Arboviral encephalomyelitides of livestock in the western hemisphere

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Arbovirus diseases have worldwide impact. The social and economic effects of insect-transmitted pathogens that cause devastating, explosive epizootics can be profound, and such diseases may be expensive to control. Often, these zoonoses are common in tropical and subtropical countries. Many of these countries are those that are least able to afford the economic burden of lost productivity or loss of life, the prohibitive costs of vaccination (if vaccines are available) and vector control, and the drugs needed by the victims of the disease. For all countries, the impact of arbovirus infections on the unrestricted international movement of livestock can be substantial. These restrictions adversely affect imports and exports of animal germplasm and live animals for genetic improvement of livestock breeds or as a source of high quality, inexpensive protein, or affect the movement of livestock for labor, pleasure, and leisure-time activities.

For more than 150 years, the equine encephalomyelitis viruses have caused severe, frequently fatal diseases in equids and human beings in the western hemisphere. The first record of eastern equine encephalomyelitis (EE) in the United States was in 1831 by a physician who described an episode of neurologic disease in horses in Massachusetts. Venezuelan equine encephalomyelitis (VEE) was first described in northern South America in the 1920s, and western equine encephalomyelitis (WEE) was first described in the western United States in 1847. The first isolation of EE virus was made from a horse in Maryland in 1933, of EE virus, from a horse in Venezuela in 1937, and of WEE virus, from a horse in California in 1930. These viruses were determined to be distinct from each other and subsequently were identified as mosquito-transmitted viruses.

Antigenic relationships—Because of their genetic, other molecular, morphologic, and physicochemical characteristics, EEE, VEE, and WEE viruses have been classified taxonomically in the family Togaviridae. They are further classified by their serologic relationships and other similarities into the Alphavirus genus. Within the EE complex, there is 1 virus (EEE), but 2 antigenic variants of it, North American and South American. Isolates of the North American variant of EE virus are antigenically homogeneous, whereas the South American variants are more antigenically heterogeneous.

The VEE complex is comprised of 1 virus, VEE, with 6 antigenically related subtypes (I, VEE; II, Everglades; III, Mucambo; IV, Pixuna; V, Cabassou; and VI, AG80-663); within subtype I are 5 variants. Epizootic VEE in equids is caused by variants A/B (originally identified as distinct variants, A and B are now considered the same variant) and C of subtype I; all other subtypes and variants of VEE virus appear to be nonpathogenic for equids and are found in sylvatic or enzootic, nonequine cycles. The epizootic variants are exotic to the United States and have not been isolated in natural cycles in the world since 1973.

Within the WEE complex are 6 viruses—WEE, Highlands J (HJ), Sindbis, Aura, Fort Morgan, and Y 62-33. Several antigenic subtypes of WEE virus from the central and western United States have been identified, but the geographic distributions of these subtypes overlap.

Geographic distribution and recent epizootic activity—Equine encephalomyelitis viruses have been isolated only in the western hemisphere. The EE virus has been isolated in the Atlantic and Gulf coastal areas, and near rivers and tributaries of the eastern United States and Canada. Virus activity or epizootics of EE have been reported in the provinces of Ontario and Quebec, in virtually all states of the United States east of the Mississippi River, and in Arkansas, Minnesota, South Dakota, and Texas, in many of the Caribbean islands, in Guatemala, Mexico, and Panama, and in Argentina, Brazil, Colombia, Ecuador, Guyana, Peru, Suriname, and Venezuela. Disease activity in equids
is annual in the southeastern United States, especially in Florida.

Epizootic (equine pathogenic) VEE virus variants have caused recurring major epizootics in northern South America from the early years of the twentieth century. Historically, epizootics were reported almost annually in many of the countries of northern South America, including Colombia, Ecuador, Peru, Trinidad, and Venezuela. An epizootic reported in Argentina was ascribed to the use of an incompletely inactivated vaccine. In 1969, the virus was transferred from an epizootic in Ecuador to Central America by an unknown means. During the next 4 years, clinical VEE was reported in equids and human beings in Belize, Costa Rica, El Salvador, Guatemala, Honduras, Mexico, Nicaragua, Peru, and in Texas (1971 only). Epizootic VEE virus was last isolated from equids and mosquitoes during an epizootic in 1973 in Venezuela. During the succeeding years, clinical encephalomyelitis without laboratory confirmation, and antibodies to epizootic VEE subtype-I-ABC viruses have been reported in equids in the United States and Central America. Although it is possible that epizootic VEE virus variants have persisted in certain areas, the absence of overt epizootic activity and of viral isolates suggest that the epizootic virus variants either have not persisted or that the epizootic character or pathogenicity of these viruses has changed. Although there is no evidence to support the possibility, it also is possible that the attenuated VEE vaccine virus strain, which was derived from a subtype-I-A/B isolate, has been introduced into a persisting mosquito cycle with some equine transmission and some reversion to virulence.

Sylvatic or enzootic VEE virus subtypes and variants may be found annually in many tropical and subtropical areas of the western hemisphere, including the United States, Mexico, throughout Central America, Panama, Trinidad, and in every South American country except Bolivia, Chile, Paraguay, and Uruguay. In the United States, sylvatic subtype-II virus, Everglades, has been isolated from mosquitoes and human beings in Florida. A subtype-III virus, Bijou Bridge, has been reported in a bird-swallow bug cycle in some of the Rocky Mountain and Northern Plains states. Although the sylvatic VEE viruses are serologically related to and will cross-protect equids against infection with the epizootic VEE virus variants, there is no known relationship between sylvatic VEE virus foci and development of epizootics.

Virus activity or epizootics of VEE have been reported in the provinces of Alberta, Manitoba, and Saskatchewan in western Canada, in the states west of the Mississippi River, and in Illinois, Indiana, Michigan, and Wisconsin, and in Mexico and South America. Surveys in Central America have not provided convincing evidence of VEE virus activity. Although VEE virus can be isolated from mosqui-

toes annually throughout the western United States, clinical disease in single animals and epizootics are less frequent than epizootics caused by VEE virus.

Ecologic characteristics—In the sylvatic cycle, VEE virus is transmitted among birds by mosquitoes in fresh water swamps frequently found along the coastal areas of the United States. The principal vector is Culiseta (Cs) melanura, a swamp-breeding mosquito species that preferentially feeds on birds. Epizootics in equids, epizootics in pheasants and quail, and cases in human beings originate in the vicinity of these swamps, often after hot, excessively rainy weather. When virus infection rates are high in birds, Aedes vexans and A canadensis mosquitoes are believed to be responsible for bird to mammal transmission. The primary epizootic vector mosquito species responsible for mammal to mammal transmission are believed to be A soricina and Coquillettidia perturbans.

Many species of mosquitoes and other hematophagous insects have been incriminated in the transmission of epizootic VEE virus, and no single genus or species is considered the primary vector. Of the potential vectors, only A taenio-
rhynchus, Psorophora confinnis, and Deinocerites pseudes mosquitoes satisfy all criteria needed to establish proven vector status. Epizootic activity has generally been in tropical and subtropical areas in which there is a definite dry season. Such areas are classified ecologically as tropical dry or tropical thorn forest.

Sylvatic VEE viruses are associated with rodent or bird/mosquito transmission cycles in typical jungle or swampy environments with a high water table or constantly available fresh or brackish water. These areas are tropical wet forest ecologic zones with no obvious dry season. The primary vector mosquitoes are species of the subgenus Culex (Melanoconion).

The natural epizootiologic cycle of VEE virus involves transmission among birds by Culex tarsa-
lis mosquitoes. This species breeds in sunlit grassy marshes, in open ground pools with emerging vegetation, and in pools of stream beds, especially in irrigated areas where seepage and improper flooding of pasture lands induce favorable breeding sites. In the spring in some areas, C tarsalis feed almost exclusively on birds. A midsummer shift in feeding pattern to mammals coincides with the onset of VEE virus infection in equids and human beings.

Clinical disease—It is difficult, if not impossible, to differentiate the diseases caused by the equine encephalomyelitis viruses on the basis of clinical signs in an infected equid. Some equids have subclinical or inapparent infections, whereas others have a mild to severe, and frequently fatal, clinical course of disease. For the first 4 to 5 days, the clinical syndrome is a nonspecific fever without obvious neurologic signs. Fever with leuko-

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nia begins 0.5 to 1.5 days after infection and continues for 3 to 6 days; with EEE virus infection, the fever may be biphasic. Equids may stand quietly with their ears and head drooping and with a somnolent appearance. Inappetence and failure to drink water may be seen; weight loss can be dramatic. Clinical signs referable to the CNS are observed at approximately 5 days after infection, coincident with the first detection of neutralizing antibodies and the termination of detectable viremia. Some equids develop signs of profound depression and stupor, are unwilling to move, have difficulty maintaining balance and stand with limbs wide apart, support their head on fixed objects, and often fall and are unable to regain their footing. Others become excited or stimulated, are hypersensitive to touch and sounds, may be aggressive or hyperactive, walk in circles or into obstacles, stand with heads pressed against obstacles or directed into dark corners, chew aimlessly, or froth from the mouth and nose. Equids may appear blind and have nystagmus. When the equid falls down in terminal convulsions and coma, a characteristic paddling motion of the limbs is commonly seen. Fatality among infected equids that develop signs of encephalomyelitis reaches 40 to 80%. Although naturally occurring cases of encephalitis are diagnosed from WEE virus infection, in the laboratory encephalomyelitis is difficult to reproduce experimentally with WEE virus by peripheral routes of infection. During an epizootic of VEE, large numbers of susceptible equids develop clinical signs in a short period and the wave of disease activity moves quickly over long distances.

Equids infected with EEE virus may have low to moderate virus titers after infection. Although most equids are probably dead-end hosts for EEE virus, some will have virus titers of 10^5 suckling mouse intracranial median lethal doses (SMICLD50)/ml, which probably is sufficient to infect the efficient vector species. With epizootic VEE virus, equids develop high viral titers that can exceed 10^8 SMICLD50/ml. Equids are the most important amplifiers and indicators of epizootic VEE virus activity. Studies have confirmed that VEE epizootics terminate when susceptible equids are no longer available to serve as definitive hosts. The interepizootic reservoir (if a reservoir exists) of epizootic VEE virus variants is unknown. Equids infected with sylvatic VEE viruses may not develop clinical signs of disease and may have virus titers less than the threshold necessary to infect mosquitoes; therefore, equids are considered dead end hosts of sylvatic VEE viruses. Virus titers after WEE virus infection are low or undetectable, and equids are dead end hosts for WEE virus.

**Diagnosis**—Although a presumptive diagnosis of encephalomyelitis is made on the basis of clinical signs of illness, a specific viral cause cannot be determined without laboratory confirmation.

Seasonality of the disease, that is, during periods of vector activity and the presence of large populations of mosquitoes, is consistent with a diagnosis of arboviral encephalomyelitis. Initial signs may go undetected, and reports of sudden death in an apparently healthy equid are not uncommon. The geographic area in which the disease is detected may help provide some indication of the identity of the etiologic agent. The differential diagnosis of equine encephalomyelitis must include EEE, VEE, WEE, and other arboviral encephalitides, African horse sickness, rhabies and other nonarboviral encephalitides, intoxications (especially mycotoxins), botulism, hepatoencephalopathy, trauma, and other causes of encephalitic signs.

A specific diagnostic confirmation can be made only by completing laboratory procedures that include virus isolation and identification, or by detecting a specific increase in antibody titer between paired acute- and convalescent-phase sera.

Virus can be isolated from brain, serum, or plasma in intracranially inoculated suckling mice, weanling mice, guinea pigs, various cell cultures, newly hatched chicks, or embryonating chicken eggs. Although VEE virus isolation from brains had been reported in horses with experimentally induced and naturally acquired VEE, during the 1969 to 1972 epizootic, virus was rarely isolated from brain tissues. Viremia persisting >5 days has not been proven, and latent infections have not been reported. By the time clinical encephalomyelitis is recognized, the viremia generally has ended. Therefore, most investigators recommend that serum for virus isolation be collected from febrile equids that appear otherwise clinically normal and are located in the same or adjacent pastures as encephalomyelitic equids. Virus isolates can be identified by using complement fixation (CF), hemagglutination inhibition (HI), and neutralization tests or antigen capture ELISA. Virus isolates can be identified to subtype and variant by using the short incubation HI test, HI with antiserum produced in rabbits to E1 or E2 envelope glycoproteins, and RNA oligonucleotide fingerprinting.

Antibodies can be measured by HI, CF, and neutralization tests as well as by antibody capture enzyme immunoassay for immunoglobulin M. Interpretation of the importance of antibody or of an increase in antibody titer to VEE virus must be made cautiously and expertly when equids are located in or near an area in which sylvatic VEE subtypes and variants are located. Detection of antibodies to VEE virus in equids located in these areas may not be a sound basis for a diagnosis of VEE, because of serologic cross reactions between epizootic and sylvatic VEE viruses. Immunity after infection or attenuated virus vaccination is long-lasting, if not life-long, and the presence of antibodies in such equids is evidence of solid immunity. Preexisting antibody to 1 equine encephalomyelitis virus (eg, EEE virus) may offer some
cross-protection to equids infected with a second virus (e.g., VEE virus).\textsuperscript{14}

\textit{Prevention and control}—Prophylaxis, rather than treatment, is the key to controlling arboviral equine encephalomyelitis.\textsuperscript{10-13} Safe and effective monovalent or bivalent formalin-inactivated VEE and VEE virus vaccines and a trivalent vaccine with VEE virus are commercially available. The VEE fraction of this vaccine was produced by formalinizing strain TC-83, an attenuated vaccine virus. Manufacturers recommend that initial vaccination include multiple injections, and an annual booster injection is recommended before onset of the vector season.

The VEE strain TC-83 vaccine has provided outstanding protection to equids,\textsuperscript{10,11} but should be used before onset of the vector season. The strain TC-83 vaccine is not recommended for use in pregnant mares, foals may be vaccinated at 3 months of age, but should be revaccinated after 6 months of age. Some vaccinated equids may develop a low-titer viremia with strain TC-83 vaccine virus and a febrile response of 1-2 days duration. Protection against virulent virus infection develops in approximately 4 days; in the face of an epizootic, equine deaths generally cease 9 to 10 days after vaccine is used. Strain TC-83 vaccine is no longer used routinely in the United States because of unsubstantiated concerns about seropositivity to VEE virus of equids offered for export, possible reversion to virulence, and possible vector transmission of vaccine virus. However, in my opinion, this vaccine should be made available and used during a VEE emergency.

Formalin-inactivated VEE virus vaccines derived from equine virulent, epizootic VEE virus should not be manufactured or used for vaccinating equids.\textsuperscript{10,11} Residual virulent virus can remain after formalin treatment and can cause severe illness, high virus titers, and death in equids. Epizootics of VEE have developed from the use of such formalin-treated viruses.\textsuperscript{11}

Although a new generation of vaccines is not currently available, on-going studies by a number of research groups are using modern molecular biologic techniques. The complete nucleotide sequences of the genomes of an equine virulent, epizootic variant and of strain TC-83 vaccine virus have been determined. Nucleotide deletion and substitution are being used to induce infectious clones for vaccinating human beings and equids. Preliminary studies in laboratory animals and equids hold great promise for new, safe and effective vaccines in the near future.\textsuperscript{13,14-17}

Experiences during epizootics and epidemics with the arboviral equine encephalomyelitides and other arbovirus diseases have shown that the best way to control them is to use an integrated disease management approach that includes vector suppression, physical protection of the susceptible host from the vectors, education, and vaccination.\textsuperscript{10-13} The 1969 to 1972 VEE epizootic was not controlled until massive aerial pesticidal spraying with ultra-low volumes of malathion or dibrom was integrated into the campaign.\textsuperscript{18} The use of adulticides, larvicides, and physical disruption of the aquatic larval developmental habitats of mosquitoes may help prevent or control epizootic activity. Equids can be protected by the use of insect repellents or by screening them from the vectors.

Restricted movement or quarantine of equids should be implemented during a VEE epizootic. Protection of febrile, viremic equids from the feeding of mosquitoes during VEE and VEE epizootics will almost certainly help to decrease the numbers of infected mosquitoes biting susceptible equids and human beings.

\textit{Other arboviruses causing encephalomyelitis}—In the eastern United States, an Alphavirus of the VEE virus complex, HJ virus, appears to overlap the geographic distribution of VEE virus and is also transmitted among birds by Culex melanura.\textsuperscript{9,10,19} Although initial reports referred to HJ virus as a variant of VEE virus, it is now recognized as a distinct member of the VEE complex. In Florida, HJ virus has been isolated from a horse with encephalitis.

Two viruses classified as flaviviruses (family Flaviviridae) have been associated with encephalomyelitis in equids.\textsuperscript{2,20,21} St Louis encephalitis (SLE) virus, a human pathogen, is geographically distributed from Canada to Argentina in the western hemisphere. This virus is transmitted among birds by Culex mosquitoes. Experimentally, SLE virus has caused encephalomyelitis in horses. Powassan virus, a human pathogen, is transmitted by Ixodes and Dermacentor tick species among rodents and carnivores in North America and Asia. Experimentally, Powassan virus has caused encephalomyelitis in horses.

Five viruses classified as bunyaviruses (family Bunyaviridae) have been associated with infection or encephalomyelitis in equids.\textsuperscript{22-28} Cache Valley virus is a Culicoides- and mosquito-transmitted virus found in rabbits in North America. Recently, Cache Valley virus has been associated with encephalomyelitis in horses and with congenital arthrogryposis and CNS malformations in lambs infected in utero. A Cache Valley complex virus subtype, Maguari, has been isolated from encephalitic horses in Argentina, Guyana, and Colombia. Lokern virus is a Culicoides variipennis-transmitted virus of large and small mammals in the western United States. This virus has been associated with encephalomyelitis in human beings, but not equids, although Lokern virus has been serologically associated with infections in equids. Main Drain virus is a Culicoides variipennis-transmitted virus of hares and rodents in the western United States. This virus has been isolated from an encephalitic horse in California. Snowshoe hare virus is transmitted
among rabbits by Culicoides and Aedes mosquitoes in the northern United States and southern Canada. This virus has been identified as a cause of encephalomyelitis in human beings and has been serologically associated with encephalomyelitis in horses.

Public health significance—Human beings can become infected by EEE virus, sylvatic and epizootic VEE virus subtypes and variants, and WEE virus. The clinical syndrome can vary from a mild influenza-like illness to a severe encephalitic disease. Deaths have been reported primarily in children and the elderly. Human disease has been reported frequently during equine epizootics, but human infections generally follow equine infections by approximately 2 weeks.

Numerous VEE virus infections have been reported among laboratory workers as a result of aerosol infections from laboratory accidents and from the handling of infected laboratory animals. The strain TC-B3 attenuated VEE virus vaccine initially was developed for use in at-risk laboratory workers. Vaccinated personnel should have demonstrable antibodies to the virus variants or subtypes with which they are working. Although immunity to the more distant related VEE virus subtypes is low, and with time, decreases more rapidly than does immunity to the variants closely related to strain TC-B3, laboratory workers previously vaccinated with strain TC-B3, and in whom immunity has waned, have developed infections. Field veterinarians and others should be cognizant of the health risks from these arboviruses when handling viremic equids and should protect themselves from the bites of hematophagous vectors during epizootics or when working in typical sylvatic virus habitats.


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