus maintenance
    - equids and humans are considered incidental or dead-end hosts
- Natural or inter-epidemic host for epizootic strains (I-AB and I-C) is yet undetermined; prevailing science indicates that the epizootic strains emerge from genetic modification of enzootic strains
    - Equidae serve as amplifying hosts for epizootic VEE virus strains; horses and donkeys produce high viraemias which, in turn, infect a wide range of mosquitoes
    - Cattle, swine, chickens and dogs have been shown to seroconvert after epizootics; also mortality has been observed in domesticated rabbits, dogs, goats and sheep
    - during VEE epizootics, virus has been isolated from cotton rats, opossums, gray fox, bats and many wild birds
    - experimentally, guinea-pigs, mice, hamsters and some non-human primates have been infected; laboratory rodents are usually subclinically infected but some isolates can be fatal

Transmission

- Haematophagous insects play a central role in the transmission of all VEE viruses
    - epizootic strains have been isolated from the genera: *Aedes, Anopheles, Culex, Deinocerites, Mansonia* and *Psorophora*
    - mechanical transmission of epizootic VEE has been demonstrated for blackflies (*Simulium* spp.)
    - role of ticks uncertain though *Amblyomma* and *Hyalomma* species have been infected experimentally with both enzootic and epizootic strains of VEE
- Enzootic/endemic or Sylvatic cycle: enzootic VEE variants and subtypes cycle in tropical ecosystems among rodents, and perhaps birds, by the feeding of mosquitoes

- Epizootic/epidemic cycle: equids are amplifying host (high, prolonged viraemias) and infect a wide range of mosquitoes which are not restricted to equid hosts
    - non-vector spread of disease via direct contact or aerosols has been proposed; horse-to-human and human-to-human transmission has not been recorded
    - role of non-equid vertebrates in transmission is not clear but likely minor
- Swiftness of spread depends on: VEE virus subtype, density of competent vectors and population of susceptible hosts; large, geographically disperse epizootics of VEE have depended on ability of virus to produce high viraemias in equids.

Sources of virus

- Equids (horses, donkeys and zebras) are a primary source of epizootic virus strains during an outbreak
    - inter-epidemic maintenance of epizootic strains is not known though three hypotheses prevail:
        - incomplete inactivation of subtype I-AB VEE vaccines (suggested by sequencing studies)
        - epizootic/epidemic subtype I-AB and I-C VEE strains arise from genetic mutation of enzootic subtype I-D strains (supported by genetic studies)
        - recent studies of epizootic I-C VEE strains in Venezuela would indicate persistent, low-level sylvatic maintenance in and yet unknown cycle (cryptic transmission cycle)
- Haematophagous vectors become infected with high titres of virus from infected horses
- Enzootic/endemic strains remain in tropical ecosystems in cycle between rodents and mosquitoes; and for some subtypes, birds also.

Occurrence

Enzootic/endemic VEE viruses are known to be circulating continually in lowland tropical and sub-tropical forests and swamps of South and Central America, Mexico, and areas of the United States. Enzootic VEE viruses do not usually produce clinical encephalomyelitis in the equine species; however, in 1993 and 1996 in Mexico, the 1-E enzootic subtype caused limited epizootics in horses. Everglades virus is a subtype II VEE virus that infects rodents and dogs in Florida.

Historically, epizootic VEE has been limited to northern and western South America (Venezuela, Colombia, Ecuador, and Peru) and the Caribbean Island of Trinidad (1944). A panzootic of VEE I-AB began spreading through Central America in 1969 reaching the United States (Texas) in 1971. 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. More recent VEE detections in equids have been reported to the OIE from Belize (1996, 1998, 2003, 2004, 2005, 2007), Colombia (1996, 1997, 1998, 1999, 2001, 2002, 2003, 2005–2007), Costa Rica (2001, 2002), Honduras (1997, 2000, 2001, 2002, 2003, 2007), Guatemala (1998, 2005-2008), Guyana (2006), Panama (1999, 2005), and Venezuela (2000, 2003, 2004, 2005) [cases may be from prior year of report]. 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.

For more recent, detailed information on the occurrence of this disease worldwide, see the *OIE World Animal Health Information Database* (WAHID) interface [http://www.oie.int/wahis/public.php?page=home] or refer to the latest issues of the *World Animal Health* and the OIE *Bulletin*.

DIAGNOSIS

Incubation period is usually recognised as 1– 5 days with high fever appearing within a day and neurologic signs manifesting themselves at approximately 5 days.

Clinical diagnosis

Although 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 can only be considered one of various possible causes and final diagnosis will require laboratory confirmation.

- Although enzootic VEE viruses tend not to cause overt signs of disease in equid hosts this may not always be the case as was seen with I-E virus in Mexico in 1993 and 1996
    - can cause clinical disease in humans
- Epizootic virus strains can result in severe disease of horses, mules, donkeys and zebras but vary in their virulence
    - some cause febrile disease and no neurologic signs.
- Infections, as measured by circulating antibody, can be as high as 90% but morbidity will vary depending on strains and immune response
- Morbidity rates can vary anywhere from 10–40% to 50–100%
- Mortality rates in horses can be 50–70% with case fatality rates of 38–90%.

Clinical disease can be characterised in four general presentations:

Subclinical

- No real manifestation of disease
- Most often associated with enzootic strains of VEE

Moderate

- Characterised by inappetance, pyrexia and depression
- The first sign observed with infection of epizootic VEE virus is fever; viraemia occurs concurrently with the onset of fever and can persist for 24 days
    - viraemia coincides with the onset of pyrexia within 12–24 hours of infection and terminates 5–6 days after infection; coinciding with the production of neutralising antibodies

Severe-non-fatal

- Continued anorexia and high fevers, with tachycardia and depression progressing to more severe central nervous system involvement
    - paresis, muscle fasciculation and spasms; incoordination, staggering, abasia resulting in open stance to prevent falling
    - blindness may result in lesions to chest
    - head pressing, bruxism, circling or rocking on limbs; paddling in animals which have fallen or are in lateral recumbency
    - stupor and/or convulsions often resulting in permanent neurologic damage
    - some animals may demonstrate diarrhoea and colic

Fatal

- Similar signs to severe disease but concluding in death
- Death can be sudden or occur within hours from the onset of neurological signs
- Prolonged disease may result in dehydration and extreme loss of condition in animals and these may also eventually perish
- Epizootics of VEE have also resulted in the deaths of other animals: rabbits, goats, dogs and sheep

Lesions

- Gross lesions of the central nervous system in horses associated with VEE are, in general, nonspecific; varying from no lesions to extensive necrosis with haemorrhages.
    - presence of ecchymotic haemorrhages may be due to self-induced ante-mortem trauma
- Lesions in other organs are also too variable to be of use diagnostically
    - they include necrotic lesions in the pancreas, adrenal cortex, heart, liver, and vasculature walls
- Histopathologically, the predominant lesions are related to a diffuse necrotising meningo-encephalitis; varying from some perivascular mixed cellular reaction to marked vascular necrosis with associated haemorrhages, gliosis, and clear neuronal necrosis
- Severity of lesions tend to be most severe in the cerebral cortex and become progressively less severe toward the cauda equina

- Evidence of central nervous system lesions is directly related to severity and duration of the clinical signs

Differential diagnosis

- Eastern or Western equine encephalomyelitis (EEE and WEE)
- Japanese encephalitis
- West Nile fever
- Leucoencephalomalacia due to mouldy corn intoxication (*Fusarium* spp.)
- Rabies
- Tetanus
- African horse sickness
- Bacterial meningitis
- Toxic poisoning

Laboratory diagnosis

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. Laboratory manupulations should be carries 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*).

A confirmatory diagnosis of VEE is based on the isolation and identification of the virus or on the demonstration of seroconversion.

Samples

Identification of the agent

- Heparinised blood of febrile animals in an early stage of infection and closely associated with clinical encephalitic cases
- Brain and piece of pancreas unfixed
    - It is often difficult to isolate VEE viruses from the brains of infected equids
- A complete set of tissues in 10% formalin from recently dead animals

Serological tests

- paired sera, if the animal survives
    - one sample at time of fever and 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

Procedures

Identification of the agent

- Virus isolation in laboratory animals
    - inoculation of blood or sera of infected animals in 1–4-day-old mice or hamsters intracerebrally or by the inoculation of other laboratory animals (guinea-pigs and weaned mice)
- Virus isolation in cell culture
    - inoculation of various cell cultures or duck or chicken embryo fibroblasts
    - inoculation of embryonated chicken eggs
- Isolates can be identified as VEE virus by reverse transcriptase-polymerase chain reaction (RT-PCR), complement fixation (CF), haemagglutination inhibition (HI), plaque reduction neutralisation (PRN), immunofluorescence
- The VEE virus isolates can be characterised by the indirect fluorescent antibody or PRN tests using monoclonal antibody or by nucleic acid sequencing
    - VEE virus characterisation should be carried out in a reference laboratory

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
    - PRN antibodies appear within 5–7 days after infection
    - CF antibodies within 6–9 days after infection
    - HI antibodies within 6–7 days after infection
- 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 non-pathogenic for equids, infection will stimulate antibody production to epizootic VEE virus variants

For more detailed information regarding laboratory diagnostic methodologies, please refer to Chapters 2.5.14 Venezuelan equine encephalomyelitis and 2.5.5 Equine encephalomyelitis (Eastern and Western) in the latest edition of the OIE *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals*: especially under the heading "Diagnostic Techniques".

PREVENTION AND CONTROL

Sanitary prophylaxis

- Epizootics of VEE are most effectively controlled by taking action on the primary amplifiers – equids
    - quarantine and movement controls of all Equidae
    - vaccination of equids
    - stabling horses in screened housing; especially during prime daily mosquito activity
    - use of repellents and fans

- Vector control measures; elimination of mosquito breeding locations (i.e. pooled or stagnant water)

Medical prophylaxis

- No specific therapy for viral encephalitides; supportive care
    - administration of fluids to horse unable to drink
    - carefully monitored application anti-inflammatory agents
    - use of anticonvulsants in cases with central nervous system involvement

- The only 2 approved vaccines against VEE are:
  Attenuated virus vaccine (made with strain TC-83)
    - should be reconstituted with physiological saline and used immediately; 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
    - immunogenic when given by intramuscular injection; may cause adverse reactions in recipient

  Inactivated virus vaccine (made with strain TC-83)
    - administered in two doses with an interval of 2–4 weeks between doses
    - annual revaccination is recommended
    - Directions for use provided with commercial products should be followed

- Formalin-inactivated virulent VEE virus preparations should never be used in equids
    - residual virulent virus can remain after formalin treatment, and thereby cause severe illness in both animals and humans.
    - epizootics of VEE have been attributed to the use of such formalin-treated viruses

For more detailed information regarding vaccines, please refer to Chapters 2.5.14 Venezuelan equine encephalomyelitis and 2.5.5 Equine encephalomyelitis (Eastern and Western) in the latest edition of the OIE *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals*: especially under the heading "Requirements for Vaccines".

For more detailed information regarding safe international trade in terrestrial animals and their products, please refer to the latest edition of the OIE *Terrestrial Animal Health Code*.

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The OIE will periodically update the OIE Technical Disease Cards. Please send relevant new references and proposed modifications to the OIE Scientific and Technical Department (scientific.dept@oie.int). Last updated April 2013.