Chapter 28

VIRAL ENCEPHALITIDES

JONATHAN F. SMITH, PH.D.[*]; KELLY DAVIS, D.V.M.[†]; MARY KATE HART, PH.D.[‡]; GEORGE V. LUDWIG, PH.D.[§]; DAVID J. MCCLAIN, M.D.[¥]; MICHAEL D. PARKER, PH.D.[¶]; AND WILLIAM D. PRATT, D.V.M., PH.D.[**]

[*]*Chief, Department of Viral Biology, Virology Division*
[†]*Lieutenant Colonel, Veterinary Corps, U.S. Army; Chief, Experimental Pathogenesis, Pathology Division*
[‡]*Department of Viral Pathogenesis and Immunology, Virology Division*
[§]*Chief, Department of Cell Culture/Hybridoma, Virology Division*
[¥]*Department of Viral Biology, Virology Division*
[¶]*Department of Viral Biology, Virology Division*
[**]*Lieutenant Colonel, Veterinary Corps, U.S. Army; Chief, Virology Division, U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, Maryland 21702-5011*

INTRODUCTION

During the 1930s, three distinct but antigenically related viruses were recovered from moribund horses and were shown to be previously unrecognized agents of severe equine encephalitis. Western equine encephalitis (WEE) virus was isolated in the San Joaquin Valley in California in 1930,[1] eastern equine encephalitis (EEE) virus in Virginia and New Jersey in 1933,[2,3] and Venezuelan equine encephalitis (VEE) virus in the Guajira peninsula of Venezuela in 1938.[4] By 1938, it was clear that EEE and WEE viruses were also natural causes of encephalitis in humans,[5–7] and naturally acquired human infections with VEE virus were documented in Colombia in 1952 in association with an equine epizootic.[8] Although these viruses cause similar clinical syndromes in horses, the consequences of the infections they cause in humans differ. EEE is the most severe of the arboviral encephalitides, with case fatality rates of 50% to 70% and with neurological sequelae common in survivors. WEE virus appears to be less neuroinvasive but has a pathology similar to that of EEE in patients with encephalitis. In contrast, severe encephalitis is rare in humans infected with VEE virus—except in children. In adults, the usual VEE syndrome is an acute, febrile, incapacitating disease with prolonged convalescence.

The three viruses under discussion in this chapter are all members of the *Alphavirus* genus of the family *Togaviridae*. As with all the alphavirus group, VEE, EEE, and WEE are transmitted in nature by mosquitoes and are maintained in cycles with various vertebrate hosts. Thus, the natural epidemiology of these viruses is controlled by environmental factors that affect the relevant mosquito and reservoir host populations and their interactions. Of the 28 viruses currently classified within this group, VEE, EEE, and WEE are the only viruses regularly associated with encephalitis. Although these encephalitic strains are restricted to the Americas, as a group, alphaviruses have worldwide distribution and include other epidemic human pathogens such as chikungunya virus (Asia and Africa), Mayaro virus (South America), O'nyong-nyong virus (Africa), Ross River virus (Australia), and Sindbis virus (Africa, Europe, and Asia). These viruses cause an acute febrile syndrome often associated with debilitating polyarthritic syndromes.

Although natural infections with the encephalitic alphaviruses are acquired by mosquito bite, the viruses are also highly infectious by aerosol. VEE virus has caused more laboratory-acquired disease than any other arbovirus. Since its initial isolation, at least 150 laboratory infections that have resulted in disease have been reported; most were known or thought to be aerosol infections.[9] Before vaccines were developed, most laboratories working with VEE virus reported disease among their personnel. In one incident at the Ivanovskii Institute in Moscow, USSR, which was reported in 1959, at least 20 individuals developed disease, most within 28 to 33 hours, after an accident in which a small number of vials containing a minute amount of lyophilized virus were dropped and broken in a stairwell.[10,11]

Perhaps as a consequence of their adaptation to dissimilar hosts in nature, the alphaviruses replicate readily, and generally to very high titers, in a wide range of cell types and culture conditions in vitro. Virus titers of 1 billion infectious units per milliliter are not unusual, and the viruses are stable in storage and in a variety of laboratory procedures. Because of the relative ease with which these viruses can be manipulated in the laboratory, they have long served as model systems by which to study various aspects of virus replication, pathogenesis, induction of immune responses, and virus–vector relationships. As a result, the alphaviruses are well described and their characteristics well defined.[12,13]

Therefore, the collective in vitro and in vivo characteristics of alphaviruses, especially the equine encephalomyelitis viruses, lend themselves very well to weaponization. This fact was recognized by the designers of offensive biological warfare programs that were initiated before or during World War II.[14] Although other encephalitic viruses could be considered as potential weapons (eg, the tick-borne encephalitis viruses), few possess as many of the required characteristics for strategic or tactical weapons development as the alphaviruses:

- These viruses can be produced in large amounts in inexpensive and unsophisticated systems.
- They are relatively stable and highly infectious for humans as aerosols.
- Strains are available that produce either incapacitating or lethal infections.
- The existence of multiple serotypes of VEE and EEE viruses, as well as the inherent

difficulties of inducing efficient mucosal immunity, confound defensive vaccine development.

The equine encephalomyelitis viruses remain as highly credible threats today, and intentional release as a small-particle aerosol, from a single airplane, could be expected to infect a high percentage of individuals within an area of at least 10,000 km^2. As a further complication, these viruses are readily amenable to genetic manipulation by modern recombinant deoxyribonucleic acid (DNA) technology. This capability is being used to develop safer and more effective vaccines,[15,16] but, in theory, could also be used to increase the weaponization potential of these viruses.

HISTORY AND SIGNIFICANCE

Descriptions of epizootics in horses, characterized by encephalitis and death and likely to have been caused by EEE virus, have been recorded as early as 1831 in Massachusetts.[17] However, it was not until the outbreaks of EEE in Delaware, Maryland, and Virginia in 1933 and 1934 that the virus was isolated, and not until a similar outbreak in North Carolina in 1935 that birds were suspected as the natural reservoir.[18] The initial isolation of EEE virus from a bird[19] and from *Culiseta melanura* mosquitoes,[20] the two major components of the EEE natural cycle, were both reported in 1951. Outbreaks of EEE virus have occurred in most eastern states and in southeastern Canada but have been concentrated along the eastern and Gulf coasts. Although only 211 cases of EEE in humans were reported[21] between 1938 (the first documented human cases[5]) and 1985, the social and economic impact of this disease has been larger than might be expected because of the high fatality rate, equine losses, extreme concern among individuals living in endemic areas during outbreaks, and the surveillance and mosquito-control measures required. Isolation of EEE virus from *Aedes albopictus* mosquitoes, which have recently been introduced into EEE endemic areas in the United States, has heightened concern because of the opportunistic feeding behavior of these mosquitoes as well as their apparent high vector competence for EEE virus.[22]

The initial isolation in 1930 of WEE virus from the brain tissues of a horse with encephalitis was made in the midst of a large and apparently unprecedented epizootic in California, which involved at least 6,000 horses and with approximate mortality of 50%.[1] Cases of human encephalitis in California were not linked to WEE until 1938, when the virus was isolated from the brain of a child. During the 1930s and 1940s, several other very extensive epizootics occurred in western and north-central states, as well as Saskatchewan and Manitoba, Canada, which affected large numbers of equines and humans. For example, it has been estimated that during 1937 and 1938, more than 300,000 equines were infected in the United States, and in Saskatchewan, 52,500 horse infections resulted in 15,000 deaths.[23,24] In 1941, unusually high numbers of human cases were reported: 1,094 in Canada and 2,242 in the United States. The attack rate in these epidemics ranged from 22.9 to 171.5 per 100,000, with case fatality rates of 8% to 15%.[24]

In the early 1940s, workers isolated WEE virus from *Culex tarsalis* mosquitoes[25] and demonstrated the presence of specific antibody to WEE virus in birds,[26] suggesting that birds are the reservoirs of the virus in nature. The annual incidence of disease in both equines and humans continues to vary widely, as would be expected of an arthropod-borne disease, and significant epidemics occurred in 1952, 1958, 1965, and 1975.[24]

The initial isolation of VEE virus was made during investigations of an epizootic occurring in horses in Venezuela in 1936, and the isolate was shown to be antigenically different from the EEE and WEE viruses isolated previously in the United States.[4,27] Over the next 30 years, many VEE outbreaks were reported among horses, and it soon became apparent that humans were also infected in large numbers in association with these epizootics.[28] Most of those infected recovered after suffering an acute, febrile episode, but severe disease with encephalitis and death also occurred, mostly in children and older individuals. In the 1960s, major epizootics occurred in Venezuela, Colombia, Peru, and Ecuador, and apparently spread to Central America in 1969.[29] These and previous epizootics were associated with immeasurable human suffering, especially among rural people, who suffered not only from disease but also from the loss of their equines, which were essential for transportation and agriculture. Between 1969 and 1971, epizootics were reported in essentially all of Central America and subsequently continued north to Mexico and into Texas. The most recent major epizootic occurred in Venezuela and Colombia in 1995.[30]

Between active epizootics it was not possible to isolate the equine virulent viruses. During the 1950s and 1960s, however, several other antigenically different VEE strains were isolated from different geographical areas; these were attenuated in equines and persisted indefinitely in endemic areas. These enzootic strains could be differentiated antigenically not only among themselves but also from the epizootic strains,[31] they utilized different mosquito vectors than the epizootic strains,[32] and they utilized rodents as reservoir hosts.[33] Many of the enzootic strains, however, proved equally pathogenic for humans.

Therefore, within 30 years of the initial isolation of the EEE, WEE, and VEE viruses, an essentially accurate picture had emerged with respect to their endemic and epidemic behavior, arthropod vectors, reservoir hosts, and the diseases produced. Although not yet understood at the molecular level (this would come with the techniques of molecular biology that were developed during the 1970s and 1980s), these three viruses were reasonably well described as agents of disease, and the basic assays for their manipulation and production were known. The development of this body of knowledge occurred during the same period of war and political instability that fostered the establishment of biological warfare programs in the United States[34] and elsewhere, and it was evident that the equine encephalomyelitis viruses were preeminent candidates for weaponization. The viruses were incorporated into these programs for both potential offensive and defensive reasons. In 1969, the offensive biological warfare program in the United States was completely disestablished and all stockpiles destroyed[14] by executive order, which stated:

> The United States shall renounce the use of lethal biological agents and weapons and all other methods of biological warfare. The United States shall confine its biological research to defensive measures such as immunization and safety measures.[35]

However, defensive concerns remained, and efforts within the defensive program in the 1960s and 1970s produced four vaccines for the encephalomyelitis viruses: live-attenuated (TC-83) and formalin-inactivated (C84) vaccines for VEE, and formalin-inactivated vaccines for EEE and WEE. These vaccines are used under Investigational New Drug (IND) status for at-risk individuals, are distributed freely under the provisions of the IND, and are recommended for use by any laboratory working with these viruses.[9] Although these vaccines have proven quite useful, they have certain disadvantages (which are discussed later in this chapter), and second-generation vaccines are under development.[16]

ANTIGENICITY AND EPIDEMIOLOGY

Antigenic Relationships

The American equine encephalitides comprise three virus complexes, VEE, EEE, and WEE, which, based on their serologic cross-reactivity, have been grouped with four additional virus complexes into the *Alphavirus* genus (Table 28-1).[13]

Venezuelan Equine Encephalitis Virus Complex

The VEE virus complex consists of six closely related subtypes that manifest different characteristics with respect to ecology, epidemiology, and virulence for humans and equines (Table 28-2). The IA/B and C varieties are commonly referred to as *epizootic* strains. These strains have been responsible for extensive epidemics in North, Central, and South America and are highly pathogenic for humans and equines. All of the epizootic strains are exotic to the United States and have been isolated from natural foci in the world only once since 1973.[36] Subtypes II, III, IV, V, and VI and varieties ID, IE, and IF are referred to as the *enzootic* strains.[37–42]

Like the epizootic strains, the enzootic strains may cause disease in humans, but they differ from the epizootic strains in their lack of virulence for equines. The enzootic viruses are commonly isolated in specific ecological habitats, where they circulate in transmission cycles primarily involving rodents and *Culex* mosquitoes of the *Melanoconion* subgenus.[43–45] Infection of equines with some enzootic subtypes leads to an immune response capable of protecting the animals from challenge with epizootic strains.[46] Limited data, acquired following laboratory exposures, suggest that cross-protection between epizootic and enzootic strains may be much less pronounced in humans.[47–49]

Eastern Equine Encephalitis Virus Complex

The EEE virus complex consists of viruses in essentially two antigenically distinct forms: the North American and the South American variants.[50] The

TABLE 28-1

ANTIGENIC CLASSIFICATION OF ALPHAVIRUSES

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TABLE 28-2

THE VENEZUELAN EQUINE ENCEPHALOMYELITIS COMPLEX

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Sources that contain original descriptions of or additional information about this strain: (1) Young NA, Johnson KM. Antigenic variants of Venezuelan equine encephalitis virus: Their geographic distribution and epidemiologic significance. *Am J Epidemiol*. 1969;89:286. (2) Walton TE. Virulence properties of Venezuelan equine encephalitis virus serotypes in horses. In: Venezuelan Encephalitis: Proceedings of the Workshop-Symposium on Venezuelan Encephalitis Virus, Washington, DC, 14–17 Sep 1971. Washington, DC: Pan American Health Organization; 1972:134. PAHO Scientific Publication 243. (3) Johnson KM, Shelokov A, Peralta PH, Dammin GJ, Young NA. Recovery of Venezuelan equine encephalomyelitis virus in Panama: A fatal case in man. *Am J Trop Med Hyg*. 1968;17:432–440. (4) Walton TE, Grayson MA. Venezuelan equine encephalitis. In: Monath TP, ed. *The Arboviruses: Epidemiology and Ecology*. Vol 4. Boca Raton, Fla: CRC Press; 1988: 203–231. (5) Chamberlain RW, Sudia WD, Coleman PH, Work TH. Venezuelan equine encephalitis virus from South Florida. *Science*. 1964;145:272. (6) Shope RE, Causey OR, de Andrade AHP, Theiler

two forms can be distinguished readily by hemagglutination-inhibition (HI) tests. All North American and Caribbean isolates show a high degree of genetic and antigenic homogeneity; they are distinct from the South American and Central American isolates, which tend to be more heterogeneous.[51,52]

EEE is endemic to focal habitats ranging from southern Canada to northern South America. The virus has been isolated as far west as Michigan but is most common along the eastern coast of the United States between New England and Florida. Enzootic transmission of EEE virus occurs almost exclusively between passerine birds (ie, the perching songbirds) and the mosquito *Culiseta melanura*. Because of the strict ornithophilic feeding behavior of this mosquito, human and equine disease requires the involvement of more general feeders, known as bridging vectors, such as members of the genera *Aedes* and *Coquilletdia*. Mosquito vectors belonging to *Culex* species are thought to play a role in maintaining and transmitting South American EEE strains.[53]

Western Equine Encephalitis Virus Complex

Six viruses—WEE, Sindbis, Y 62-63, Aura, Fort Morgan, and Highlands J—make up the WEE com-

plex. Several antigenic subtypes of WEE virus have been identified, but their geographical distributions overlap.[36] Most of the members of the WEE complex are distributed throughout the Americas, but subtypes of Sindbis virus and Y 62-33 have strictly Old World distributions.[13] The New World WEE complex viruses can be distinguished readily by neutralization tests. In addition, WEE complex viruses isolated in the western United States (ie, WEE) are antigenically and genetically distinct from those commonly found in the eastern United States (ie, Highlands J).[52,54]

The best studied member of the WEE virus complex—in terms of its epidemiology—is WEE virus itself. The virus is maintained in cycles involving passerine birds and the mosquito *Culex tarsalis*. Humans (and equines) become involved only tangentially and are considered to be dead-end hosts,[55] indicating that they do not normally contribute to further spread of the virus in nature. Recent studies have isolated WEE virus from male *Aedes dorsalis* mosquitoes reared in the laboratory from larvae collected in salt marsh habitats.[56] These data indicate that vertical transmission (ie, direct transmission from one generation to the next) in mosquitoes may be an important mechanism for persistence and overwintering in endemic areas.

Epidemiology

The epidemiology of the equine encephalitides in humans is closely tied to the ecology of these viruses in naturally occurring endemic foci. Most commonly, human involvement occurs following intrusion into geographical regions where natural transmission cycles are in progress, or following perturbation of those cycles by environmental changes[57] or the addition of other vectors.[22] The dramatic exception to this is epizootic VEE, in which the spreading waves of the epizootic among equines can move rapidly over large distances, and humans become infected by mosquitoes that have fed on viremic equines. The high levels of viremia in equines infected with epizootic VEE make them efficient amplifying hosts, with the result that equine infections normally precede human infections by days to weeks.[58] Medical officers should view with some suspicion evidence of widespread human VEE infections outside of endemic areas, in the absence of mosquito vectors, or in the absence of equine disease, as this combination of circumstances could indicate an unnatural release of virus into the environment.

Enzootic VEE virus subtypes, as described above, are maintained quite efficiently in transmission cycles involving mainly mosquitoes belonging to the subgenus *Melanoconion*. These mosquitoes often occur in very humid localities with abundant open spaces—such as sunny, swampy pastures cut by slowly flowing streams. The mosquitoes are ground feeders, seldom found higher than 8 m above ground, and prefer feeding on mammals rather than birds.[59] In part because their ecologies are similar to that of the mosquito vector's, ground-dwelling rodents serve as the primary vertebrate hosts for the enzootic forms of VEE virus. Following infection, these animals develop viremias of sufficient magnitude and duration to infect mosquitoes during their acquisition of a blood meal.[60] Other animals such as bats and certain birds may play a secondary role.[61]

Seroprevalence rates among human populations living in or near endemic VEE areas vary but can approach 100%, suggesting that continuous transmission occurs.[58] However, virus activity within endemic zones can also be highly focal. In one incident at the Fort Sherman Jungle Operations Training Center in the Panama Canal Zone in December 1967, 7 of 12 U.S. soldiers camped in one area developed VEE disease within a 2-day period, but another group camped only a few yards away showed no disease.[62,63]

The incidence of disease during epizootics also varies, but it is often very high. During an outbreak in Venezuela, attack rates of 119 per 1,000 inhabitants per month were reported.[64] Following an epizootic in Guatemala and El Salvador, overall seroprevalence was estimated at 20%.[65]

Unlike the enzootic strains, the fate of the epizootic strains during interepidemic periods is unclear. Of several theories on how epizootic strains arise, the most appealing suggests that they evolve by genetic drift from enzootic strains. Results from oligonucleotide fingerprinting and sequence analysis of I-D isolates from Colombia and Venezuela reveal a close similarity to the epizootic strains, suggesting that the equine virulent epizootic strains arise naturally from variants present in populations of I-D virus.[66,67]

While the genetic evidence suggests that genetic drift of enzootic strains may lead to the development of epizootic strains, ecological data suggest that there is also a strong selective pressure to maintain the enzootic genotype in certain habitats. The enzootic VEE vector *Culex* (*Melanoconion*) *taeniopus* is fully susceptible to both I-AB and I-E strains following intrathoracic inoculation. Orally exposed mosquitoes, however, are fully competent vectors of the enzootic strain, but they fail to develop disseminated infection and transmit epizootic virus.[32,68] This observation suggests that genetic drift of en-

Fig. 28-1. This photograph was taken in 1995 near Buena Vista, Colombia. During large Venezuelan equine encephalitis (VEE) epizootics, typical morbidity rates among unvaccinated equines are 40% to 60%, with at least half of the affected animals progressing to lethal encephalitis. Note the disruption of the ground surface, which is caused by the characteristic flailing or swimming syndromes of moribund animals. Although clinically indistinguishable from the syndromes produced by eastern equine encephalitis (EEE) and western equine encephalitis (WEE) viruses, the capability of VEE to initiate explosive and rapidly expanding epizootics makes reliable diagnostic tests essential for the initiation of appropriate veterinary and public health measures.

zootic strains may be selected against with this combination of vector and virus. Mosquito resistance to epizootic strains of VEE virus is rare; epizootic strains have been isolated from a large number of mosquito species, and many have been shown to be efficient vectors.[69] Thus, host switching from enzootic to epizootic vectors may be an important factor in the evolution of epizootic VEE strains. The introduction of mosquito species into previously unoccupied geographical ranges (eg, *Aedes albopictus* into North America) may, therefore, offer the opportunity for epizootic strains to reemerge.

A major outbreak of epizootic VEE occurred in the late 1960s and early 1970s. Epizootic virus first reached North America in 1966 but did not reach the United States until 1971. Studies of this epizootic demonstrated that the virus easily invaded territories in which it was formerly unknown,[64] presumably as a result of (1) the availability of large numbers of susceptible equine amplifying hosts and (2) the presence of competent mosquito vectors. The initial outbreak in North America, and the first recorded such epizootic, occurred in 1966 in Tampico, Mexico, involving approximately 1,000 equines. By the end of 1969 and the beginning of 1970, the outbreak had expanded to such an extent that the Mexican government requested the TC-83 vaccine from the U.S. Army through the U.S. Department of Agriculture.[70] Despite the immunization of nearly 1 million equines, the epizootic continued to spread and reached the United States in June 1971. The nature of the virus and the number of human and equine cases prompted the secretary of agriculture to declare a national emergency on July 16, 1971.[71] Subsequent immunization of over 2 million horses and unprecedented mosquito abatement efforts eventually stopped the epizootic before it was able to spread from Texas. Epizootic VEE has not been isolated in the United States since the 1971 outbreak.

The first large outbreak since the 1969–1971 epizootic occurred in 1995 (Figures 28-1 and 28-2). The epizootic began in northwestern Venezuela and spread across the Guajira peninsula into northeastern Colombia. An estimated 75,000 to 100,000 humans were infected, with more than 20 deaths reported. This outbreak was caused by an IC strain of VEE virus. By sequence analysis, this strain proved to be essentially identical to a virus that caused an outbreak in Venezuela in 1963.[30]

Fig. 28-2. This photograph was taken in 1995 near Maicao, Colombia. Equine vaccination is the most effective means available to prevent Venezuelan equine encephalitis (VEE) epizootics as well as to control emerging outbreaks. Equines are the major amplifying hosts, and maintaining a high rate of immunity in the equine population will largely prevent human infection with the epizootic strains of VEE. Both inactivated and live-attenuated vaccines are available for veterinary use, but the ability of the live-attenuated vaccine to induce immunity in 7 to 10 days with a single inoculation makes it the only practical vaccination strategy in the face of an outbreak. Other measures used to control outbreaks include using insecticides to reduce mosquito populations and prohibiting the transportation of equines from affected areas.

STRUCTURE AND REPLICATION OF ALPHAVIRUSES

Virion Structure

The alphavirus virion, a spherical particle approximately 60 to 65 nm in diameter, is typically composed of three different structural proteins enclosing a single molecule of single-stranded RNA. The RNA genome is packaged within an icosahedral nucleocapsid, which is constructed from multiple copies of a single species of capsid (C) protein (Figure 28-3). The nucleocapsid is, in turn, surrounded by a lipid envelope that is derived from areas of the host cell plasma membrane that had previously been modified by the insertion of two viral glycoproteins. These envelope glycoproteins, E1 and E2, form heterodimers that associate further into trimers[72,73] to form the short spikes on the surface of the virion. The glycoproteins are the primary targets of the neutralizing antibody response[74] and are the determinants of tropism and virulence.[75] Semliki Forest virus contains a third glycoprotein, E3, which is associated with the E1–E2 dimers on the virion surface. With other alphaviruses, the E3 protein is shed from the infected cell and does not appear in the mature virion.

Replication

Viral Infection. The infection cycle is initiated when the glycoprotein spikes on the virion bind to receptors on the cell surface. The virus is localized initially to coated pits, where it is engulfed in a coated vesicle and transported to the endosomal compartment within the interior of the cell. A decrease in the pH in the interior of the vesicle induces a conformational change in the glycoprotein spikes, and rearrangement of the E1 glycoprotein mediates fusion of the virion envelope with the endosomal membrane.[76] This fusion results in the release of the nucleocapsid into the cytoplasm, where disassembly of the nucleocapsid releases the viral RNA genome to the synthetic apparatus of the cell.

Genomic RNA. The viral genome is a positive-stranded RNA of approximately 11,700 nucleotides and has the structural features of messenger RNA (ie, mRNA, a 5′ methylated cap [m^7GpppA] and a poly-A tract at the 3′ end).[77] As a complete and functional mRNA, genomic RNA purified from virions is fully infectious when artificially introduced (ie, transfected) into susceptible cells. Similarly, RNA transcribed from a full-length complementary DNA (cDNA) clone of an alphavirus is also infectious, and it is this property that allows genetic manipulation of these viruses. Mutations introduced into a cDNA clone by site-directed mutagenesis will be reflected in the RNA transcribed from the altered clone and in the virus obtained from transfected cells. These procedures are being utilized to develop

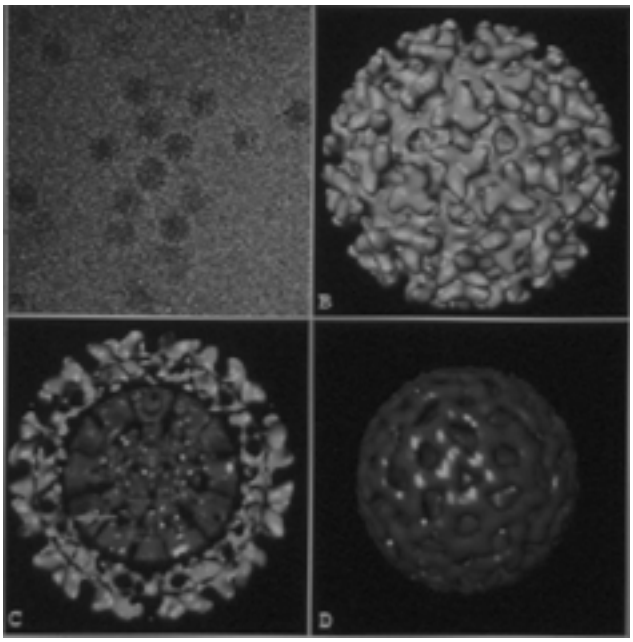

Fig. 28-3. Structure of an alphavirus. Shown is the three-dimensional reconstruction of Sindbis virus at 28 Å resolution from computer-processed images taken by electron cryomicroscopy. (**a**) The original electron micrograph shows virus particles in vitreous ice. (**b**) The surface view of the virus shows details of the 80 trimeric spikes, which are arranged in a T=4 icosahedron. Each spike protrudes 50 Å from the virion surface and is believed to be composed of three E1–E2 glycoprotein heterodimers. (**c**) The cross-sectional view shows the outer surface spikes (yellow) and the internal nucleocapsid (blue), composed of the capsid and viral RNA. The space between the spikes and the nucleocapsid would be occupied by the lipid envelope. The green arrows mark visible points of interaction between the nucleocapsid and transmembranal tails of the glycoprotein spikes. (**d**) The reconstructed capsid also exhibits a T=4 icosahedral symmetry. Computer models: Courtesy of Angel M. Paredes, Cell Research Institute and Department of Microbiology, The University of Texas at Austin, Austin, Tex. Similar but not identical versions of these computer models were published in Paredes AM, Brown DT, Rothnagel R, et al. Three-dimensional structure of a membrane-containing virus. *Proc Natl Acad Sci USA*. 1993; 90:9095–9099.

improved vaccines,[16] but conceivably could be used also to enhance specific characteristics required for weaponization.

Glycoprotein Synthesis. The alphavirus genome contains two protein coding regions. The 5′ proximal 7,500 nucleotides encode a 220,000-dalton precursor polypeptide, which is proteolytically processed to produce four components of the viral RNA polymerase. The polymerase genes are followed by a second coding region of approximately 3,800 nucleotides, which contains the information that directs the synthesis of the viral structural proteins. Soon after release of the viral genome from the nucleocapsid, the 5′ 7,500 nucleotides of the genome RNA are translated to produce the viral RNA polymerase. Early in infection, the incoming viral genome is also utilized as a template for the synthesis of a negative-stranded 45S RNA, identical in length to the genome RNA but of opposite polarity. The negative-stranded 45S RNA subsequently serves as a template for the synthesis of additional genomic RNA. The negative-stranded RNA is also utilized as a template for transcription of a 26S subgenomic mRNA, which is identical to the 3′ third of the genome. The 26S RNA is capped and polyadenylated, and is then translated to yield a precursor polypeptide that is proteolytically processed by cotranslational and posttranslational cleavages to produce the viral structural proteins. The order of the structural proteins within the precursor is C-E3-E2-6K-E1.

As the 26S mRNA is translated, the C protein is produced first and catalyzes its own cleavage from the nascent polypeptide soon after the ribosome transits into the sequences that encode E3. After release of the C protein, the free amino terminus of E3 is bound to the membranes of the rough endoplasmic reticulum. As the synthesis of nascent E3 and E2 continues, the polypeptide is translocated into the lumen of the endoplasmic reticulum, where oligosaccharides and fatty acids are added.[78] A domain of hydrophobic amino acids near the carboxyl terminus of E2 inhibits further transmembranal movement so that the last 30 to 40 amino acids of the E2 polypeptide remain exposed on the cytoplasmic side of the membrane. It is thought that the 6K polypeptide serves as an internal signal for membrane insertion of the second glycoprotein, E1, and is subsequently cleaved from both E2 and E1 by the signal peptidase.[79] A hydrophobic anchor sequence present near the carboxyl terminus of E1 secures the protein in the membrane.

Budding and Release of Progeny Virus Particles. Soon after synthesis, the precursor E2 (pE2) and E1 interact to form multimeric complexes,[80] which are then transported through the Golgi apparatus, where the final modifications of the oligosaccharide are made. The precursor pE2 is cleaved to the mature E2 and E3 glycoproteins soon after the glycoproteins leave the Golgi apparatus,[81] and the mature viral spikes assume an orientation in the plasma membrane with the bulk of the E2 and E1 polypeptides exposed on the exterior surface of the cell. Final assembly, or budding, of progeny virus particles takes place exclusively at the plasma membrane in vertebrate cells,[82] whereas in arthropod cells, budding can also occur at intracellular membranes.[83]

Budding is initiated when intracellular nucleocapsids bind to the 30– to 40–amino acid cytoplasmic domain of the E2 glycoprotein,[84–86] inducing the formation of a locally ordered array of glycoprotein spikes and excluding most of the cellular membrane proteins from the region. Additional lateral associations between the individual spikes stabilize the lattice and promote additional E2–C protein interactions. The growing lattice is thought to draw the membrane around the nucleocapsid, completing the process of envelopment with the release of the spherical virus particle.

Maximal amounts of virus are typically produced from mammalian cells within 8 to 10 hours after infection, and disintegration of the infected cell is likely due to programmed cell death (apoptosis) rather than direct effects of the virus on cellular function.[87] In contrast, alphaviruses initially replicate to high titer in arthropod cells with little or no evidence of cytopathology. The surviving cells continue to produce lesser amounts of virus, often for weeks or months. The ability of the virus to replicate without causing cell death in arthropod cells may be critical for maintenance of the virus in the mosquito vector in nature.

PATHOGENESIS

In humans, the pathogenesis of VEE, EEE, and WEE infections acquired by aerosol—the route of biological defense concern—is unknown. Indeed, little is known of the pathogenesis following natural vector-borne infections of humans, mainly because of the limited availability of autopsy material. Much of the information on VEE pathogenesis in humans is based on a histological review of 21 human fatalities from the 1962–1963 VEE epidemic in Zulia, Venezuela.[88] With few exceptions, the his-

topathological lesions in these cases were comparable to those observed in experimentally infected animals. Tissues that were commonly affected in both humans[88] and animals[89–97] include those of the lymphoid and reticuloendothelial systems and the central nervous system (CNS). Interestingly, widespread hepatocellular degeneration and interstitial pneumonia, not ordinarily seen in experimental animals, were frequent histological findings in these cases of severe human disease.

The clinical and pathological responses of the host to VEE infection are highly dependent on a number of host and viral factors. These factors include

- the species, immune status, and age of the host animal;
- the route of infection; and
- the strain and dose of virus.

Most of the existing experimental data have come from studies using rodent models challenged subcutaneously with the Trinidad donkey (TrD) strain of VEE, an epizootic IA serotype virus. The lymphatic system and the CNS appear to be universal target organs in experimental animal models, as was seen in humans. However, the relative degree of injury caused by the TrD strain of VEE to these tissues varied among the species. TrD caused only mildly severe and reversible lesions to the lymphoid organs in the mouse and monkey,[89,90] but was extremely destructive and irreversible to those organs in the guinea pig[90] and hamster.[89,90,94,97] The virus causes lymphatic necrosis within the nodes associated with the gut; normal gut flora escape, leading to systemic bacterial infections. The severity of the viral infection in the lymphoreticular tissues (in particular the Peyer's patches of the distal intestine in hamsters) appears to contribute to the bacteremia and endotoxic shock syndrome that lead to early death.[97]

The effects of virus infection with the TrD strain of VEE on the CNS also demonstrated considerable species variability. Mice exhibited a severe paralytic episode prior to death from diffuse encephalomyelitis.[89,90] Monkeys, however, showed few if any clinical signs of CNS involvement following peripheral inoculation, and only modest pathological changes in the CNS (found mainly in the thalamus, hypothalamus, and olfactory areas of the brain).[90] However, the extent of neuroinvasion in animals is also a function of both the strain of VEE and the route of infection. Cynomolgus monkeys infected by the intranasal route developed immunoglobulin (Ig) M and IgG antibodies in the cerebrospinal fluid (CSF) and showed moderate areas of perivascular cuffing and nodular and diffuse gliosis, especially in the cortex and hypothalamus.[98] A Colombian epizootic strain of VEE given by the aerosol route caused severe clinical and pathological CNS signs and resulted in death in approximately 35% of rhesus monkeys.[91] Mice and cynomolgus monkeys challenged intracerebrally with TrD or a serologically related strain of VEE developed severe and lethal neurological signs with moderate to severe brain histopathology.[98]

VEE virus can infect the CNS directly through the olfactory nervous system. In rhesus monkeys intranasally inoculated with VEE virus, the virus gained access to the olfactory bulb within 24 hours after infection and before the onset of viremia, suggesting direct neuroinvasion via olfactory neurons.[99] However, in inoculated monkeys whose olfactory nerves had been surgically removed, VEE virus was nonetheless able to reach the olfactory bulb by 36 hours after infection, presumably by the vascular route. Although the olfactory bulb and tract were sites of early viral replication, viral infection did not appear to spread to the rest of the brain along the neural tracts in these monkeys. In 1991, researchers[100] concluded from studies carried out in outbred mice that aerosolized VEE virus can enter the CNS of nonimmunized mice by both the vascular and the olfactory pathways. Morphologic evidence of virus multiplication was observed in olfactory epithelial cells and in secretory cells of Bowman's glands. These researchers suggest two possible routes for viral spreading from the nasal mucosa: (1) virus entry into the blood stream through the fenestrated capillaries of the olfactory zone, with subsequent systemic infection; and (2) axonal transport along the olfactory nerves, allowing direct virus entry into the olfactory bulbs.

In more recent studies, BALB/c mice were challenged either subcutaneously or by aerosol with the V3000 strain[16] of VEE, and brain and nasal tissues taken from animals sacrificed at daily intervals were examined immunocytochemically for viral antigen.[101] In mice challenged by aerosol, both the nasal olfactory epithelium and the olfactory nerve axon bundles in the underlying connective tissue were immunoreactive for VEE virus antigen within 24 hours after infection (Figure 28-4). Within 48 hours after infection, olfactory nerves, nasal-associated lymphoid tissue (NALT), and olfactory bulbs were immunoreactive (Figure 28-5). In a bilaterally symmetrical pattern, the prepiriform area and the piriform cortex were also immunoreactive by 48 hours after infection. Other areas of the brain were

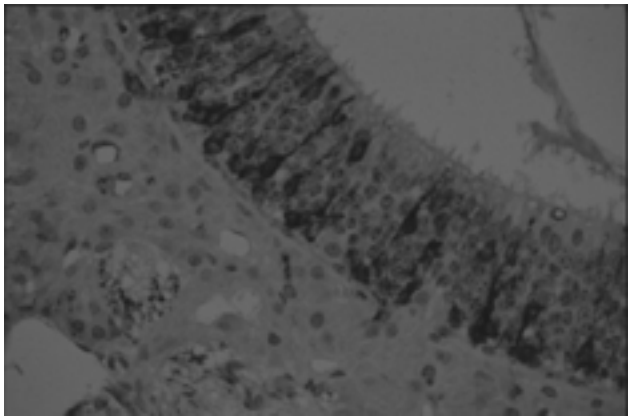

Fig. 28-4. Nasal tissue, BALB/c mouse, 2 days after exposure to aerosolized VEE virus. Note immunoreactive olfactory epithelium and olfactory nerves. Alkaline phosphatase-labeled streptavidin method using rabbit antiserum to VEE virus (Mayer's hematoxylin counterstain, original magnification x 300).

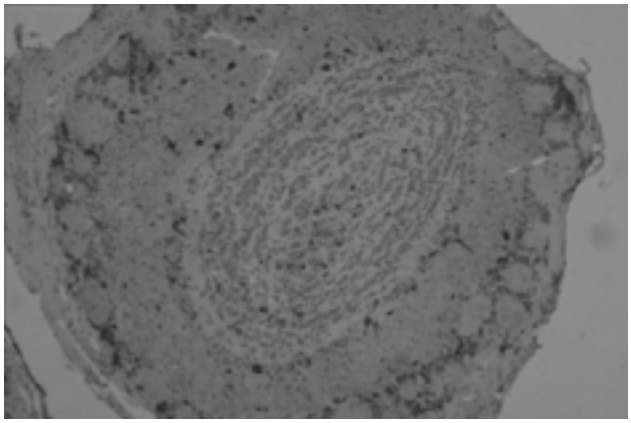

Fig. 28-5. Olfactory bulb, BALB/c mouse, 2 days after exposure to aerosolized VEE virus. Note immunoreactive cells. Alkaline phosphatase-labeled streptavidin method using rabbit antiserum to VEE virus (Mayer's hematoxylin counterstain, original magnification x 150).

immunoreactive at 4 days after infection. In subcutaneously challenged mice, the olfactory nerves and nasal olfactory epithelium were not immunoreactive at any time, and the olfactory bulbs, prepiriform area and piriform cortex, and NALT were not positive until 3 days after infection. By day 4 after infection, subcutaneously challenged mice also had immunoreactive cells in other areas of the brain. These immunocytochemical findings are consistent with previous studies carried out in rodents and primates; they indicate not only that aerosolized VEE virus may enter the mouse brain by means of the olfactory nerves but also that this process is very rapid. The efficiency with which this process occurs with the equine encephalomyelitis viruses will put very high demands on the vaccines used for immunoprophylaxis (vaccines are discussed later in this chapter).

CLINICAL DISEASE AND DIAGNOSIS

The three equine encephalomyelitis virus complexes within the *Alphavirus* genus—EEE, WEE, and VEE—are also recognized for their potential for neuroinvasion and encephalitis in humans, sometimes in epidemic proportions. However, many of the infections caused by these viruses are manifested as systemic viral febrile syndromes, and infections by EEE and WEE viruses may remain subclinical. Furthermore, these alphaviruses vary markedly in both their neurotropism and the severity of their neurological sequelae. Depending on the virus, patients presenting with the general syndrome of alphavirus encephalitis have a varying combination of fever, headache, confusion, obtundation, dysphasia, seizures, paresis, ataxia, myoclonus, and/or cranial nerve palsies.

Venezuelan Equine Encephalitis

The IA, IB, and IC variants of VEE virus are pathogenic for equines and have the capacity for explosive epizootics with epidemic human disease. Epidemics of VEE affecting 20,000 to 30,000 people, or more, have been documented in Venezuela and Ecuador. In contrast to the other alphavirus encephalitides, EEE and WEE, epizootic strains of VEE are mainly amplified in equines, rather than birds, so that equine disease normally occurs prior to reports of human disease. Enzootic VEE strains (variants ID, IE, and IF and subtypes II, III, IV, V, and VI) are not recognized as virulent for equines, but disease has been documented with most of these variants in humans who reside in or move into enzootic foci, or after laboratory infections (see Table 28-2). The resulting syndromes appear to be similar, if not indistinguishable, from the syndrome produced by epizootic variants, which ranges from undifferentiated febrile illness to fatal encephalitis.

Following an incubation period that can be as short as 28 hours[10] but is usually 2 to 6 days, patients typically develop a prostrating syndrome of chills, high fever (38°C–40.5°C), headache, and mal-

aise.[102] Photophobia, sore throat, myalgias, and vomiting are also common symptoms. Frequent signs noted on physical examination include conjunctival injection, erythematous pharynx, and muscle tenderness. Although essentially all human infections with VEE virus are symptomatic,[62,63] only a small percentage manifest neurological involvement.[103] In one epidemic, it was estimated that the ratio of encephalitis to infections is less than 0.5% in adults, although possibly as high as 4% in children.[104] Mild CNS involvement is evidenced by lethargy, somnolence, or mild confusion, with or without nuchal rigidity.[105] Seizures, ataxia, paralysis, or coma herald more severe CNS involvement. In children with overt encephalitis, case fatalities range as high as 35% compared with 10% for adults.[106] However, for those who survive encephalitic involvement, neurological recovery is usually complete.[107] School-age children are believed to be more susceptible to a fulminant form of disease, in which depletion of lymphoid tissues is prominent and which follows a lethal course over 48 to 72 hours.[88,108,109]

In the first 3 days of illness, leukopenia and elevated serum glutamic-oxaloacetic transaminase (SGOT) are common. For those with CNS involvement, a lymphocytic pleocytosis of up to 500 cells per microliter will be observed in the CSF. The CSF pleocytosis may acutely be polymorphonuclear but soon becomes predominantly lymphocytic.

Specific diagnosis of VEE can be accomplished by virus isolation, serologic testing, or both.[110] During the first 1 to 3 days of symptoms of nonspecific febrile illness, VEE virus may be recovered from either the serum or the nasopharynx.[111] Despite the theoretical possibility of person-to-person transmission of virus present in the nasopharynx, no evidence of such occurrences has been reported. Identification of the VEE subtype of an isolate involved can be accomplished by cross-neutralization tests. HI, enzyme-linked immunosorbent assay (ELISA), or plaque reduction neutralization (PRN) antibodies appear as viremia diminishes. Complement-fixing (CF) antibodies make their appearance later during convalescence. VEE IgM antibodies are present in acute phase sera,[63] and it has been reported that the VEE IgM tests do not react with sera from patients with EEE or WEE.[112] Since patients with encephalitis typically come to evaluation later in the course of clinical illness, virus is recovered less often from them,[112] and they usually have serum antibody by the time of clinical presentation.[113]

Immunity after infection is probably lifelong to the homologous serotype, but cross-immunity is weak or nonexistent to heterologous serotypes.[47–49] Thus, when viewed either as an endemic disease threat or as a potential biological warfare threat, adequate immunization will require polyvalent vaccines.

Eastern Equine Encephalitis

EEE is maintained in a natural transmission cycle between *Culiseta melanura* mosquitoes and passerine birds in swampy and forested areas. EEE outbreaks are typically recognized when severe equine or human encephalitis occurs near such areas.[114] During vector-borne EEE epidemics, the incidence of human infection is low (< 3% of the population at risk),[115] and the neurological attack rate in one outbreak was estimated as 1 in every 23 cases of human infection.[116] However, the effect on morbidity and mortality of aerosol-acquired EEE infection (which would be the expected route of infection in a biological warfare offensive) is unknown. The incubation period in humans varies from 5 to 15 days. Adults typically exhibit a febrile prodrome for up to 11 days before the onset of neurological disease[117]; however, illness in children exhibits a more sudden onset.[118] Viremia occurs during the febrile prodrome,[119] but is usually undetectable by the time clinical encephalitis develops, when HI and neutralizing antibodies become evident.[120] Despite the development of a prompt and neutralizing humoral response, virus is not eradicated from the CNS, and progressive neuronal destruction and inflammation continue.

EEE is the most severe of the arboviral encephalitides, with high mortality and severe neurological sequelae.[121] During outbreaks of EEE, the attack, morbidity, and fatality rates are highest in young children[122] and the elderly.[123] Case fatality rates are estimated to be from 50% to 75%, but asymptomatic infections and milder clinical illness are certainly underreported. The illness is characterized by rapid onset of high fever, vomiting, stiff neck, and drowsiness. Children frequently manifest generalized, facial, or periorbital edema. Motor involvement with paresis is common during the acute phase of the illness. Major disturbances of autonomic function, such as impaired respiratory regulation or excess salivation, may dominate the clinical picture. Up to 30% of survivors are left with neurological sequelae such as seizures, spastic paralysis, and cranial neuropathies. Cognitive impairment ranges from minimal brain dysfunction to severe dementia.

Clinical laboratory findings in patients with EEE often demonstrate an early leukopenia followed by

a leukocytosis. Elevated opening pressure is commonly noted on lumbar puncture, and in children, especially, the CSF lymphocytic pleocytosis may reach a cell count of thousands of mononuclear cells per microliter. Specific diagnosis of EEE depends on virus isolation or serologic testing in which rising titers of HI, CF, or neutralizing antibodies are observed. IgM antibodies are usually detectable in acute-phase sera.[112] As with other alphaviruses, neutralization tests are considered to be the most specific.

Western Equine Encephalitis

Like VEE, WEE is less virulent for adult humans than it is for equines and children, with lower rates of fatalities and neurological sequelae.[124] As with EEE, infants and the elderly are especially susceptible to severe clinical illness and neurological sequelae, with case fatality rates of about 10%. Highlands J (HJ) virus, an antigenically related member of the WEE complex that is isolated frequently in the *eastern* United States, rarely infects humans.

The incubation period is 5 to 10 days. A large percentage of patients with vector-borne infections are either asymptomatic or present with a nonspecific febrile illness or aseptic meningitis. The ratio of encephalitis cases per infection has been estimated to vary from 1:1,150 in adults, to 1:58 in children, to 1:1 in infants.[57] However, the severity of the syndrome and the incidence of inapparent infection almost certainly depend on the strain and dose of virus and the route of infection. Some unusual isolates show very high virulence in laboratory animals,[125] and in one study of laboratory-acquired infections in adults, 2 of 5 patients died.[126] Symptoms usually begin with malaise, headache, and fever, followed by nausea and vomiting.[127] Over the next few days the symptoms intensify, and somnolence or delirium may progress into coma. The severity of neurological involvement is inversely related to age, with more than 90% of children younger than 1 year old exhibiting focal or generalized seizures.[128] Physical examination typically reveals nuchal rigidity, impaired sensorium, and upper motor neuron deficits with pathologically abnormal reflexes.

Patients with the severest infections usually die within the first week of clinical illness, with overall case fatalities averaging 10%. Other patients begin a gradual convalescence after the first week of encephalitic symptoms. Most adults recover completely, but may take months to years to recuperate from fatigability, recurrent headaches, emotional lability, and impaired concentration.[129] Some patients are left with permanent residua of motor weakness, cognitive deficits, or a seizure disorder. Children carry a higher incidence of neurological sequelae, ranging from less than 1% in those older than 1 year old, to 10% in infants 2 to 3 months old, to more than 50% in newborns. Congenital infection in the last trimester of pregnancy has been described, with resultant encephalitis in the infants.[130]

Viremia is rarely detectable by the time patients present with encephalitic symptoms, but IgM, HI, and neutralizing antibodies can generally be found by the end of the first week of illness, and they increase in titer during the next week.[112,131,132] CF serologic responses generally appear in the second week and rise thereafter. Isolation of virus or 4-fold titer rises are diagnostic, but because of serologic cross-reactions with other alphaviruses, neutralization tests are preferred. Examination of the CSF reveals a lymphocytic pleocytosis ranging from 10 to 400 mononuclear cells per microliter. WEE virus may occasionally be isolated from the CSF or throat swabs taken within the first 2 days of illness, and is frequently recovered from brain tissue on postmortem examination.[133] Natural infection presumably confers long-term immunity.

Differential Diagnosis of Alphavirus Encephalitis

Most acute infections with VEE and WEE produce a moderately severe but nonspecific clinical illness consisting of fever, headache, and myalgias. Therefore, in a potential biological warfare scenario, alphaviruses should be considered in the differential diagnosis whenever epidemic febrile illness occurs, especially if a number of patients progress to neurological disease. Sick or dying equines in the vicinity of an epidemic febrile illness among troops should immediately suggest the possibility of large-scale alphavirus exposure. Other potential biowarfare agents that may infrequently produce or imitate a meningoencephalitic syndrome include *Brucella* species, *Yersinia pestis*, *Salmonella typhi*, *Coxiella burnetii*, and botulinum toxin. As with any diagnosis of meningoencephalitis, it is imperative to rule out any potential cause that may be specifically treatable.

For encephalitis cases that are more sporadic in their occurrence, other important viral etiologies that might not be readily discriminated from the alphaviruses by clinical features are listed in Table 28-3. This list is not all-inclusive but suggests other viral encephalitides that should be considered if a

TABLE 28-3

SOME IMPORTANT VIRAL CAUSES* OF ENDEMIC ENCEPHALOMYELITIS

Virus *Family*	Genus	Species
Togaviridae	Alphavirus	Eastern equine
		Western equine
		Venezuelan equine
Flaviviridae	St. Louis	
	Murray Valley	
	West Nile	
	Japanese	
	Dengue	
	Tick-borne complex	
Bunyaviridae	LaCrosse	
	Rift Valley	
	Toscana	
Paramyxoviridae	Paramyxovirus	Mumps
	Morbillivirus	Measles
Arenaviridae	Arenavirus	Lymphocytic choriomeningitis
		Machupo
		Junin
Picornaviridae	Enterovirus	Poliovirus
		Coxsackievirus
		Echovirus
Reoviridae	Colorado tick fever	
Rhabdoviridae	Rabies	
Herpesviridae	Herpesvirus	Herpes simplex virus types 1 and 2
		Epstein-Barr virus
		Cytomegalovirus
Adenoviridae	Adenovirus	

*Not all-inclusive

patient presents, a priori, with an encephalitic syndrome. Epidemiological, historical, and laboratory information remain critical to differential diagnosis. Immediate and careful consideration must be given to treatable infections that may mimic viral encephalitis (Exhibit 28-1), since prompt and appropriate intervention can be lifesaving. In addition, it should be kept in mind that vascular, autoimmune, and neoplastic diseases may imitate infectious meningoencephalitis.

EXHIBIT 28-1

NONVIRAL CAUSES OF ENCEPHALOMYELITIS

Treatable infectious conditions that can mimic viral encephalitis:

- Partially treated bacterial meningitis
- Brain abscess
- Subdural empyema
- Embolic encephalitis associated with bacterial endocarditis
- Lyme disease
- Tuberculous meningitis
- Fungal meningitis
- Rocky Mountain spotted fever
- Cat scratch disease
- Cerebral malaria
- Trypanosomiasis
- Toxoplasmosis

Vascular, autoimmune, and neoplastic diseases that can mimic infectious meningoencephalitis:

- Lupus cerebritis
- Cerebral and granulomatous arteritis
- Lymphomatous cerebritis
- Whipple's disease
- Behçet syndrome
- Carcinomatosis meningitis

For endemic meningoencephalitic disease that occurs outside biowarfare theaters, the geographical locale and the patient's travel history are of preeminent importance in diagnosing an arboviral encephalitis. Risk for disease is increased relative to the patient's amount of arthropod contact near swampy or forested areas during the summer. Encephalitic illness of equines in the surrounding locale is an important indication of ongoing transmission of encephalitic alphaviruses. Examination of the CSF, to include viral cultures, is critical in differentiating bacterial from viral infections, and infectious from noninfectious etiologies. Serum and CSF tests based on polymerase chain reaction (PCR) techniques hold great promise in more-rapid diagnosis of infectious encephalitis. In some instances it will be necessary to (*a*) institute chemotherapy for possible, treatable, infecting organisms and (*b*) await definitive laboratory diagnostic tests.

Medical Management and Prevention

No specific therapy exists for the togaviral encephalitides; hence, treatment is aimed at management of specific symptoms (eg, anticonvulsant medication, protection of the airway). The extremes of high fever occasionally produced by WEE infection in humans is a special problem among the arboviral encephalitides that may require aggressive antihyperthermia measures.

The U.S. Army has extensive experience with a live-attenuated vaccine for VEE (TC-83) in humans. However, this vaccine would be expected to protect efficiently against only IA/B and IC serotypes. The TC-83 vaccine is also reactogenic, with more than 20% of vaccine recipients experiencing fever, malaise, and headache after the vaccination. Half of these patients experience symptoms severe enough to warrant bed rest for 1 to 2 days.

Use of an effective vaccine in horses would prevent outbreaks of epizootic VEE, as equines are the major amplifying species for VEE virus. Vaccination of horses is not a useful public health tool for EEE, WEE, or enzootic VEE, however, since horses are not important as amplifying hosts for these diseases. Investigational formalin-inactivated vaccines for humans are available for WEE and EEE, but they require multiple injections and are poorly immunogenic. Insecticide measures of vector control may also have an impact on ameliorating epidemic transmission.

IMMUNOPROPHYLAXIS

Relevant Immune Effector Mechanisms

The equine encephalomyelitis viruses constitute both an endemic disease threat as well as a biological warfare threat; therefore, adequate immunoprophylaxis of military personnel will require protection against both vector-borne and aerosol-acquired infection. The requirements for protection against parenteral infection are well described, but the requirements for protection against infectious aero-

sols are certainly more stringent, and are largely unidentified. Within a few days of infection with an alphavirus, specific antibodies can be detected in the serum of animals or humans. Within 7 to 14 days, a virus-neutralizing antibody response develops, as measured by the ability of serum antibodies to block virus infectivity in vitro or in vivo. Protection from mosquito-vectored alphavirus disease is believed to be primarily mediated by this virus-specific neutralizing antibody response, which is largely directed against epitopes on the E2 glycoprotein. Protection mediated by nonneutralizing antibodies to alphaviruses, directed largely at epitopes on the E1 glycoprotein, has also been described.[134–136] However, it has proven more difficult to correlate protection from aerosol exposure with serum neutralization or antibody titers.[137]

Other nonspecific or immune responses that occur following alphavirus infection include the induction of secretion of interferon[138–141] and the activation of cytotoxic macrophages.[142] There have also been reports of virus-specific cytotoxic T cell responses induced against alphaviruses,[143–146] although it has proven difficult to show that these T cell responses play a significant role in protection.

Passive Immunization

Passive transfer of neutralizing antisera or monoclonal antibodies to naive recipients protects animals from subsequent parenteral challenge with homologous VEE strains.[136,140,147] Passive transfer of nonneutralizing, anti-E1 monoclonal antibodies directed against appropriate epitopes is also protective against Sindbis,[134] WEE,[135] and VEE[136] viruses. However, for the respiratory route of infection, uniform protection was not observed after passive transfer of hyperimmune serum to hamsters[137] or neutralizing monoclonal antibodies to mice,[148] suggesting that either additional immune mechanisms or the presence of protective antibodies along the respiratory tract may be needed. The time between the administration of immune serum and virus exposure may also be relevant. Protection of mice from intracerebral inoculation with WEE virus was observed if immune serum was given no more than 3 days prior to virus exposure.[149,150] Similarly, monkeys passively immunized with horse antiserum to EEE or WEE resisted intranasal challenge from homologous virus 24 hours later, but they were unable to resist a second challenge with the same virus 7 weeks later.[151] However, as the immune serum given in both studies was xenogeneic, the loss of protective capacity was presumably related in part to active clearance of the immune serum by the recipients.

The effect of giving immune serum to animals after the establishment of intracerebral infections has also been evaluated. Several studies, employing different alphaviruses, have demonstrated at least partial protection if the immune serum was administered within 24 hours of infection.[149,150,152–154] Other studies have suggested that postinfection serum transfer may also cause a more severe pathology, or may merely delay the onset of disease symptoms.[152,155] Aggressive serotherapy following infections of two laboratory workers who developed acute WEE encephalitis resulted in the survival of one patient[156] but was ineffective in the second patient.[157]

In an EEE outbreak in New Jersey in 1959, 22 of 32 diagnosed patients died. Most patients had demonstrable antibody during the onset or progression of encephalitis, and neutralizing antibody titers in sera from patients who died were generally similar to those observed in patients who recovered.[116] This finding, coupled with animal studies indicating that transfer of virus-neutralizing antisera was unable to prevent progression of disease if infection of the brain was firmly established (described above[149,150,152–154]), suggests that serotherapy would be an ineffective means of treatment for these virus infections, unless initiated very early in the course of disease.

Active Immunization

Vaccines currently available for use against the equine encephalomyelitis viruses include TC-83, which is a live attenuated vaccine for VEE, and inactivated vaccines for VEE, EEE, and WEE. All are used under IND status. The characteristics of these vaccines and the responses induced in human vaccinees are summarized in Table 28-4.

Live Vaccines

The TC-83 VEE vaccine was developed in 1961 by serial passage of the virulent TrD strain in fetal guinea pig heart cells,[158] and is administered subcutaneously (0.5 mL) at 1×10^4 to 2×10^4 plaque-forming units (pfu) per dose. The vaccine was used initially in laboratory and field personnel at risk for exposure to VEE,[159] and more than 6,000 people received the vaccine between 1964 and 1972.[160] For

TABLE 28-4

VACCINES CURRENTLY AVAILABLE FOR VEE, EEE, AND WEE VIRUSES

Vaccine	Form/Strain	Dose (mL)/ Route of Administration	Schedule	Responding %	Duration*	Booster Dose/ Route
VEE (TC-83)	Attenuated TrD	0.5 mL/sc	Day 0	82%	92%	C-84/sc
VEE (C-84)†	Inactivated TC-83	0.5 mL/sc	After TC-83	76% NR‡	60%	0.5 mL/sc
				100% WT§	100%	
EEE	Inactivated PE-6¥	0.5 mL/sc	Days 0, 28	58%	75%	0.1 mL/id
WEE	Inactivated CM-4884¥	0.5 mL/sc	Days 0, 7, 28	50%	20%	0.5 mL/sc

*% of responders whose virus-neutralizing titers persist for at least 1 y
†current IND protocols specify use of C-84 only as a booster vaccine
‡TC-83 nonresponders
§TC-83 responders given C-84 to boost waning titers
¥laboratory designation
EEE: eastern equine encephalitis
id: intradermal
IND: investigational new drug
sc: subcutaneous
TC: tissue culture
TrD: Trinidad donkey
VEE: Venezuelan equine encephalitis
WEE: western equine encephalitis

reasons that remain unclear, approximately 20% of the people who receive TC-83 fail to make a minimum neutralizing antibody response and presumably would not be protected should they be exposed to the virus. Another 25% of vaccine recipients experience clinical reactions ranging from mild transient symptoms to fever, chills, sore throat, and malaise sufficient to require bed rest.[161] However, for recipients who respond with postvaccination titers of at least 1:20, long-term follow-up studies have shown that titers persist for several years.[162] In humans, documented vaccine-breakthrough infections have been attributed largely to exposure to heterologous, enzootic strains of VEE virus.[47–49] Although pregnant mares were not adversely affected by TC-83,[163] pregnant women are advised not to receive the TC-83 vaccine, as wild-type VEE may have been associated with spontaneous abortions or stillbirths during an epidemic in Venezuela in 1962.[164]

In animals, TC-83 vaccination will protect hamsters from a lethal VEE subcutaneous or aerosol challenge,[137] although up to 20% of hamsters may die of reactions to the vaccine.[95,165] Subcutaneous immunization of monkeys[98] with the vaccine produces (*a*) neutralizing antibody responses in serum and (*b*) protection from virulent VEE virus delivered by peripheral or intranasal challenge. However, TC-83 provides only partial protection against aerosol challenge in outbred mice.[100] TC-83 has been extensively administered to horses, burros, and mules, in part because large numbers of equines were vaccinated during the 1969–1970 epizootic. TC-83 immunization produces febrile responses and leukopenia in some equines,[166,167] but neutralizing antibody responses to homologous (serotype IA) virus eventually develop in 90% of these animals.[166,168] Although it was difficult to accurately assess vaccine efficacy under the conditions of an ongoing epizootic, herds of animals known to have been immunized at least 2 weeks prior to any disease occurrence in the area did not sustain any VEE-related deaths, whereas unimmunized herds experienced up to 60% mortality rates.[161]

An unresolved problem with the use of TC-83, and presumably with other live-attenuated alphavirus vaccines, is the phenomenon of vaccine interference, in which prior immunity to heterologous alphaviruses inhibits vaccine virus replication and subsequent immune responses. This occurrence has been observed in horses,[169,170] in which preexisting antibodies to EEE and WEE may have interfered with TC-83 vaccination. Interference has also been observed in humans, in whom prior vaccination with Chikungunya virus has reduced the response to TC-83, and vice versa (D.J.McC., unpublished research, 1994).

Inactivated Vaccines

Against VEE (C-84). Early attempts to develop an inactivated vaccine against VEE resulted in preparations that contained residual live virus and caused disease in 4% of those who received it.[158,171] Because of the problems associated with incomplete inactivation, development of an inactivated VEE vaccine (C-84) was begun, using the TC-83 attenuated strain of virus.[172] Initial clinical trials with the C-84 inactivated vaccine were begun in 1976 in 14 volunteers previously immunized with TC-83, and subsequently in 14 naive volunteers.[173] The vaccine was found to be safe and elicited only mild tenderness at the site of injection. Although C-84 was immunogenic, three doses were required to maintain neutralizing antibody titers in recipients. A subsequent study has shown that most TC-83 nonresponders and 100% of individuals with waning titers from TC-83 immunizations respond to a booster dose of C-84 and have a high probability of maintaining a titer for 3 years.[160]

The observation that hamsters given C-84 vaccine were protected from subcutaneous challenge but not from an aerosol exposure to VEE virus[137] raised concerns that C-84 vaccination may not protect at-risk laboratory workers from aerosol exposure. Therefore, C-84 is currently administered only as a booster immunogen.

Against EEE and WEE. The PE-6 strain of EEE virus was passed in primary chick-embryo cell cultures, and then was formalin-treated and lyophilized to produce an inactivated vaccine for EEE.[174] This vaccine is administered as a 0.5-mL dose subcutaneously on days 0 and 28, with 0.1-mL intradermal booster doses given as needed to maintain neutralizing antibody titers. Mild reactions to the vaccine were observed, and immunogenicity was demonstrated in initial clinical trials.[175] The vaccine was given to 896 at-risk laboratory workers between 1976 and 1991. No significant clinical reactions have been observed. A long-term follow-up study of 573 recipients indicated a 58% response rate after the primary series, and a 25% chance of failing to maintain adequate titers for 1 year. Response rates and persistence of titers increased with the administration of additional booster doses.[176]

The WEE vaccine was similarly prepared using the B-11 or CM-4884) virus strain, and caused only mild clinical reactions when administered to WEE-naive individuals.[177] Between 1976 and 1990, 359 laboratory workers were immunized with this vaccine. Long-term follow-up studies have indicated that administration of three doses of 0.5 mL subcutaneously on days 0, 7, and 28 results in a 50% responder rate (neutralization titer > 1:40) after the primary series. Only 20% of recipients maintain a titer for 1 year, although this level can be increased to 60% to 70% with additional booster immunizations.[176]

SUMMARY

The equine encephalomyelitis viruses consist of three antigenically related viruses within the *Alphavirus* genus of the family *Togaviridae*: Venezuelan equine encephalomyelitis (VEE), western equine encephalomyelitis (WEE), and eastern equine encephalomyelitis (EEE). These viruses are vectored in nature by various species of mosquitoes and cause periodic epizootics among equines. Infection of equines with virulent strains of any these viruses produces a similar clinical course of severe encephalitis with high mortality. However, the clinical course following infection of humans differs. EEE is the most severe of the arbovirus encephalitides, with case fatality rates of 50% to 70%. WEE virus is generally less virulent for adults, but the infection commonly produces severe encephalitis in children, with case fatality rates approaching 10%. In contrast, encephalitis is rare following VEE virus infection, but essentially all infected individuals develop a prostrating syndrome of high fever, headache, malaise, and prolonged convalescence.

Although natural infections are acquired by mosquito bite, these viruses are also highly infectious in low doses as aerosols. They can be produced in large amounts in inexpensive and unsophisticated systems, are relatively stable, and are readily amenable to genetic manipulation. For these reasons, the equine encephalomyelitis viruses are classic biological warfare threats.

No specific therapy exists for infections caused by these viruses. A live-attenuated vaccine for VEE (TC-83) and inactivated vaccines for VEE, EEE, and WEE have been developed and are used under IND status. Although these vaccines are useful in protecting at-risk individuals, they have certain disadvantages, and improved vaccines are under development.

REFERENCES

1. Meyer KF, Haring CM, Howitt B. The etiology of epizootic encephalomyelitis of horses in the San Joaquin Valley, 1930. *Science*. 1931;74:227.

2. Giltner LT, Shahan MS. The 1933 outbreak of infectious equine encephalomyelitis in the eastern states. *North Am Vet*. 1933;14:25.

3. Ten Broeck C, Hurst EW, Traub E. Epidemiology of equine encephalitis in the eastern United States. *J Exp Med*. 1935;62:677.

4. Kubes V, Rios FA. The causative agent of infectious equine encephalitis in Venezuela. *Science*. 1939;90:20.

5. Webster LT, Wright FH. Recovery of eastern equine encephalomyelitis virus from brain tissue of human cases of encephalitis in Massachusetts. *Science*. 1938;88:305.

6. Howitt BE. Recovery of the virus of equine encephalomyelitis from the brain of a child. *Science*. 1938;88:455.

7. Fothergill LD, Dingle JH, Farber S, et al. Human encephalitis caused by a virus of eastern variety of equine encephalitis. *N Engl J Med*. 1983;219:411.

8. Sanmartin-Barberi C, Groot H, Osborn-Mesa E. Human epidemic in Colombia caused by the Venezuelan equine encephalitis virus. *Am J Trop Med Hyg*. 1954;3:283.

9. Richmond JY, McKinney RW. *Biosafety in Microbiological and Biomedical Laboratories*. 3rd ed. Washington, DC: US Department of Health and Human Services; May 1993. HHS Publication (CDC) 93–8395.

10. Slepushkin AN. An epidemiological study of laboratory infections with Venezuelan equine encephalomyelitis. *Vopr Virusol*. 1959;3:311–314.

11. Shubladze AK, Gaidmovich SYa, Gavrilov VI. A virological study of laboratory infections with Venezuelan equine encephalomyelitis. *Vopr Virusol*. 1959;3:305–310.

12. Strauss JH, Strauss EG. The alphaviruses: Gene expression, replication, and evolution. *Microbiological Reviews*. 1994;58(3):491–562.

13. Peters CJ, Dalrymple JM. Alphaviruses. In: Fields BM, Knipe DM, eds. *Virology*. 2nd ed, Vol 1. New York, NY: Raven Press; 1990: 713–761.

14. Huxsoll DL, Patrick WC, Parrott CD. Veterinary services in biological disasters. *J Am Vet Med Assoc*. 1987;190(6):714–722.

15. Davis NL, Willis LV, Smith JF, Johnston RE. In vitro synthesis of infectious Venezuelan equine encephalitis virus RNA from a cDNA clone: Analysis of a viable deletion mutant. *Virology*. 1991;171:189–204.

16. Davis NL, Powell N, Greenwald GF, et al. Attenuating mutations in the E2 glycoprotein gene of Venezuelan equine encephalitis virus: Construction of single and multiple mutants in a full-length cDNA clone. *Virology*. 1991;183:20–31.

17. Hanson RP. An epizootic of equine encephalomyelitis that occurred in Massachusetts in 1831. *Am J Trop Med Hyg*. 1957;6:858.

18. Ten Broeck C. Birds as possible carriers of the virus of equine encephalomyelitis. *Arch Pathol*. 1938;25:759.

19. Kissling RE, Rubin H, Chamberlain RW, Edison ME. Recovery of the virus of eastern equine encephalomyelitis from the blood of a purple grackle. *Proc Soc Exp Biol Med*. 1951;77:398.

20. Chamberlain RW, Rubin H, Kissling RE, Eidson, ME. Recovery of eastern equine encephalomyelitis from a mosquito, *Culiseta melanura (Coquillett)*. *Proc Soc Exp Biol Med*. 1951;77:396.

21. Morris CD. Eastern equine encephalitis. In: Monath TP, ed. *The Arboviruses: Epidemiology and Ecology*. Vol 3. Boca Raton, Fla: CRC Press; 1988: 203–231.

22. Mitchell CJ, Niebylski ML, Smith GC, et al. Isolation of eastern equine encephalitis virus from *Aedes albopictus* in Florida. *Science*. 1992;257:526–527.

23. Davison RO. Encephalomyelitis in Saskatchewan, 1941. *Can J Public Health*. 1942;33:83.

24. Reisen WK, Monath TP. Western equine encephalitis. In: Monath TP, ed. *The Arboviruses: Epidemiology and Ecology*. Vol 5. Boca Raton, Fla: CRC Press; 1988: 203–231.

25. Hammon W McD, Reeves WC, Brookman B, Izumi EM. Isolation of viruses of western equine and St. Louis encephalitis from *Culex tarsalis* mosquitoes. *Science*. 1941;94:328.

26. Hammon W McD, Gray JA, Evans FC, Izumi EM. Western equine and St. Louis encephalitis antibodies in the sera of mammals and birds from an endemic area. *Science*. 1941;94:305.

27. Beck CE, Wyckoff RW. Venezuelan equine encephalomyelitis. *Science*. 1938;88:530.

28. Walton TE, Grayson MA. Venezuelan equine encephalitis. In: Monath TP, ed. *The Arboviruses: Epidemiology and Ecology*. Vol 4. Boca Raton, Fla: CRC Press; 1988: 203–231.

29. Franck PT, Johnson KM. An outbreak of Venezuelan equine encephalomyelitis in Central America: Evidence for exogenous source of a virulent virus subtype. *Am J Epidemiol*. 1971;94:487.

30. Weaver SC, Salas R, Rico-Hesse R, et al. Re-emergence of epidemic Venezuelan equine encephalitis in South America. *Lancet*. 1966;348:436–440.

31. Young NA, Johnson KM. Antigenic variants of Venezuelan equine encephalitis virus: Their geographic distribution and epidemiologic significance. *Am J Epidemiol*. 1969;89:286.

32. Scherer WF, Cupp EW, Dziem GM, Breener RJ, Ordonez JV. Mesenteronal infection threshold of an epizootic strain of Venezuelan encephalitis virus in *Culex (Melanoconion) taeniopus* mosquitoes and its implication to the apparent disappearance of this virus strain from an enzootic habitat in Guatemala. *Am J Trop Med Hyg*. 1982;31:1030–1037.

33. Jonkers AH. Silent hosts of Venezuelan equine encephalitis (VEE) virus in endemic situations: Mammals. In: Venezuelan Encephalitis: Proceedings of the Workshop-Symposium on Venezuelan Encephalitis Virus; 14–17 September 1971. Washington, DC: Pan American Health Organization; 1972: 263–268. PAHO Scientific Publication 243.

34. Bernstein BJ. The birth of the US Biological Warfare Program. *Sci Am*. 1987;256(6):116–121.

35. National Security Agency. *National Security Decision*. Washington, DC: NSA; Nov. 25, 1969. Memorandum 35.

36. Walton TE. Arboviral encephalomyelitides of livestock in the western hemisphere. *J Am Vet Med Assoc*. 1992;200:1385–1389.

37. Contigiani MS, De Basualdo M, Camara A, et al. Presencia de anticuerpos contra el virus de la encefalitis equina Venezolana subtipo VI en pacientes con enfermedad aguda febril [in Spanish]. *Revista Argentina de Microbiologia*. 1993;25:212–220.

38. Karabatsos N. *International Catalogue of Arboviruses Including Certain Other Viruses of Vertebrates*. 3rd ed. San Antonio, Tex: American Society for Tropical Medicine and Hygiene; 1985.

39. Shope RE, Causey OR, de Andrade AHP, Theiler M. The Venezuelan equine encephalitis complex of group A arthropodborne viruses, including *Mucambo* and *Pixuna* from the Amazon region of Brazil. *Am J Trop Med Hyg*. 1964;13:723.

40. Chamberlain RW, Sudia WD, Coleman PH, Work TH. Venezuelan equine encephalitis virus from South Florida. *Science*. 1964;145:272.

41. Scherer WF, Anderson K. Antigenic and biological characteristics of Venezuelan encephalitis virus strains including a possible new subtype isolated from the Amazon region of Peru in 1971. *Am J Epidemiol*. 1975;101:356.

42. Walton TE. Virulence properties of Venezuelan equine encephalitis virus serotypes in horses. In: Venezuelan Encephalitis: Proceedings of the Workshop-Symposium on Venezuelan Encephalitis Virus; 14–17 September 1971. Washington, DC: Pan American Health Organization; 1972: 134. PAHO Scientific Publication 243.

43. Cupp EW, Scherer WF, Ordonez JV. Transmission of Venezuelan encephalitis virus by naturally infected *Culex (Melanoconion) opisthopus*. *Am J Trop Med Hyg*. 1979;28:1060–1063.

44. Scherer WF, Dickerman RW, Cupp EW, Ordonez JV. Ecologic observations of Venezuelan encephalitis virus in vertebrates and isolations of Nepuyo and Patois viruses from sentinel hamsters at Pacific and Atlantic habitats in Guatemala, 1968–1980. *Am J Trop Med Hyg*. 1985;34:790–798.

45. Scherer WF, Dickerman RW, Diaz-Najera A, Ward BA, Miller MH, Schaffer PA. Ecologic studies of Venezuelan encephalitis virus in southeastern Mexico, III: Infection of mosquitoes. *Am J Trop Med Hyg*. 1971;20:969–979.

46. Walton TE. Equine arboviral encephalomyelitides: A review. *J Equine Vet Sci*. 1988;8:49–53.

47. De Mucha-Macias J, Sanchez-Spindola I. Two human cases of laboratory infection with Mucambo virus. *Am J Trop Med Hyg*. 1965;14(3):475–478.

48. Franck PT. In discussion: Round table on epidemic control. In: Venezuelan Encephalitis: Proceedings of the Workshop-Symposium on Venezuelan Encephalitis Virus; 14–17 September 1971. Washington, DC: Pan American Health Organization; 1972: 400. PAHO Scientific Publication 243.

49. Dietz WH, Peralta PH, Johnson KM. Ten clinical cases of human infection with Venezuelan equine encephalomyelitis virus, subtype I-D. *Am J Trop Med Hyg.* 1979;28:329–334.

50. Casals J. Antigenic variants of eastern equine encephalitis virus. *J Exp Med*. 1964;119:547.

51. Weaver SC, Bellew LA, Gousset L, Repik PM, Scott TW, Holland JJ. Diversity within natural populations of eastern equine encephalomyelitis virus. *Virology*. 1993;195:700–709.

52. Weaver SC, Rico-Hesse R, Scott TW. Genetic diversity and slow rates of evolution in New World alphaviruses. *Curr Top Microbiol Immunol*. 1992;176:99–117.

53. Dietz WH, Galindo P, Johnson KM. Eastern equine encephalitis in Panama: The epidemiology of the 1973 epizootic. *Am J Trop Med Hyg*. 1980;29:133–140.

54. Trent DW, Grant JA. A comparison of new world alphaviruses in the western equine encephalomyelitis complex by immunochemical and oligonucleotide fingerprint techniques. *J Gen Virol*. 1980;47:261–282.

55. Hardy JL. The ecology of western equine encephalomyelitis virus in the central valley of California, 1945–1985. *Am J Trop Med Hyg*. 1987;37(suppl):18S–32S.

56. Fulhorst CF, Hardy JL, Eldridge BF, Pressor SV, Reeves WC. Natural vertical transmission of western equine encephalomyelitis virus in mosquitoes. *Science*. 1994;263:676–678.

57. Reeves WC, Hammon WM. Epidemiology of the arthropod-borne viral encephalitides in Kern County, California, 1943–52. *Univ Calif Pub Health*. 1962;4:257.

58. Johnson KM, Martin DH. Venezuelan equine encephalitis. *Adv Vet Sci Comp Med*. 1974;18:79.

59. Galindo P. Endemic vectors of Venezuelan encephalitis. In: Venezuelan Encephalitis: Proceedings of the Workshop-Symposium on Venezuelan Encephalitis Virus; 14–17 September 1971. Washington, DC: Pan American Health Organization; 1972: 249–252. PAHO Scientific Publication 243.

60. Young NA, Johnson KM, Gauld LW. Viruses of the Venezuelan equine encephalomyelitis complex. *Am J Trop Med Hyg*. 1969;18:290–296.

61. Seymour C, Dickerman RW, Martin MS. Venezuelan encephalitis virus infection in neotropical bats, II: Experimental infections. *Am J Trop Med Hyg*. 1978;27:297–306.

62. Franck PT, Johnson KM. An outbreak of Venezuelan encephalitis in man in the Panama Canal Zone. *Am J Trop Med Hyg*. 1970;19:860–863.

63. Sanchez JL, Lednar WM, Macasaet FF, et al. Venezuelan equine encephalitis: Report of an outbreak associated with jungle exposure. *Milit Med*. 1984;149:618–621.

64. Groot H. The health and economic impact of Venezuelan equine encephalitis (VEE). In: Venezuelan Encephalitis: Proceedings of the Workshop-Symposium on Venezuelan Encephalitis Virus; 14–17 September 1971. Washington, DC: Pan American Health Organization; 1972: 7–27. PAHO Scientific Publication 243.

65. Hinman AR, McGowan JE, Henderson BE. Venezuelan equine encephalomyelitis: Surveys of human illness during an epizootic in Guatemala and El Salvador. *Am J Epidemiol*. 1971;93:130–136.

66. Kinney RM, Tsuchiya KR, Sneider JM, Trent DW. Genetic evidence that epizootic Venezuelan equine encephalitis (VEE) viruses may have evolved from enzootic VEE subtype 1-D virus. *Virology*. 1992;191:569–580.

67. Weaver SC, Bellew LA, Rico-Hesse R. Phylogenetic analysis of alphaviruses in the Venezuelan equine encephalitis complex and identification of the source of epizootic viruses. *Virology*. 1992;191:282–290.

68. Weaver SC, Scherer WF, Cupp EW, Castello DA. Barriers to dissemination of Venezuelan encephalitis viruses in the middle American enzootic vector mosquito, *Culex (melanoconion) taeniopus*. *Am J Trop Med Hyg*. 1984;33:953–960.

69. Turell MJ, Ludwig GV, Beaman JR. Transmission of Venezuelan equine encephalomyelitis virus by *Aedes sollicitans* and *Aedes taeniorhynchus* (Diptera: Culicidae). *J Med Entomol*. 1992;29:62–65.

70. Reta G. Equine disease: Mexico. In: Venezuelan Encephalitis: Proceedings of the Workshop-Symposium on Venezuelan Encephalitis Virus; 14–17 September 1971. Washington, DC: Pan American Health Organization; 1972: 209–214. PAHO Scientific Publication 243.

71. Sharman R. Equine disease. In: Venezuelan Encephalitis: Proceedings of the Workshop-Symposium on Venezuelan Encephalitis Virus; 14–17 September 1971. Washington, DC: Pan American Health Organization; 1972: 221–224. PAHO Scientific Publication 243.

72. Anthony RP, Brown DT. Protein-protein interactions in an alphavirus membrane. *J Virol.* 1991;65:1187–1194.

73. Rice CM, Strauss JH. Association of Sindbis virus glycoproteins and their precursors. *J Mol Biol.* 1982;154:325–348.

74. Dalrymple JM. Antigenic characterization of two Sindbis envelope glycoproteins separated by isoelectric focusing. *Virology.* 1976;69:93–102.

75. Davis NL, Fuller FJ, Dougherty WG, Olmsted RA, Johnston RE. A single nucleotide change in the E2 glycoprotein gene of Sindbis virus affects penetration rate in cell culture and virulence in neonatal mice. *Proc Natl Acad Sci. USA* 1986;83:6771–6775.

76. Bron RJ, Wahlberg M, Garoff H, Wilschut J. Membrane fusion of Semliki Forest virus in a model system: Correlation between fusion kinetics and structural changes in the envelope glycoprotein. *EMBO J*. 1993;12:693–701.

77. Strauss EG, Strauss JH. Structure and replication of the alphavirus genome. In: Schlesinger S, Schlesinger MJ, eds. *The Togaviridae and Flaviviridae*. New York, NY: Plenum Press; 1986: 350–390.

78. Sefton B. Immediate glycosylation of Sindbis virus membrane proteins. *Cell*. 1977;10:659–668.

79. Liljestrom P, Garoff H. Internally located cleavable signal sequences direct the formation of Semliki Forest virus membrane proteins from a polyprotein precursor. *J Virol*. 1991;65:147–153.

80. Ziemiecki AH, Simons GK. Formation of the Semliki Forest virus membrane glycoprotein complexes in the infected cell. *J Gen Virol*. 1980;50:111–123.

81. de Curtis IA, Simons K. Dissection of Semliki Forest virus glycoprotein delivery from the trans-Golgi network to the cell surface in permeabilized BHK cells. *Proc Natl Acad Sci USA*. 1988;85:8052–8056.

82. Simons K, Garoff H. The budding mechanisms of enveloped viruses. *J Gen Virol.* 1980;50:1–21.

83. Gliedman JB, Smith JF, Brown DT. Morphogenesis of Sindbis virus in cultured *Aedes albopictus* cells. *J Virol*. 1975;16:913–926.

84. Metsiikko K, Garoff H. Oligomers of the cytoplasmic domains of the p62/E2 membrane protein of Semliki Forest virus bind to the nucleocapsid in vitro. *J Virol*. 1990;64:4678–4683.

85. Paredes AM, Brown DT, Rothnagel R, et al. Three-dimensional structure of a membrane-containing virus. *Proc Natl Acad Sci USA*. 1993;90:9095–9099.

86. Lopez S, Yao J-S, Kuhn RJ, Strauss EG, Strauss JH. Nucleocapsid–glycoprotein interactions required for alphavirus assembly. *J Virol*. 1994;68:1316–1323.

87. Levine V, Huang Q, Isaacs JT, Reed JC, Griffin DE, Hardwick JM. Conversion of lytic to persistent alphavirus infection by the bcl-2 cellular oncogene. *Nature*. 1993;361:739–741.

88. de la Monte S, Castro F, Bonilla NJ, Gaskin de Urdaneta A, Hutchins GM. The systemic pathology of Venezuelan equine encephalitis virus infection in humans. *Am J Trop Med Hyg.* 1985;34:194–202.

89. Jackson AC, SenGupta SK, Smith JF. Pathogenesis of Venezuelan equine encephalitis virus infection in mice and hamsters. *Vet Pathol*. 1991;28:410–418.

90. Gleiser CA, Gochenour WS, Berge TO, Tigertt WD. The comparative pathology of experimental Venezuelan equine encephalomyelitis infection in different animal hosts. *J Infect Dis.* 1962;110:80–97.

91. Gochenour WS Jr. The comparative pathology of Venezuelan encephalitis virus infection in selected animal hosts. In: Venezuelan Encephalitis: Proceedings of the Workshop-Symposium on Venezuelan Encephalitis Virus; 14–17 September 1971. Washington, DC: Pan American Health Organization; 1972: 113–117. PAHO Scientific Publication 243.

92. Austin FJ, Scherer WF. Studies of viral virulence, I: Growth and histopathology of virulent and attenuated strains of Venezuelan encephalitis virus in hamsters. *Am J Pathol*. 1971;62:195–210.

93. Walker DH, Harrison A, Murphy K, Flemister M, Murphy FA. Lymphoreticular and myeloid pathogenesis of Venezuelan equine encephalitis in hamsters. *Am J Pathol*. 1976;84:351–370.

94. Jahrling PB, Scherer WF. Histopathology and distribution of viral antigens in hamsters infected with virulent and benign Venezuelan encephalitis viruses. *Am J Pathol*. 1973;72:25–38.

95. Jahrling PB, Scherer WF. Growth curves and clearance rates of virulent and benign Venezuelan encephalitis viruses in hamsters. *Infect Immunol*. 1973;8:456–462.

96. Gorelkin L, Jahrling PB. Pancreatic involvement by Venezuelan equine encephalomyelitis virus in the hamster. *Am J Pathol*. 1974;75:349–362.

97. Gorelkin L, Jahrling PB. Virus-initiated septic shock: Acute death of Venezuelan encephalitis virus-infected hamsters. *Lab Invest*. 1975;32:78–85.

98. Monath TP, Cropp CB, Short WF, et al. Recombinant vaccinia-Venezuelan equine encephalomyelitis (VEE) vaccine protects nonhuman primates against parenteral and intranasal challenge with virulent VEE virus. *Vac Res*. 1992;1:55–68.

99. Danes L, Kufner J, Hruskova J, Rychterova V. The role of the olfactory route on infection of the respiratory tract with Venezuelan equine encephalomyelitis virus in normal and operated Macaca rhesus monkeys, I: Results of virological examination. *Acta Virol (Praha).* 1973;17:50–56.

100. Ryzhikov AB, Tkacheva NV, Sergeev AN, Ryabchikova EI. Venezuelan equine encephalitis virus propagation in the olfactory tract of normal and immunized mice. *Biomed Sci*. 1991;2:607–614.

101. Vogel P, Abplanalp D, Kell W, et al. Venezuelan equine encephalitis in BALB/c mice. *Arch Pathol Lab Med*. 1996;120:164–172.

102. Sanmartin C, Mackenzie RB, Trapido H, et al. Encefalitis equina Venezolana en Colombia, 1967 [in Spanish]. *Bol Of Sanit Panam*. 1973;74:104–137.

103. Martin DH, Eddy GA, Sudia WD, Reeves WC, Newhouse VF, Johnson KM. An epidemiologic study of Venezuelan equine encephalomyelitis in Costa Rica, 1970. *Am J Epidemiol*. 1972;95:2565–2578.

104. Sanmartin C. Diseased hosts: Man. In: Venezuelan Encephalitis: Proceedings of the Workshop-Symposium on Venezuelan Encephalitis Virus; 14–17 September 1971. Washington, DC: Pan American Health Organization; 1972: 186–188. PAHO Scientific Publication 243.

105. Sanmartin-Barberi C, Groot H, Osborno-Mesa E. Human epidemic in Colombia caused by the Venezuelan equine encephalomyelitis virus. *Am J Trop Med Hyg*. 1954;3:283–293.

106. Bowen GS, Fashinell TR, Dean PB, Gregg MG. Clinical aspects of human Venezuelan equine encephalitis in Texas. *Bull Pan Am Health Organ*. 1976;10:46–57.

107. Leon CA, Jaramillo R, Martinez S, et al. Sequelae of Venezuelan equine encephalitis in humans: A four year follow-up. *Int J Epidemiol*. 1975;4:131–140.

108. Avilán Rovira J. In discussion: Sanmartin C. Diseased hosts: Man. In: Venezuelan Encephalitis: Proceedings of the Workshop-Symposium on Venezuelan Encephalitis Virus; 14–17 September 1971. Washington, DC: Pan American Health Organization; 1972: 189–195. PAHO Scientific Publication 243.

109. Johnson KM, Shelokov A, Peralta PH, Dammin GJ, Young NA. Recovery of Venezuelan equine encephalomyelitis virus in Panama: A fatal case in man. *Am J Trop Med Hyg*. 1968;17:432–440.

110. Briceño Rossi AL. Rural epidemic encephalitis in Venezuela caused by a group A arbovirus (VEE). In: Melnick JL, ed. *Progress in Medical Virology*. Vol 9. Basel, Switzerland: Karger; 1967: 176–203.

111. Briceño Rossi AL. The frequency of VEE virus in the pharyngeal material of clinical cases of encephalitis. *Gac Med Caracas*. 1964;72:5–22.

112. Calisher CH, El-Kafrawi AO, Al-Deen Mahmud MI, et al. Complex specific immunoglobulin M antibody patterns in humans infected with alphaviruses. *J Clin Microbiol*. 1986;23:155–159.

113. Madalengoitia J, Palacios O, Ubiliuz JC, Alva S. An outbreak of Venezuelan encephalitis virus in man in the Tumbes department of Peru. In: Venezuelan Encephalitis: Proceedings of the Workshop-Symposium on Venezuelan Encephalitis Virus; 14–17 September 1971. Washington, DC: Pan American Health Organization; 1972: 198–200. PAHO Scientific Publication 243.

114. Monath TP. Arthropod-borne encephalitides in the Americas. *Bull WHO*. 1979;57:513–533.

115. Goldfield M, Welsh JN, Taylor BF. The 1959 outbreak of eastern encephalitis in New Jersey, VI: The frequency of prior infection. *Am J Epidemiol*. 1968;87:39–49.

116. Goldfield M, Welsh JN, Taylor BF. The 1959 outbreak of eastern encephalitis in New Jersey, V: The inapparent infection:disease ratio. *Am J Epidemiol*. 1968;87:32–38.

117. Hart KL, Keen D, Belle EA. An outbreak of eastern equine encephalomyelitis in Jamaica, West Indies, Nov–Dec 1962, I: Description of human cases. *Am J Trop Med Hyg*. 1964;13:331–334.

118. Farber S, Hill A, Connerly MI, Dingle JH. Encephalitis in infants and children caused by the virus of the eastern variety of equine encephalitis. *JAMA*. 1940;114:1725–1731.

119. Clarke DH. Two non-fatal human infections with the virus of eastern encephalitis. *Am J Trop Med Hyg*. 1961; 10:67–70.

120. Goldfield M, Taylor BF, Welsh JN. The 1959 outbreak of eastern encephalitis in New Jersey, III: Serologic studies of clinical cases. *Am J Epidemiol*. 1968;87:18–22.

121. Feemster RF. Equine encephalitis in Massachusetts. *N Engl J Med*. 1958;257:107–113.

122. Ayres JC, Feemster RF. The sequelae of eastern equine encephalomyelitis. *N Engl J Med*. 1949;240:960–962.

123. McGowan JE, Bryan JA, Gregg MB. Surveillance of arboviral encephalitis in the United States, 1955–1971. *Am J Epidemiol*. 1973;97:199–207.

124. Hayes RO. Eastern and western encephalitis. In: Beran GW, ed. *Handbook Series in Zoonoses: Viral Zoonoses*. Vol. 1. Boca Raton, Fla: CRC Press; 1981: 29–57.

125. Bianchi TI, Aviles G, Monath TP, Sabatini MS. Western equine encephalomyelitis: Virulence markers and their epidemiologic significance. *Am J Trop Med Hyg*. 1993;49(3):322–328.

126. Hanson RP, Sulkin SE, Buescher EL, Hammon W McD, McKinney RW, Work TH. Arbovirus infections of laboratory workers. *Science*. 1967;158:1283.

127. Sciple GW, Ray CG, Holden P, La Motte LC, Irons JV, Chin TDY. Encephalitis in the high plains of Texas. *Am J Epidemiol*. 1968;87:87–98.

128. Finley KG. Postencephalitis manifestations of viral encephalitides. In: Fields NS, Blattner RF, eds. *Viral Encephalitis*. Springfield, Ill: Charles C Thomas; 1959: 69–91.

129. Earnest MP, Goolishian HA, Calverly JR, Hayes RO, Hill HR. Neurologic, intellectual, and psychologic sequelae following western encephalitis. *Neurology*. 1971;21:969–974.

130. Shinefield HR, Townsend TE. Transplacental transmission of western equine encephalomyelitis. *J Ped*. 1953;43:21–25.

131. Calisher CH, Emerson JK, Muth DJ, Lazuick JS, Monath TP. Serodiagnosis of western equine encephalitis infections: Relationships of antibody titer and test to observed onset of Illness. *J Am Vet Med Assoc*. 1983;183:438.

132. Calisher CH, Karabatsos N. Arbovirus serogroups: Definition and geographic distribution. In: Monath TP, ed. *The Arboviruses: Epidemiology and Ecology*. Vol 1. Boca Raton, Fla: CRC Press; 1988: 19–57.

133. Rozdilsky B, Robertson HE, Chorney J. Western encephalitis: Report of eight fatal cases: Saskatchewan epidemic, 1965. *Can Med Assoc J*. 1968;98:79–86.

134. Schmaljohn AL, Johnson ED, Dalrymple JM, Cole GA. Non-neutralizing monoclonal antibodies can prevent lethal alphavirus encephalitis. *Nature*. 1982;297:70–72.

135. Hunt AR, Roerhig, JT. Biochemical and biological characteristics of epitopes on the E1 glycoprotein of western equine encephalitis virus. *Virology*. 1985;142:334–346.

136. Mathews JH, Roehrig JT. Determination of the protective epitopes on the glycoproteins of Venezuelan equine encephalomyelitis virus by passive transfer of monoclonal antibodies. *J Immunol*. 1982;129(6):2763–2767.

137. Jahrling PB, Stephenson EH. Protective efficacies of live attenuated and formaldehyde-inactivated Venezuelan equine encephalitis virus vaccines against aerosol challenge in hamsters. *J Clin Microbiol*. 1984;19(3):429–431.

138. Vilcek J. Production of interferon by newborn and adult mice infected with Sindbis virus. *Virology*. 1964;22:651–652.

139. Finter NB. Interferon as an antiviral agent in vivo: Quantitative and temporal aspects of the protection of mice against Semliki Forest virus. *Br J Exp Pathol*. 1966;47:361–371.

140. Rabinowitz SG, Adler WH. Host defenses during primary Venezuelan equine encephalomyelitis virus infection in mice, I: Passive transfer of protection with immune serum and immune cells. *J Immunol*. 1973;110(5):1345–1353.

141. Tazulakhova EB, Novokhatsky AS, Yershov FI. Interferon induction by, and antiviral effect of, poly(rI)-poly (rC) in experimental viral infection. *Acta Virol*. 1973;17:487–492.

142. Rodda SJ, White DO. Cytotoxic macrophages: A rapid nonspecific response to viral infection. *J Immunol*. 1976;117(6):2067–2072.

143. McFarland HF. In vitro studies of cell-mediated immunity in an acute viral infection. *J Immunol*. 1974;113(1):173–180.

144. Mullbacher A, Blanden RV. Murine cytotoxic T-cell response to alphavirus is associated mainly with H-2Dk. *Immunogenetics*. 1978;7:551–561.

145. Mullbacher A, Blanden RV. H-2-linked control of cytotoxic T-cell responsiveness to alphavirus infection: Presence of H-2Dk during differentiation and stimulation converts stem cells of low responder genotype to T cells of responder phenotype. *J Exp Med*. 1979;149:786–790.

146. Peck R, Brown A, Wust CJ. In vitro heterologous cytotoxicity by T effector cells from mice immunized with Sindbis virus. *J Immunol*. 1979;123(4):1763–1766.

147. Roerhig JT, Mathews JH. The neutralization site on the E2 glycoprotein of Venezuelan equine encephalitis (TC-83) virus is composed of multiple conformationally stable epitopes. *Virology*. 1985;142:346–356.

148. Kinney RM, Esposito JJ, Mathews JH, et al. Recombinant vaccinia virus/Venezuelan equine encephalitis (VEE) virus protects mice from peripheral VEE virus challenge. *J Virol*. 1988;(62):4697–4702.

149. Howitt BF. Equine encephalomyelitis. *J Infect Dis*. 1932;51:493–510.

150. Olitsky PK, Schlesinger RW, Morgan IM. Induced resistance of the central nervous system to experimental infection with equine encephalomyelitis virus, II: Serotherapy in western virus infection. *J Exp Med*. 1943;77:359–375.

151. Wyckoff RWG, Tesar WC. Equine encephalomyelitis in monkeys. *J Immunol*. 1939;37:329–343.

152. Berge TO, Gleiser CA, Gochenour WS, Miesse ML, Tigertt WD. Studies on the virus of Venezuelan equine encephalomyelitis, II: Modification of specific immune serum of response of central nervous system of mice. *J Immunol*. 1961;87:509–517.

153. Griffin DE, Johnson RT. Role of the immune response in recovery from Sindbis virus encephalitis in mice. *J Immunol*. 1978;118(3):1070–1075.

154. Igarashi A, Fukuoka T, Fukai K. Passive immunization of mice with rabbit antisera against Chikungunya virus and its components. *Biken J*. 1971;14:353–355.

155. Seamer JH, Boulter EA, Zlotnik I. Delayed onset of encephalitis in mice passively immunised against Semliki Forest virus. *Br J Exp Path*. 1971;52:408–414.

156. Gold H, Hampil B. Equine encephalomyelitis in a laboratory technician with recovery. *Ann Intern Med*. 1942;16: 556–569.

157. Helwig FC. Western equine encephalomyelitis following accidental inoculation with chick embryo virus. *JAMA*. 1940;115:291–292.

158. Berge TO, Banks IS, Tigertt WD. Attenuation of Venezuelan equine encephalomyelitis virus by in vitro cultivation in guinea-pig heart cells. *Am J Hyg*. 1961;73:209–218.

159. McKinney RW, Berge TO, Sawyer WD, Tigertt WD, Crozier D. Use of an attenuated strain of Venezuelan equine encephalomyelitis virus for immunization in man. *Am J Trop Med Hyg*. 1963;12:597–603.

160. Pittman PR, Makuch RS, Mangiafico JA, Cannon TL, Gibbs PH, Peters CJ. Long-term duration of detectable neutralizing antibodies after administration of live-attenuated VEE vaccine and following booster vaccination with inactivated VEE vaccine. *Vaccine*. 1996;14(4):337–343.

161. McKinney RW. Inactivated and live VEE vaccines—A review. In: Venezuelan Encephalitis: Proceedings of the Workshop-Symposium on Venezuelan Encephalitis Virus; 14–17 September 1971. Washington, DC: Pan American Health Organization; 1972: 369–377. PAHO Scientific Publication 243.

162. Burke DS, Ramsburg HH, Edelman R. Persistence in humans of antibody to subtypes of Venezuelan equine encephalitis (VEE) virus after immunization with attenuated (TC-83) VEE virus vaccine. *J Infect Dis*. 1977;136(3):354–359.

163. Eddy GA, Martin DH, Reeves WC, Johnson KM. Field studies of an attenuated Venezuelan equine encephalomyelitis vaccine (strain TC-83). *Infect Immunol*. 1972;5(2):160–163.

164. Wenger F. Venezuelan equine encephalitis. *Teratology*. 1977;16:359–362.

165. Austin FJ, Scherer WF. Studies of viral virulence, I: Growth and histopathology of virulent and attenuated strains of Venezuelan encephalitis virus in hamsters. *Am J Pathol*. 1971;62:195–209.

166. Spertzel RO, Kahn DE. Safety and efficacy of an attenuated Venezuelan equine encephalomyelitis vaccine for use in equidae. *J Am Vet Med Assoc*. 1971;159(6):731–738.

167. Monlux WS, Luedke AJ, Browne J. Central nervous system response of horses to Venezuelan equine encephalomyelitis vaccine (TC-83). *J Am Vet Med Assoc*. 1972;161(3):265–269.

168. Walton TE, Alvarez O, Buckwalter RM, Johnson KM. Experimental infection of horses with an attenuated Venezuelan equine encephalomyelitis vaccine (strain TC-83). *Infect Immunol*. 1972;5(5):750–756.

169. Jochim MM, Barber TL, Luedke AJ. Venezuelan equine encephalomyelitis: Antibody response in vaccinated horses and resistance to infection with virulent virus. *J Am Vet Med Assoc*. 1973;162(4):280–283.

170. Calisher CH, Sasso DR, Sather GE. Possible evidence for interference with Venezuelan equine encephalitis virus vaccination of equines by pre-existing antibody to eastern or western equine encephalitis virus, or both. *Appl Microbiol*. 1973;26:485–488.

171. Sutton LS, Brooks CC. Venezuelan equine encephalomyelitis due to vaccination in man. *JAMA*. 1954;155(17):1473–1478.

172. Cole FE Jr, May SW, Eddy GA. Inactivated Venezuelan equine encephalomyelitis vaccine prepared from attenuated (TC-83 strain) virus. *Appl Microbiol*. 1974;27(1):150–153.

173. Edelman R, Ascher MS, Oster CN, Ramsburg HH, Cole FE, Eddy GA. Evaluation in humans of a new, inactivated vaccine for Venezuelan equine encephalitis virus (C-84). *J Infect Dis.* 1979;140(5):708–715.

174. Maire LF III, McKinney RW, Cole FE Jr. An inactivated eastern equine encephalomyelitis vaccine propagated in chick-embryo cell culture, I: Production and testing. *Am J Trop Med Hyg*. 1970;19(1):119–122.

175. Bartelloni PJ, McKinney RW, Duffy TP, Cole FE Jr. An inactivated eastern equine encephalomyelitis vaccine propagated in chick-embryo cell culture, II: Clinical and serologic responses in man. *Am J Trop Med Hyg*. 1970;19(1):123–126.

176. Pittman PR. Lieutenant Colonel, Medical Corps, US Army. Chief, Special Immunizations Program, Medical Division, US Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, Md. Personal communication, October 1996.

177. Bartelloni PJ, McKinney RW, Calia FM, Ramsburg HH, Cole FE Jr. Inactivated western equine encephalomyelitis vaccine propagated in chick embryo cell culture: Clinical and serological evaluation in man. *Am J Trop Med Hyg*. 1971;20(1):146–149.