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ENCEPHALITIC ALPHAVIRUSES

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SHORT SUMMARY

This review will cover zoonotic, encephalitic alphaviruses in the family *Togaviridae*. Encephalitic alphaviruses, i.e. western- (WEEV), eastern- (EEEV), Venezuelan equine encephalitis virus (VEEV) and, more rarely, Ross River virus, Chikungunya virus and Highlands J virus (HJV), are neuroinvasive and may cause neurological symptoms ranging from mild (e.g., febrile illness) to severe (e.g., encephalitis) in humans and equines. Among the naturally occurring alphaviruses, WEEV, EEEV and VEEV have widespread distributions in North, Central and South America. WEEV has found spanning the U.S. from the mid-West (Michigan and Illinois) to the West coast and extending to Canada with human cases reported in 21 states. EEEV is found along the Gulf (Texas to Florida) and Atlantic Coast (Georgia to New Hampshire), as well as in the mid-West (Wisconsin, Illinois and Michigan) and in Canada, with human cases reported in 19 states. In contrast, transmission of VEEV occurs predominantly in Central and South America. As with their geographical distribution, equine encephalitis viruses differ in their main mosquito vector species and their zoonotic potential.

INTRODUCTION

In the 1930s, previously unrecognized viruses were isolated from diseased horses in California, in Virginia and New Jersey, and from an infected child in Caracas, Venezuela. These subsequently were named Western equine encephalomyelitis virus (WEEV), Eastern equine encephalomyelitis virus (EEEV) and Venezuelan equine encephalomyelitis virus (VEEV), respectively. Since then, these viruses have been isolated from infected mosquitoes, horses, humans, and other vertebrate species, predominantly birds and rodents. These viruses are transmitted naturally by hematophagous arthropods (Smith et al., 1997; Strauss et al., 1995). The respective history of the isolation of the infectious agent and description of the disease development in man and animals will be presented by virus in each section below. Table 1 presents a summary of the WEEV, EEEV, and VEEV distribution, epidemiological features and clinical outcomes in humans and horses.

WESTERN EQUINE ENCEPHALITIS VIRUS (WEEV)

In 1930, WEEV was first isolated from the brain of an encephalitic horse in California (Meyer et al., 1931). WEEV is maintained in an enzootic cycle between its natural vertebrate hosts, passerine birds, and its most common mosquito vector, *Culex tarsalis*, a species associated

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RONFLICT OF INTEREST STATEMENT
None.

with irrigated agriculture and stream drainages in the western U.S. Transmission to horses and humans is mediated by so-called bridging mosquito vector species including *Ochlerotatus melanimon* in California, *Aedes (Ae.) dorsalis* in Utah and New Mexico and *Ae. campestris* in New Mexico. Depending on the climate, the natural transmission cycle may be maintained throughout the year. In more moderate climate areas, WEEV may overwinter in yet unidentified hosts, or may be reintroduced annually by migratory birds. Interestingly, except for the Veracruz region of Mexico, WEEV has not been found in Central America.

Genetic analyses of WEEV isolates from South America (Brazil and northern Argentina) suggest that WEEV has a monophyletic lineage with nucleotide identity >90% in the E2/6K/E1 coding region when compared to isolates from California, Texas and as far north as Montana. Detailed phylogenetic analysis indicates that WEEV viruses are recombinants of EEEV-like (5' two-thirds of the genome) and Sindbis virus-like (3'one-third of the genome) parental viruses (Hahn et al., 1988; Weaver et al., 1997).

DISEASE IN ANIMALS AND MAN

According to the U.S. Centers for Disease Control and Prevention, 639 confirmed human cases of WEE occurred in the U.S. from 1964 to 2005. A decline in the annual number of cases to fewer than 10 cases per year since 1988 has been attributed to changes in irrigation practices and to successful mosquito control programs in the western U.S. WEEV infections tend to be asymptomatic or cause mild disease after a short incubation period of 2–7 days with non-specific symptoms, e.g., sudden onset of fever, headache, nausea, vomiting, anorexia and malaise. In some cases, additional symptoms of altered mental status, weakness and signs of meningeal irritation occur. In a minority of infected individuals, encephalitis or encephalomyelitis occurs and may lead to neck stiffness, confusion, tonic-clonic seizures, somnolence, coma and death. The overall case-fatality rate in humans is estimated to be in the range of 3–7%. However, the ratio of inapparent to apparent infections changes with age: 1:1 in children <1 year old; 58:1 for children between age 1 and 4; and >1,000:1 in children >14 years old. Fifteen to 30% of the survivors of encephalitis are estimated to experience severe neurological sequelae, especially younger children (<1 year old). Encephalitis in humans due to WEEV is characterized by a vasculitis and focal hemorrhages in the basal ganglia and the nucleus of the thalamus. Small hemorrhages that are sometimes observed in the white and gray matter may be mistaken for resolved infarcts in elderly patients (Reeves et al., 1958).

DIAGNOSIS AND TREATMENT

In WEEV-exposed horses and humans, WEEV-specific IgM can usually be detected at the time of disease onset via IgM antibody-capture enzyme-linked immunosorbent assay (MAC-ELISA) or a few days after onset via hemagglutination-inhibition and neutralization assay (Martin et al., 2000). Infection with WEEV can also be diagnosed as a four-fold or greater rise in IgG antibody to WEEV comparing paired acute- and convalescent-phase samples taken at least ten days apart. A neutralization assay may be required to exclude cross-reaction against VEEV, EEEV, HJV or other alphaviruses depending upon the geographical origin or travel history. Virus can be isolated directly in a variety of vertebrate and mosquito cell lines; however, Vero cells (African Green Monkey kidney cells) are preferentially used. Amplification of virus-specific RNA can also be performed using reverse transcription-polymerase chain reaction (RT-PCR) and real-time RT-PCR (Lambert et al., 2003; Linssen et al., 2000). Finally, indirect peroxidase assay can be used for immunohistochemical detection of viral antigen in brain tissues from dead horses using polyclonal antiserum. Among potential clinical indicators of disease, cerebrospinal fluid (CSF) show elevated levels of protein (90–110 mg/dl) and leucocytosis (up to 500/cm^3, mainly lymphocytes).

The WEEV mortality rate in horses is higher (up to 50%) than for humans (Table 1). Horses are vaccinated with formalin-inactivated virus that is available as a double vaccine in combination with EEEV. Due to the poor immunogenicity of the inactivated vaccine, two vaccinations per year are necessary to maintain protection. The development of neutralizing antibodies correlates with protection and can be used to monitor the success of immunization.

EASTERN EQUINE ENCEPHALITIS VIRUS

EEEV was first isolated in 1933 from infected horses in Virginia and New Jersey (Giltner and Shahan, 1933; TenBroeck and Merrill, 1933). The primary EEEV transmission cycle occurs between birds and mosquitoes (*Culiseta melanura*). However, the principal arthropod vector for transmission of EEEV to humans or to horses are; *Aedes*, *Coquillettidia*, and *Culex* species which, unlike *Culiseta melanura*, tend to feed on both birds and mammals. Virus transmission occurs most commonly in and around freshwater hardwood swamps in the Atlantic and Gulf Coast states and in the Great Lakes region. Most cases of EEE have been reported from Florida, Georgia, Massachusetts, and New Jersey. In horses, some bird species, and in dogs, EEEV can cause severe disease; however, it is believed that horses do not serve as amplifying hosts during epidemics. Nevertheless, horses tend to be the first to develop overt disease in a natural focus and thus often serve as an indicator of a starting epidemic situation.

DISEASE IN ANIMALS AND MAN

According to the U.S. Centers for Disease Control and Prevention, 220 confirmed human cases of EEE have occurred in the U.S. from 1964 to 2004. Although generally more prevalent in the southeastern U.S., recently horse deaths have been reported from further north on the eastern U.S. coast, e.g., New Hampshire, Maine, and in Canada. This virus is probably the most virulent of the encephalitic alphaviruses, with a case-fatality rate in humans estimated in the range of 50–70%. After a 4–10 day incubation period, symptoms begin with sudden onset of fever, general muscle pains, and headache of increasing severity. In human cases of encephalitis, fever, headache, vomiting, respiratory symptoms, leucocytosis, hematuria, seizures and coma may occur. Clinical studies of serologically confirmed and human EEEV infections using magnetic resonance imaging and computed tomography have shown changes in the basal ganglia and thalami, suggesting brain edema, ischemia and hypoperfusion are present in the early stage of disease. Brain edema with necrosis, facial or generalized edema, vascular congestion and hemorrhage in the brain and visceral organs are seen in gross pathological examination of fatal human cases; histopathological examination reveals vasculitis, hemorrhage and encephalitis (Deresiewicz et al., 1997).

Studies of the pathogenesis of EEEV have been performed using experimental models, e.g., mice, hamsters, guinea pigs and rhesus monkeys, and using histopathological studies of equine and porcine cases. The hamster model of EEE was recently described for its advantage in reproducing the vascular component of human disease that is lacking in the murine model. Neuroinvasion and encephalitis development is rapid in both the murine and hamster models with infection of periventricular and perivascular neuronal cells in the basal ganglia and hippocampus. In contrast to VEEV (see below), EEEV appears to rapidly invade the brain of infected animals via blood, and the first antigen-positive neuronal cells are located in the basal ganglia and brain stem in the hamster model. The inflammatory response in the brain is prominent in cases in which animals had survived for at least five days, and is produced by macrophages, lymphocytes and neutrophils (Charles et al., 1995).

DIAGNOSIS AND TREATMENT

EEEV infection is diagnosed using serological assays, especially by detection of IgM in serum and CSF in the presence of CNS or febrile disease, and neutralizing antibody testing of acute-

and convalescent-phase samples. Virus isolation and detection of viral nucleic acid is possible, as described above for WEEV.

The EEEV mortality rate in horses is higher than for WEEV, but is relatively similar in humans and horses (Table 1). Horses are vaccinated with formalin-inactivated virus that is available as a double vaccine in combination with WEEV (described in EEEV section).

VENEZUELAN EQUINE ENCEPHALITIS VIRUS

VEEV was first isolated in 1938 from the brain of an infected animal in Venezuela (Beck and Wyckoff, 1938). Like WEEV and EEEV, VEEV is a zoonotic pathogen, transmitted between vector mosquitoes and vertebrate hosts, namely rodents and humans in enzootic cycles, and horses and humans in epidemic or epizootic cycles. Although essentially any mosquito can be found infected with VEEV during epizootics, *Ochlerotatus taeniorhynchus* is believed to be the principal vector responsible for transmission of VEEV during outbreaks, whereas *Culex (Melanoconion*) species mosquitoes transmit enzootic strains of VEEV.

VEEV viruses are highly infectious via the aerosol route. Thus, VEEV has been responsible for numerous laboratory accidents (>150 cases without an associated perforating injury), and has been developed as a biological weapon in the U.S and in the former Soviet Union. VEEV infection also has been associated with abortion and fetal death in humans. Upon infection with epizootic viruses equids develop high titer viremias, which serve as sources of infection for subsequently feeding mosquitoes. In recent years, spillover to humans during equine epizootics has resulted in epidemics of VEEV (Weaver, 1998).

DISEASE IN ANIMALS AND MAN

The VEEV antigenic complex, of which VEE virus is the prototype member, is divided into six distinct antigenic subtypes (I–VI). The major human epidemics and equine epizootics have been associated almost exclusively with subtypes IAB and IC. Enzootic transmission is generally associated with subtypes ID and IE, which are less virulent for horses. In contrast, epizootic subtypes IAB and IC are highly pathogenic for horses, with case-fatality rates between 20–80% reported. The most recent major outbreak occurred in 1995 in Venezuela and Colombia in which 75,000–100,000 human cases occurred, and more than 300 fatal encephalitis cases were recorded. In 1993, equine disease was associated with VEEV-IE in Mexico and in the period between 1993 and 1995, human cases of VEEV ID-associated disease occurred in Peru (Weaver et al., 2004).

Generally, severe encephalitis in humans infected with VEEV is less common than with EEEV and WEEV infections. In adults, VEEV infection usually results in flu-like symptoms and encephalitis is rare. In humans, while the case-fatality rate is low (≤1%), neurological disease, including disorientation, ataxia, mental depression, and convulsions can be detected in up to 14% of infected individuals, especially children. Neurological sequelae in humans are also common. The predominant pathological findings in fatal human VEE cases include: 1) in the CNS: edema, congestion, hemorrhages, vasculitis, meningitis and encephalitis; 2) in the lungs: interstitial pneumonia, alveolar hemorrhage, congestion and edema; 3) in lymphoid tissue: follicular necrosis and lymphocyte depletion; and 4) in the liver: diffuse hepatocellular degeneration (Johnson and Martin, 1974).

A murine model that mimics human and equine disease, e.g., encephalitis and lymphotropism is characterized by biphasic, lethal disease that starts with productive infection of lymphoid tissue and ends in the destruction of the CNS. At the later phase of encephalitis development, infectious virus is low to undetectable in peripheral organs and blood, but high virus levels are found in the brain and death occurs 5–7 days after infection. Recent studies on genetically

modified mice with pre-existing anti-VEEV immunity have shown that a high level of replication in the brain over a period of 28 days is not lethal indicating that the virus infection alone is not sufficient to cause death (Paessler et al., 2006; Paessler et al., 2007).

The brain has been proposed to be a distinct immune regulatory tissue, given its status as an immune privileged site and that T-lymphocyte entry into the CNS under normal homeostatic conditions is thought to be restricted to memory T-cells. Once, the blood-brain barrier is breached by the virus, local antibody production by B cells in the brain plays a role in prevention of viral entry into cells of the CNS and facilitates virus clearance via Fc receptors. Murine studies of the immune response to VEEV indicates that T-cells are critical to the host defense against alphavirus infection, survival, encephalitis, and also in repair of neural damage and homeostasis in the brain. In particular, protection from lethal encephalitis appears to be dependent on the presence of alpha-beta T-cell receptor (TCR)-bearing cells but not on gamma-delta TCR-bearing cells. The relative degree of immune-mediated inflammation in various TCR-deficient mice does not fully correlate with viral clearance. Remarkably, vaccinated gamma-delta TCR-deficient mice are protected from lethal viral challenge by intranasal inoculation but VEEV can persist to 28 days post inoculation. Virus clearance is not affected in vaccinated, immunocompetent mice or in surviving animals lacking functional IFN-gamma receptor. The viral replication pattern of gamma-delta T-cell deficient mice resembles that of unvaccinated, immunocompetent mice; however, the latter “wild type” mice become paralyzed and succumb to infection, whereas gamma-delta T-cell deficient mice are asymptomatic and survive to 28 days. Virus can persist in the brain despite a moderate level of inflammation and cellular infiltration at the site of infection (Paessler et al., 2006; Paessler et al., 2007)

DIAGNOSIS AND TREATMENT

Currently, VEEV infection is diagnosed principally by direct detection, e.g., nucleic acid or virus isolation from acute-phase serum or spinal fluid or by serological assay, e.g., detection of VEEV-specific IgM in the CSF using MAC-ELISA or monoclonal antibody-based antigen-capture ELISA (Calisher et al., 1986; CDC, ; Deresiewicz et al., 1997; Martin et al., 2000; Sahu et al., 1994). The plaque reduction neutralization test (PRNT), which like MAC-ELISA is useful in distinguishing VEEV infections from infections with other alphaviruses (see above), cannot be used to identify the serotype. Recently, a VEEV-specific blocking ELISA was described that also identifies serotype-specific antibodies against VEEV in sera of humans, equids or rodents (Wang et al., 2005).

No effective antiviral treatment exists for any of these encephalitic arboviruses, so that treatment remains supportive, as indicated for EEE and WEE.

CONCLUSIONS AND FUTURE PROSPECTS

Vaccines to limit infection and/or fatal encephalitis are currently lacking, however several are under development for VEEV, WEEV and EEEV using a variety of approaches (Barabe et al., 2007; Charles et al., 1997; Davis et al., 1995; Fine et al., 2008; Paessler et al., 2006; Phillpotts et al., 2005; Pratt et al., 2003; Schoepp et al., 2002; Wang et al., 2007; Wu et al., 2007a). The live attenuated vaccine strain, TC83 was developed over four decades ago by serial passaging of the Trinidad Donkey VEEV strain in guinea pig myocytes and remains the only available vaccine for humans (United States Food and Drug Administration *Investigational New Drug* status). However, this strain has residual pathogenicity in humans, which corresponds to that seen in the animal model (Anishchenko et al., 2006; Jahrling and Scherer, 1973; Paessler et al., 2006). Recent new generation live-attenuated vaccines that incorporate the use of alphavirus vectors expressing VEEV and EEEV proteins are highly immunogenic and have been shown to be both safe and effective in protecting mice and/or hamsters from lethal disease

(Ni et al., 2007; Paessler et al., 2003; Paessler et al., 2006; Paessler et al., 2007; Wang et al., 2007) and may also be useful for protection of humans and equines.

Antiviral drug development is another area in need of a breakthrough. A better understanding of the pathogenesis of VEE, WEE and EEE and, in particular, features of the host immune response and tissue (CNS)-specific responses that may actually contribute to fatal outcomes, as opposed to recovery, following the development of encephalitis could be useful to the development of antiviral strategies. Some examples are: delivery of cytokines or cytokine blocking agents (Julander et al., 2007; Lukaszewski and Brooks, 2000; Phillpotts et al., 2003; Wu et al., 2007b), treatment with agents that block specific stages of the virus life cycle (Julander et al., 2008), and antibody-based therapeutics.

Numerous studies of human infections demonstrate that VEEV elicits an antibody response, although it has not clear if the antibody response alone would protect individuals from the development of encephalitis and consequent death. Pathogenesis studies using the model alphavirus Sindbis virus (Byrnes et al., 2000; Griffin et al., 1997) as well as treatment and related studies with VEEV specific antibody (VEEV ab) suggest that VEEV ab therapy may be useful (Hunt and Roehrig, 1995). Recent studies indicate that high titer VEEV ab is partially protective against lethal intranasal VEEV infection (Paessler, unpublished data). However, the limited ability of these mice to survive when treated at a lower VEEV ab dose and the ability of some mice with a deficiency in mature B cells to survive VEEV infection (Paessler et al., 2007) indicates that additional studies are needed to strengthen the rationale for this approach.

Another promising approach is the use of antisense technology, recently shown to be effective against VEEV in the animal model (O'Brien, 2006; Paessler et al., 2008).

These approaches may also be applicable to other neurovirulent viruses for which little or no treatment or vaccination options are available.

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Table 1

Overview epidemiology and clinical aspects

	WEEV	EEEV	VEEV (epizootic)	VEEV (enzootic)
Distribution	Western U.S.; South America	Eastern and Northern U.S.; South America	South and Central America	Southern U.S. (Florida); South and Central America
Transmission-cycle	Birds-*Culex tarsalis*	Birds-*Culiseta melanura*	Unknown	Rodents-*Culex species* (sp.)
Vector for horses and humans	*Culex tarsalis*	*Aedes* and *Coquillettidia* sp.	Various mosquito genera	*Culex* sp.
Equine amplifiers	No	1 out of 20	Yes	Unknown
Human mortality	3–7%	50–75%	1%	Unknown
Horse mortality	3–50%	70–90%	20–80%	Mostly none
Average number of sick horses per year	0–5	120	During epidemics: thousands	Unknown