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Medically Important Arboviruses of the United States and Canada

CHARLES H. CALISHER*

Arthropod-borne Infectious Diseases Laboratory, Colorado State University, Foothills Campus, Ft. Collins, Colorado 80523

INTRODUCTION AND HISTORICAL BACKGROUND

As recently as a decade ago, writing a review such as this would have been a relatively straightforward task. The geographic distributions of the North American arboviruses considered here already were well established, the main vectors and vertebrate hosts had been determined with relative certainty, and nearly as much was known then as now about the diseases they cause. However, rapid advances in molecular virology are revealing the molecular correlates of virus functions and are affecting our views of arbovirus-vector-host interactions and their epidemiological consequences. Particularly, this newer knowledge is being applied in viral taxonomy to determine the bases for variation in virus strains, i.e., determining gene sequences and the products those sequences specify. At a dizzying pace, sophisticated techniques are being developed for rapid diagnosis of arboviral diseases and for rapid and highly specific detection of both viruses and antibody. I anticipate that both prevention and treatment of arboviral diseases will progress in parallel with these advances and that continuing efforts to understand virus and vector evolution will provide useful insight into the possible emergence and reemergence of viruses. I attempt to bring these new aspects into perspective in the sections that follow. Fortunately, a number of excellent and comprehensive review articles already published provide additional perspectives and greater detail in specific areas than can be presented here (6, 16, 20, 25, 52, 80, 102, 105, 112, 136, 161, 163, 165, 166, 176, 186, 191, 200–202, 234, 236, 241).

Arboviruses (arthropod-borne viruses) are maintained in nature in cycles involving hematophagous arthropod vectors and susceptible vertebrate hosts. Simply stated, an infected

* Phone: (303) 223-8586. Fax: (303) 225-1903.

vector transmits virus to the vertebrate host during feeding. The host becomes infected and develops viremia of sufficient level and duration to infect other vectors, and so on. Mosquitoes are the main vectors for most known arboviruses, possibly due to intentional bias in collecting and processing. However, some arboviruses are transmitted exclusively by other biting flies or ticks. Of the mosquito vectors, the female is the blood feeder, requiring certain protein substances in blood to produce eggs. This fact explains why arboviruses usually are isolated only from female mosquitoes in field studies. The rare isolation of virus from larval mosquitoes is taken as evidence of transovarial infection; isolations from male mosquitoes give evidence that the virus was acquired either transovarially or venereally from an infected female. Infection of the vector has been considered to be lifelong, with no observable pathologic changes or untoward effects. However, some exceptions may exist. Weaver et al., for example, have reported cytopathologic changes in the midgut of *Culiseta melanura* mosquitoes after oral infection with eastern equine encephalitis (EEE) virus (250).

Certain arboviruses, particularly those of the California (CAL) serogroup, are notable among those arboviruses that may be transmitted transovarially and venereally by their arthropod vectors, as well as by the usual vector-host-vector cycle. Also, arboviruses occasionally can be transmitted mechanically by arthropods. In nature, two types of vertebrate hosts are of particular note: those that serve as main sources of infections for vectors, and those that do not but in which overt disease can occur. The latter are important to us in relation to human disease and domestic animal loss; epidemiologically, the former are more significant because, together with the vector, they serve as arbovirus reservoirs, disseminators, and amplifiers. Many factors help to determine the effectiveness of an animal species for this role, but among those of greatest importance are the presence of a high-titer viremia of duration adequate to infect a critical number of the vector species, attractiveness to the arthropod vector, and a continuing availability of additional nonimmune individuals. With few exceptions, humans are dead-end or tangential hosts and do not play a significant role in the maintenance and dissemination of the arboviruses indigenous to North America. Their infection usually is incidental and does not affect the main cycle. The vertebrate host spectrum varies for each virus; generally, particular species of smaller vertebrates with high population replacement rates, such as birds or rodents, serve as hosts, but in some instances larger mammals can be involved. Reptiles, amphibians, and bats have been suspected of serving as overwintering hosts for a few of these viruses, but there still is doubt of their significance in this role.

The first arbovirus isolated in the United States was vesicular stomatitis Indiana virus in 1925; since then, more than 58 other arboviruses have been isolated in the United States and 5 more arboviruses have been isolated in Canada. However, only six of these are known to cause significant human illness (129), namely, western equine encephalitis (WEE), EEE, St. Louis encephalitis (SLE), Powassan (POW), LaCrosse (LAC), and Colorado tick fever (CTF) viruses.

The first comprehensive studies in the United States of an arbovirus causing human illness were begun more than 60 years ago in California by Karl Meyer of the Hooper Foundation. In the summer of 1930, he and coworkers investigated an extensive epizootic of equine encephalitis in the San Joaquin Valley and isolated a virus from the brain of a sick horse (160). He wondered at that time whether the same disease might account for a polioencephalomyelitis concurrently being noted in some people of the region. The next summer, the disease reappeared in epizootic form in the San Joaquin Valley and spread to other areas of California. It recurred in the summer of 1932 throughout much of California and in most western states (189, 239). In each of those years the outbreaks ceased with the onset of cool weather. This finding and that of high-level viremias in acutely infected horses led to the suspicion that the virus was arthropod-borne. Occurrence of a similar illness in humans who were in contact with sick horses tended to substantiate Meyer's earlier hunch and gave impetus to further studies of the etiologic agent, the WEE virus Meyer had isolated in 1930. The virus subsequently was shown in the laboratory to replicate in and be transmitted by *Aedes aegypti* mosquitoes, and serologic surveys indicated widespread infection of birds and a variety of other animals in the wild. The detailed clinical studies, epidemiologic investigations, and data analyses are classics in the history of both arbovirology and virology in general and did much to establish arbovirology as a significant field of research. In 1938, WEE virus was isolated from the brain of a child who died from encephalitis (120), and in 1941 the virus was isolated from naturally infected *Culex tarsalis* mosquitoes (109, 188), first in the Yakima Valley of Washington and later in the San Joaquin Valley of California, where it had all begun.

It is logical to assume that both WEE and EEE, and indeed the other North American arboviruses as well, existed in natural cycles long before they were recognized. Although old reports suggest that outbreaks of a horse disease compatible with EEE occurred in the eastern coastal United States as early as 1831 (176), epizootics and epornitics were attributed definitively to EEE virus only since 1933, at which time EEE virus was first isolated from equine brains (224). Isolation of the virus from human brain tissue followed in 1938 (95, 253). The epidemiology of EEE virus soon was seen to differ from that of WEE virus, both in geographic distribution and in ecologic and epidemiologic characteristics. The disease was found to occur in horses annually along the eastern seaboard, with infrequent infections noted as far inland as the upper Midwest and as far north as southeastern Canada in association with freshwater swamps.

The third arbovirus shown to be a human pathogen in the United States was SLE virus. SLE first was recognized as a human encephalitic disease during an outbreak in Paris, Ill., in 1932 (166). The causative virus was isolated the following year from human brain tissue in a similar but larger epidemic in St. Louis, Mo. (178, 252). Since its discovery, SLE virus has been responsible for more than 1,000 deaths, at least 10,000 severe illnesses, and no fewer than a million mild or subclinical infections (58, 164). In September of 1933, the Surgeon General, H. S. Cumming, detailed L. L. Lumsden, a Public Health Service field officer, to make an epidemiologic study of the ongoing St. Louis outbreak. Lumsden reported that the epidemiologic characteristics of the disease indicated mosquito transmission, and he provided evidence that *Culex pipiens* breeding in local sewage ditches was the species involved. However, other investigators thought that the data were insufficient to prove such a hypothesis and favored the idea of person-to-person transmission instead. Lumsden's report was shelved at the time, but his conclusions were corroborated by later studies.

In 1941, in the same Yakima Valley studies in which WEE virus was first isolated from *Cx. tarsalis*, Hammon et al.

(109, 188) isolated SLE virus from the same mosquito species. Pathologic and clinical characteristics of SLE disease in concomitant human cases and the incubation period were defined. Laboratory animal host systems were developed, studies of disease epidemiology were begun, and the geographic distribution was demarcated. Their work indicated that *Cx. tarsalis* was to SLE virus in the western environment what *Cx.pipiens* was to this virus in the East. SLE virus subsequently was shown to be a member of the group B arboviruses (i.e., genus *Flavivirus*, family *Flaviviridae*). To avoid confusion at this point, it seems appropriate to clarify the taxonomy of these arboviruses.

Studies mainly by Havens et al. in the early 1940s (113), later extended by workers at the Rockefeller Foundation Virus Laboratory (225), demonstrated that the viruses then recognized as arthropod-borne could be separated by serologic tests into at least two groups. Using hemagglutination inhibition and neutralization assays, Casals and coworkers showed that WEE and EEE viruses are related but distinct and suggested that they, as well as Venezuelan equine encephalitis (VEE) and Sindbis viruses, form a group they called group A. A second interrelated group of viruses, including dengue-1, dengue-2, Ilheus, Japanese encephalitis, Ntaya, SLE, Uganda S, West Nile, and yellow fever, were categorized as group B (55). These seminal antigenic studies were the foundation of the group concept and the first steps taken toward a logical, Linnaean-like taxonomy of viruses in general. Subsequent electron microscopic, physicochemical, and genetic studies led to placement of the group A and group B arboviruses together in a virus family, *Togaviridae*. Later, more advanced studies revealed that differences in virion structure, gene sequences, and replication strategy of these viruses justified placement of the former group A arboviruses into a new genus, *Alphavirus*, of the family *Togaviridae* and the establishment of a new family, *Flaviviridae*, and genus, *Flavivirus*, to embrace the former group B arboviruses (254, 255).

In 1943, Hammon, Reeves, and coworkers (108, 110) isolated a new virus from *Aedes melanimon* (formerly *Aedes dorsalis*) mosquitoes in Kern County, Calif., and gave it the name California encephalitis virus. This virus was associated with three cases of encephalitis in humans living in the San Joaquin Valley (108). At the time, the new virus was considered to be one of a kind, as it was unrelated to either group A or group B arboviruses. Eventually, it was shown to be related to a virus isolated later by Eklund and coworkers from *Aedes trivittatus* mosquitoes collected near Bismarck, N.D. (trivittatus virus), providing a basis for a CAL serogroup (family *Bunyaviridae*, genus *Bunyavirus*). Because, by taxonomic convention, the first discovered virus of a serogroup becomes the prototype member of that group, California encephalitis virus is the prototype of the CAL serogroup. Slovakian arbovirologists isolated the CAL serogroup virus Tahyña from mosquitoes and from febrile humans (14), but it was the isolation of LAC virus by Thompson and his associates in 1964 that established CAL serogroup viruses as important human pathogens. The original isolate of LAC virus was from the brain of a 4-year-old Minnesota girl who was hospitalized and died in LaCrosse, Wis., 4 years earlier (229). LAC virus is now recognized as a human pathogen of considerable importance and has been isolated many times from a variety of mosquito species, but principally *Aedes triseriatus*, in the upper midwestern and eastern United States.

Two other arboviruses indigenous to North America have been found to cause serious human illness. One is POW virus, and the other is CTF virus; both are transmitted by ticks but each has quite distinct characteristics. POW virus is a flavivirus, more closely related to the tick-borne flaviviruses of Europe (Central European encephalitis and louping ill) and Asia (Russian spring-summer encephalitis) than to the mosquito-borne flaviviruses, such as SLE (53). Although POW virus was first isolated from a pool of *Dermacentor andersoni* ticks collected in 1952 in Colorado (227), it was not named and identified until it had been isolated from the brain of a 5-year-old boy who died with encephalitis in Ontario, Canada, in 1958 (155). CTF virus, although also isolated from *D. andersoni* ticks, is totally unrelated to POW virus. It is, instead, a member of the family *Reoviridae*, to be discussed later.

In the mid-19th century, settlers and visitors to the Rocky Mountain region of North America mentioned "mountain fever" as a cause of morbidity and occasional mortality. Mountain fever likely was any of a number of illnesses, including typhoid fever, Rocky Mountain spotted fever, CTF, and other febrile illnesses (25). The subsequent association of fever, chills, headache, myalgia, and arthralgia with onset in spring and summer in the Rocky Mountain region suggested a connection between the illness and transmission by ticks (21). Florio and coworkers isolated CTF virus from humans and from *D. andersoni* ticks and demonstrated that this virus is the etiologic agent of the disease (91–94). When the maintenance cycles of CTF virus in natural foci in Colorado (82) and Montana (31) were better understood, it was apparent that the virus and, likely, the disease were more widespread than first recognized. Table 1 lists, by taxon, serogroup, principal vector, geographic area, disease syndrome, and recommended biosafety level, the indigenous arboviruses of Canada and the United States causing human disease.

GEOGRAPHIC DISTRIBUTION

WEE Virus

WEE virus has been found only in the Western Hemisphere. Although serologic surveys suggested that WEE virus is distributed throughout the Americas, and perhaps elsewhere, precise identifications have shown that many of these serologic reactions were due to antibody to closely related viruses (40). Neutralization tests and molecular studies have shown that the WEE complex is composed of at least four closely related viruses in North America: WEE, Highlands J, Fort Morgan, and Buggy Creek. When mentioned here, WEE signifies WEE virus in the strict sense and no other unless specifically mentioned by name. Highlands J, Fort Morgan, and Buggy Creek viruses are not known to cause human disease, although Highlands J virus was once isolated from the brain of an encephalitic horse in Florida (130).

WEE virus has been isolated from Argentina (45, 191) to western Canada (243), with antibody studies suggesting that the virus also occurs in northern Canada (32). Some Argentine virus isolates were shown to differ substantially from the prototype strain of WEE virus by neutralization tests and to be distinct by RNA oligonucleotide mapping. The vector relationships of these strains indicate that they are enzootic viruses, not associated with equine disease. The true WEE virus in South America has been experimentally transmitted by *Aedes albifasciatus* mosquitoes, thus implicating members of this species as vectors (11).

Periodic epizootics since 1908 in Argentina and isolation

TABLE 1. Arboviruses indigenous to Canada or the United States causing human disease, listed by taxon, serogroup, principal vector, geographic area of disease or virus isolation, disease syndrome, and recommended biosafety level

Serogroup and virus	Vectors	Geographic area	Disease syndrome	Recommended biosafety level[a]
Group A (family *Togaviridae*, genus *Alphavirus*)				
EEE	Mosquitoes	East Canada, east U.S.	Encephalitis	2V
Everglades	Mosquitoes	Florida	Fever, encephalitis	3V[b]
WEE	Mosquitoes	West Canada, west U.S.	Encephalitis, fever	2V
Group B (family *Flaviviridae*, genus *Flavivirus*)				
POW	Ticks	Canada, north U.S.	Encephalitis	3
SLE	Mosquitoes	Canada and U.S.	Encephalitis, fever	3
CAL (family *Bunyaviridae*, genus *Bunyavirus*)				
California encephalitis	Mosquitoes	West U.S., west Canada	Fever, encephalitis	2
Jamestown Canyon	Mosquitoes	U.S., Canada	Fever, encephalitis	2
LAC	Mosquitoes	Midwest, east, south U.S.	Fever, encephalitis	2
Snowshoe hare	Mosquitoes	Canada, north U.S.	Fever, encephalitis	2
CTF (family *Reoviridae*, genus *Coltivirus*)				
CTF	Ticks	West U.S., west Canada	Fever, myalgia	2

[a] V, It is suggested that work with this virus be done by individuals vaccinated against and with demonstrated antibody to the virus. Without such vaccination, the next higher containment level is recommended.

[b] Work with this virus should be done at containment level 3, using HEPA filtration of all exhaust air prior to discharge to the outside.

of virulent WEE virus strains indicate that, throughout its range, WEE virus can be an equine pathogen, but human disease in South America occurs less frequently and with less severity than in North America. Isolations of WEE virus from mosquitoes, wild birds and mammals, horses, and humans in California, Washington, North Dakota, South Dakota, Minnesota, Wyoming, Colorado, Nebraska, Montana, New Mexico, Utah, and Kansas in the United States and the provinces of Manitoba, Saskatchewan, Alberta, and British Columbia in Canada (7, 64, 191, 239) attest to the wide distribution of this virus in western North America. Isolates of WEE virus have been obtained from naturally infected snakes, frogs, and tortoises in Saskatchewan (Canada), Utah, and south Texas (33, 100, 219), and experimentally infected garter snakes developed viremias that persisted through hibernation and were of sufficient titer to infect mosquitoes that fed on them (226). Further efforts to extend these findings in field and laboratory studies have given mixed results, and studies to determine whether WEE virus actually overwinters in various poikilotherms have not provided convincing evidence (189).

EEE Virus

EEE virus has been isolated from humans, equines, wild birds, a variety of small mammals, and mosquitoes in Quebec and Ontario, Canada, and from Florida to New England in essentially all of the United States east of the Mississippi River (154, 176); it also has been isolated in Minnesota, South Dakota, Texas, and Mexico. The first recorded outbreak of EEE in Ohio horses occurred in 1991, suggesting the possibility that human disease could also occur there (180). Periodic outbreaks of EEE have been recorded in many of the Caribbean islands, including Cuba (215) and Hispaniola (41). EEE virus infections in equines have been recognized in Cuba for more than 50 years (175); however, associated human illnesses are rare. The virus has been isolated in Cuba from equids, birds, rodents, and mosquitoes during epizootic and interepizootic periods but never from human cases. EEE virus isolates also have been obtained from various sources throughout much of South America, including Venezuela (240), Brazil (78), Peru, and Argentina, but, as mentioned below, South American varieties of EEE virus are of less antigenic uniformity and appear to be less virulent for, or less likely to infect, humans.

VEE Virus

Isolation of the causative agent of VEE virus was reported in 1939 (142). This was the first of a series of closely related viruses forming the VEE complex, which occur only in the Americas. They may be distinguishable antigenically by use of kinetic hemagglutination tests with infection-immune sera from spiny rats (*Proechimys semispinosus*) or other infection-immune sera (167, 258) or with antibody prepared against envelope glycoproteins; anti-E1 is group reactive, and anti-E2 is type specific (96, 134). There are at least six VEE complex virus subtypes, which have been numbered I through VI; varieties IAB, IC, ID, IE, IF, IIIA, IIIB, and IIIC also are recognized. VEE IAB and VEE IC viruses are epidemic varieties. On the basis of both epidemiologic evidence and experimental infections, other VEE complex viruses generally are considered enzootic viruses. Substantial and extensive studies of these viruses, far too extensive to be covered in a review such as this, have described the epidemiology and natural history of the VEE complex viruses. They have been well reviewed (186).

VEE II virus, also known as Everglades virus, appears to be limited to the Everglades of south Florida, where it is maintained in an enzootic habitat in a cycle involving wild rodents and *Culex* mosquitoes of the subgenus *Melanoconion*. Experimentally, it failed to produce overt disease in horses (114). Only two or three human cases of Everglades virus infection have been recognized thus far, despite high antibody prevalence in long-term residents of south Florida, suggesting that the low virulence for horses also applies to humans.

The only known incursion into the United States (Texas) of an epidemic VEE virus (subtype IAB) was in 1971, by extension of an epidemic of huge proportions, involving thousands of humans and equids (241), including zebras in zoos (35), that appeared to have begun in 1969 at the

Peru-Ecuador border. Because of the comprehensive equine vaccination campaign of 1971 in south Texas, cross-protection by antibody against WEE and EEE viruses (48), a combination of both, or other factors, the epidemic was extinguished. VEE IAB virus continued to cause isolated outbreaks and cases continued to be reported in Mexico until 1972, but only anecdotal and unsubstantiated accounts of VEE were reported in the hemisphere until early in 1993, when a VEE outbreak occurred in Venezuela. In recent months, VEE IE virus, heretofore considered equid avirulent, was isolated from equids during an outbreak in Chiapas, Mexico (4). It is unlikely that this occurrence has immediate implications for the United States and Canada because of the absence of VEE IE virus here and the peculiarity of the natural cycle of this virus. Nevertheless, the reemergence of VEE as an equid disease, after its apparent nearly 20-year absence, must be considered instructive because, at present, there is no plausible explanation for the periodic reappearance of these viruses, although there have been many hypotheses.

SLE Virus

Widely distributed in the Americas, SLE virus has been isolated from southern Canada to Argentina, but most human illness due to this virus has been seen in the central and eastern states of the United States. Virus isolations from humans, wild birds, and mosquitoes have correlated with outbreaks of human disease, with increases in antibody in wild or sentinel birds, or with both. Human infections in North America have been documented in western and eastern Canada, in Mexico, and in every state in the United States except Maine, New Hampshire, Massachusetts, Rhode Island, and South Carolina (164, 174). During the 1940s, disease caused by SLE virus was recognized in patients in the Pacific coastal states, and the virus was isolated from mosquitoes collected there (109).

LAC Virus

Several of the CAL serogroup of bunyaviruses occur in North America (5, 10, 220), but LAC virus is medically the most important (233). The upper midwestern states of the United States continue to report most of the human LAC virus infections and virus isolations. Whereas this may in part reflect greater surveillance efforts, it is likely that the ecology and natural history of this virus limit its distribution to areas with hardwood forests, eastern chipmunks (*Tamias striatus*) or other small mammals, and *Ae. triseriatus* mosquitoes (see below), all attributes of the states reporting high incidences of LAC virus isolations. Even within states, distribution is related to availability of breeding sites for the arthropod vector. LAC virus isolations thus far have been made in 13 states; laboratory-confirmed LAC virus infections with illness have been shown to occur in 24 states, with decreasing frequency from north to south. Human infections with LAC virus have not been diagnosed, and LAC virus has not been isolated outside the United States (34, 128). The closely related snowshoe hare virus causes rare symptomatic infections of humans in Canada; most of the fewer than 20 known cases have been diagnosed in Quebec and Ontario (5).

POW Virus

Although POW virus was first isolated from two humans (the original isolate in Ontario and one in New York State) and from *Ixodes* ticks in Canada and the United States (6, 7), and thus is considered a New World virus, its subsequent isolation from ticks and mosquitoes in the former U.S.S.R., including the maritime region (145), has led us to reconsider its natural history and geographic distribution. Isolates of POW virus have been obtained in the United States from hard ticks, principally *Ixodes marxi* and *Ixodes cookei* in the East and *Ixodes spinipalpus* (and *D. andersoni*) in the West, and from various wild and domestic vertebrates collected in Ontario, Canada, and in Colorado, California, Connecticut, Massachusetts, New York, South Dakota, and West Virginia (6, 181). Human cases of POW disease have been documented in Quebec and New Brunswick, Canada, and in Pennsylvania, and human serologic surveys have suggested past infections (possibly subclinical) with POW or a closely related virus in Alberta, British Columbia, and Nova Scotia, Canada, in Maine, and perhaps in Mexico and the People's Republic of China and some other parts of Asia. Despite this evidence of widespread activity, however, overt human disease appears to be limited to or recognized only in southern Canada and the northern tier of states of the United States.

CTF Virus

The distribution of CTF virus roughly approximates that of the vector tick, *D. andersoni*. It has been isolated from humans, ticks, or both in Colorado, Utah, Montana, California, Wyoming, Idaho, Oregon, South Dakota, Washington, New Mexico, and Nevada and also in southern Alberta and British Columbia, Canada (25, 63). Many of these areas include vacation meccas, frequented during the tick season (spring and early summer) by vast numbers of tourists. Furthermore, CTF virus has been isolated from humans vacationing or travelling through these states. Physicians should be aware of the possibility of CTF in febrile patients returning from these regions.

LABORATORY VIROLOGY

DNA virus replication has an absolute requirement for complementarity of nucleic acid strandedness. However, when single-stranded RNA viruses replicate, errors in translation can occur without correction. The diversity of sequences of closely related RNA viruses attests to the frequency of single base substitutions among these viruses, attributed to poor fidelity because of the lack of proofreading enzymes (117). This poor fidelity of replication allows mutations to occur at a much higher rate in RNA than in DNA viruses (117). Because WEE, EEE, SLE, POW, LAC, and CTF viruses are RNA viruses, genetic variation is common, and there are increased opportunities for selection of more fit genotypes, as well as diversity due to chance alone. Under such circumstances, it is not surprising to find genotypic, and therefore phenotypic, variations. Selective pressures brought to bear by ecologic circumstances and characteristics of the natural cycles apparently limit the variants that can persist. Nevertheless, differences in genotypes, antigenicity, and virulence have been recognized for these viruses. These differences and their possible significance for humans will be discussed separately, by virus. Mention of virus names follows the guidelines established by the International Committee for Taxonomy of Viruses (97, 237). The following antigenic terms also are used (50). A serogroup is composed of two or more viruses, distinct from each other by quantitative serologic criteria (fourfold or greater differences be-

tween homologous and heterologous titers of both sera) in one or more tests but related to other viruses of the serogroup by some serologic method. In current practice, the first discovered virus of a newly recognized serogroup lends its name to that serogroup. A complex is a subset of closely related viruses within a serogroup. Individual agents, referred to as viruses or types, are antigenically related but easily separable by one or more serologic tests (fourfold or greater differences between homologous and heterologous titers of both sera). Subtypes are virus isolates separable from the type virus by at least a fourfold difference between the homologous and heterologous titers of one, but not both, of the two serum samples tested. Varieties are those isolates that can be differentiated only by the application of special tests or reagents, such as kinetic hemagglutination inhibition, monoclonal antibody assays, and infection-immune sera from experimentally infected animals.

WEE Virus

There are at least 26 members of the virus family *Togaviridae*, genus *Alphavirus* (50). Many of the New World alphaviruses cause encephalitis; most of the Old World alphaviruses more typically cause fever, rash, and arthralgia. As with other members of the genus, virions of WEE virus are spherical, enveloped particles, 60 to 70 nm in diameter. They contain one segment of single-stranded, positive-sense RNA (molecular weight, 4×10^6) and proteins arranged in an icosahedral configuration. The nucleocapsid envelope is a lipid bilayer derived from the plasma membrane of the host cell; the nucleocapsid (approximately 35 nm) includes the genome associated with the capsid protein. Projecting from and embedded in the bilayer are two virus-encoded envelope glycoproteins (E1 and E2) with molecular weights of 50×10^3 to 59×10^3. WEE virus also has a nonglycosylated capsid protein of 30×10^3 to 34×10^3 molecular weight.

Extensive information about the structure and replicative mechanisms of alphaviruses is available (200). The WEE virus genome contains about 11,700 nucleotides. The 5′ end is capped with 7-methylguanosine, and the 3′ end is polyadenylated. A subgenomic mRNA species formed during replication contains about 4,100 nucleotides identical in sequence to the 3′-terminal third of the genomic RNA. This mRNA is also capped and polyadenylated and serves as mRNA for the synthesis of viral structural proteins.

Certain regions of alphavirus genomes are highly conserved. Only sequences at the 3′ and 5′ ends are required for the genome to be amplified and packaged, and 19 nucleotides upstream of the mRNA and 5 nucleotides downstream are required for production of subgenomic RNAs. The 19 upstream nucleotides are conserved among all alphaviruses, but only 2 nucleotides downstream are conserved.

Glycoprotein spikes on the virion function in attachment to cells. Entry to the cytoplasm usually is by endocytosis: bound virus accumulates in depressions that are endocytosed to form coated vesicles. These vesicles then are uncoated and form endosomes which, by their acidity, prompt fusion of the viral and vesicle membranes. Once released from the endosome, alphaviral genomic RNA is translated, transcribed, and replicated. An AUG codon 59 nucleotides from the 5′ cap site of the 49S (nonstructural) RNA is the site for initiation of translation.

Trent and Grant (230) compared WEE complex viruses by immunochemical and oligonucleotide fingerprinting techniques; their results supported and extended previous serologic findings. Hahn and coworkers (107) sequenced the 3′-terminal 4,288 nucleotides of the RNA of WEE virus. The sequences of the capsid protein and of the (untranslated) 3′-terminal 80 nucleotides of WEE virus were shown to be closely related to the corresponding sequences of the New World alphavirus EEE virus, whereas the sequences of glycoproteins E2 and E1 of WEE virus were determined to be more closely related to those of the Old World alphavirus, Sindbis virus. From their determinations and the results of elegant comparisons of molecular sequences of several other alphavirus genomes, they concluded that alphaviruses have descended from a common ancestor by divergent evolution. They deduced that WEE virus is a recombinant of a predecessor of Sindbis virus and a predecessor of EEE virus and that WEE virus has the encephalitogenic properties of EEE virus and the antigenic specificity of Sindbis virus. In addition, comparisons of numerous alphavirus genomic sequences and the proteins they specify have provided sufficient information to propose a "family tree" for viruses of this genus (147). Until the relationship of the WEE complex representative in the eastern United States, Highlands J virus, is understood, however, the picture will not be complete.

EEE Virus

EEE virus shares many of its characteristics with the alphavirus WEE virus. Chang and Trent (59) have cloned the 26S mRNA and most of the nsP4 encoding regions of the EEE viral genome, and Volchkov et al. (238) have cloned its 42S RNA. Excluding the poly(A) tail, the 26S mRNA region is 4,139 nucleotides long and has the same general organization as that of other alphaviruses, as does the 42S RNA. A highly conserved region composed of 19 nucleotides, the putative transcriptase recognition site for 26S mRNA synthesis, is present at the 26S/42S junction region of the 42S genomic RNA. The four putative posttranslational cleavage sites used to generate the proteins are conserved. Comparison of the deduced amino acid sequences of the polyproteins of five alphaviruses, not including WEE virus, suggested that EEE virus is more closely related to VEE viruses than to the other three viruses.

Phylogenetic analyses of RNA nucleotide sequences of VEE complex viruses by Weaver et al. (247) suggest that all were descended from a common ancestor. These authors further argue that subtype II (Everglades) and variety ID enzootic viruses form a monophyletic group that also includes the IAB and IC (epizootic) viruses. Everglades virus evidently diverged from this ID lineage and colonized North America about 100 to 150 years ago, after which variety IAB and IC epizootic viruses diverged. These results suggest that the source of epizootic VEE viruses was the variety ID enzootic virus lineage, which occurs in northern South America and Panama. Even if variety IAB and IC viruses are extinct, recent multiple emergences of epizootic viruses from an enzootic lineage suggest that other epizootic VEE viruses may evolve again in the future. The close genetic relationship of Everglades virus to the variety ID lineage also implies the potential for emergence of equine-virulent VEE viruses in Florida.

SLE and POW Viruses

Virions of viruses of the family *Flaviviridae*, genus *Flavivirus*, are spherical, enveloped, and approximately 40 nm in diameter with a 30-nm core. The genome is composed of

positive-sense, single-stranded RNA. The virion envelope contains one protein (E) that may be glycosylated and one nonglycosylated protein (M). The RNA is enclosed in a capsid composed of a single polypeptide (C). The E protein is associated with hemagglutinating and neutralizing activities and contains at least three antigenic determinants: one is serotype specific, one is complex specific, and one is flavivirus group reactive (166, 195, 200, 255). The 66 recognized flaviviruses are divided into seven serologic complexes on the basis of cross-reactivities in neutralization tests.

Most flaviviruses of medical importance are in one of three complexes: Japanese encephalitis complex (including Japanese encephalitis, West Nile, SLE, and Murray Valley encephalitis viruses); tick-borne encephalitis complex (including POW, Russian spring-summer encephalitis, Kyasanur forest disease, and Omsk hemorrhagic fever viruses); and dengue complex (dengue-1 to -4 viruses). Yellow fever virus is antigenically distinct.

LAC Virus

Five genera make up the family *Bunyaviridae*: *Bunyavirus*, *Nairovirus*, *Phlebovirus*, *Hantavirus*, and *Tospovirus*. Among the members of this family are LAC, Oropouche, and Akabane viruses (genus *Bunyavirus*), Congo-Crimean hemorrhagic fever and Nairobi sheep disease viruses (genus *Nairovirus*), Rift Valley fever and the sandfly fever viruses (genus *Phlebovirus*), Hantaan virus (Korean hemorrhagic fever) and the emerging viruses of the current western U.S. epidemic (genus *Hantavirus*), and tomato spotted wilt (genus *Tospovirus*). There are more than 258 recognized members of this remarkably diverse family of viruses, at least 167 of which are members of the genus *Bunyavirus* (bunyaviruses). Members of the family *Bunyaviridae* have three segments (large, L; medium, M; and small, S) of single-stranded RNA; intact virions are spherical or oval, enveloped, and 90 to 100 nm in diameter. They have glycoprotein surface projections; lipid makes up 20 to 30% of the virion by weight and forms part of the lipoprotein envelope, which is cell derived. Carbohydrate makes up 2 to 7% of the weight of the virion and is incorporated as a component of the glycoproteins and glycolipids (201).

Virions consist of a unit membrane envelope with spikes surrounding a somewhat unstructured interior from which a helical, 2.5-nm-wide nucleocapsid can be extracted. Constituent synthesis takes place in the cytoplasm, and morphogenesis occurs without prior core formation by budding directly into the Golgi complex and vesicles of infected cells (179). Host RNA sequences prime viral mRNA translation, involving a process similar to cap snatching in orthomyxoviruses (138). No enzymatic function has been associated with the envelope glycoproteins.

The three negative-sense RNA species of bunyaviruses are L RNA (2.7×10^6 to 3.1×10^6 [≈7,000 bases]), M RNA (1.8×10^6 to 2.3×10^6 [4,450 to 4,540 bases]); and S RNA (0.28×10^6 to 0.50×10^6 [850 to 990 bases]); the RNA makes up 1 to 2% of the total virion by weight. Differences exist between terminal nucleotide sequences of gene segments of viruses of different genera within the family.

The 3′-terminal nucleotide sequences of L, M, and S gene segments of bunyaviruses are (3′ to 5′) UCAUCACA UGA . . . , and the 5′-terminal nucleotide sequences of M and S gene segments are (5′ to 3′) AGUAGUGUGCU. . . . Thus, the terminal 11 bases are complementary in inverted order, so that the two ends of each segment can form a hydrogen-bonded panhandle, at least under laboratory conditions. Sequence (genotypic) differences between individual viruses are reflected in antigenic (phenotypic) differences between them and allow for serologic characterizations.

Virions usually contain four structural proteins: two external glycoproteins (G1 and G2), a nucleocapsid protein (N), and a large protein (L), which is presumed to be a transcriptase. A single open reading frame in the M RNA encodes the glycoproteins, which are cotranslationally cleaved to G1 and G2. Estimated M_rs are as follows: G1, 108×10^3 to 120×10^3; G2, 29×10^3 to 41×10^3; N, 19×10^3 to 25×10^3; and L, $\approx 200 \times 10^3$. Both glycoproteins and a 15×10^3- to 18×10^3-molecular-weight nonstructural protein are derived from M RNA; the N protein and a nonstructural protein are coded in overlapping reading frames by the S RNA. The L protein is coded by the L RNA.

Considerable efforts have been made to understand the molecular and genetic bases and mechanisms of LAC virus virulence (102). Biological characterizations indicate that virulence variants exist among selected progeny virus. In the laboratory, strains selected from LAC virus isolates have been shown to differ in titer, pathogenicity by route of inoculation, neuroadaptability, ability to replicate in vertebrate versus invertebrate cell cultures at different temperatures, plaque morphology, thermal stability, and infectivity for mosquitoes. The use of monoclonal antibodies for selecting LAC virus variants has provided a tool for constructing biological maps of antigenic sites on the G1 glycoprotein, which is involved in hemagglutination, neutralization, and fusion.

Segment reassortants have been used to determine the biological role of each segment and have shown that the M RNA segment is a major determinant of peripheral virulence for mice and infectivity for mosquitoes. Distinct sites within the M RNA code for different genetic determinants of biological markers, including subcutaneous and intracranial mouse virulence and oral and intrathoracic infection of mosquitoes (19, 101).

Reassortment can occur between the three segments of two different bunyaviruses. However, reassortment has not been detected between bunyaviruses of different serogroups, and available evidence suggests that bunyavirus reassortment is limited to viruses belonging to the same serogroup. Naturally occurring reassortants of LAC virus have been detected by genotype analyses of field isolates (135). There is inadequate information regarding the molecular mechanisms of persistent infections of arthropod vectors with LAC virus, other bunyaviruses, and other arboviruses. These infections are lifelong in the vectors and may exert little or no untoward effect on them. Lifelong infection of the vector provides substantial opportunity for bunyaviruses to evolve by genetic drift and, under suitable circumstances (i.e., mixed infection), by segment reassortment (15).

CTF Virus

Eight genera and one proposed genus make up the family *Reoviridae*. More than 138 viruses have been arranged in the genera *Aquareovirus*, *Coltivirus*, *Cypovirus*, *Fijivirus*, *Orthoreovirus*, *Orbivirus*, *Phytoreovirus*, and *Rotavirus* or the proposed genus of plant reoviruses. All members of the family are composed of 10 to 12 segments of double-stranded RNA, 6 to 10 proteins, and carbohydrate. Members of this virus family replicate in cytoplasm, and genetic recombination occurs very efficiently by genome segment reassortment. CTF virus is the prototype member of the genus *Coltivirus* (Colorado tick fever virus), established because it

has 12 RNA segments compared with 10 RNA segments in members of the genus *Orbivirus* in which it had been placed. Also, the surface capsomeric structure of the core particles differs from that of the orbiviruses (97). The coltiviruses are spherical particles 80 nm in diameter with two outer capsid shells and a core with no projections. They are labile at pH 3 and are scarcely sensitive to lipid solvents (as opposed to most other arboviruses, which are extremely sensitive to lipid solvents [24]). Replication occurs in cytoplasmic viroplasms; during morphogenesis, regularly structured filaments and tubules form.

Two recognized and nine probable serotypes of this genus are known. The recognized serotypes are Eyach and CTF viruses. Eyach virus has been isolated from adult female *Ixodes ricinus* ticks, *I. ricinus* males, and *Ixodes ventalloi* larvae in Germany and France (129). Comparisons of numerous strains of CTF virus from ticks, humans, and other mammals by neutralization (131), hybridization (23), and polyacrylamide gel electrophoresis (29) indicated that, although multiple genotypes and some antigenic variations exist and, therefore, reassortment of gene segments must occur, most of the 12 genes were highly conserved over the 33-year period represented.

EPIDEMIOLOGY

Viruses evolve by a variety of genetic mechanisms: (i) substitution of single nucleotides by point mutation; (ii) deletion of one or more nucleotides; (iii) duplication of existing sequences; (iv) insertions of nucleotides; (v) substitution of nucleotides, termed recombination when the genome is nonsegmented; (vi) reassortment of segmented genomes; and (vii) rearrangement of nucleotides. When any of such changes take place, genetic variation, a potentially powerful tool for evolution, is generated. Such changes can be silent, in that they have no effect on virus replication and survival; they can be lethal, in that they eliminate a function critical to the ability of the virus to replicate; or they can change the ability of the virus to replicate or confer on it a new ability, such as to extend its host range or to cross phyla, replicate at higher or lower temperatures, escape the effects of the immune system of the host, or otherwise become more competitive than before.

The evolution of arboviruses can, at least in part, be attributed to their vectors, to their vertebrate hosts, and to the viruses themselves. In addition to the possibility of reassortment and recombination in arthropods, genetic rearrangements can occur in vertebrate hosts. Evolution is the combined result of mutation and natural selection. Most mutations that occur in an already balanced system usually are deleterious, but silent or neutral mutations, or adaptive mutations, certainly have an effect on viral genetic variation. Whereas the rates of mutations are relatively consistent and may have little to do with opportunities for virus evolution, most selection is variable and may have much to do with virus evolution. Selection involves the host and its ecology, as well as the virus. A perfect virus-host system would be in complete equilibrium and would be expected to result in production of selectively neutral and homogeneous virus progeny. Whereas reassortment may account for sudden and marked genetic changes in reassortant viruses, nothing new has been created; however, the trafficking of viruses leads to trafficking of viral genes (177).

For adaptive virus evolution to increase, and particularly for us to better notice that it has increased, changes must first occur in the virus-host ecosystem, and then conditions under which genotypic selection could occur can be optimized. Changes in the virus environment represent expanding opportunities for viruses to adapt successfully. Certainly, viruses change due to chance factors such as genetic drift. However, such chance changes often may also provide expanding opportunities to generate new viral gene combinations that will increase the role of genotype selection. It is likely that in the future virus evolution will continue as before, but the opportunities for successful adaptation of evolved viruses, an adaptation dependent on the interaction between genetic drift and natural selection, will increase because of the increasing opportunities we have made available by our poor management practices. In addition, procedures that reduce virus populations may create population bottlenecks that actually increase viral genetic drift and chance gene fluctuations, thereby creating more genetic diversity, on which selection can act to increase viral adaptation. As they should be, evolution and emergence of viruses and viral diseases currently are topics of research (20), policy (144), and conversation.

WEE Virus

WEE virus uses birds as principal vertebrate hosts in its natural maintenance cycle, and birds likely spread progenitor WEE viruses north and south and from continent to continent. This probably accounts for the widespread geographic distribution of the virus and its antigenic and genetic subtypes and variants. When 14 WEE complex viruses were cross-tested by neutralization, it was shown that North American strains McMillan and R-43738, Argentine strain AG80-646, Brazilian strain BeAr 102091, and Siberian strain Y62-33 are antigenic subtypes or varieties of the North American WEE virus Fleming strain. Results of comparisons of these and other WEE complex viruses from other parts of the world indicate parallels between geographic distribution and antigenic relatedness. Viruses of the WEE complex with lesser antigenic differences may develop in discrete ecologic conditions (40).

By 1945, it was thought that during the summer WEE virus is amplified in a silent transmission cycle involving mosquitoes, domestic chickens, and possibly wild birds, from which it can be transmitted tangentially to, and cause disease in, humans and equines. Field and laboratory studies in California have more clearly defined the specific invertebrate and vertebrate hosts involved in the basic virus transmission cycle. The basic transmission cycle involves *Cx. tarsalis* as the primary vector mosquito species and house finches (*Carpodacus mexicanus*) and house sparrows (*Passer domesticus*) as the primary amplifying hosts. Secondary amplifying hosts, on which *Cx. tarsalis* frequently feeds, include other passerine species, chickens, and possibly pheasants in areas where they are abundant. Another transmission cycle that most likely is initiated from the *Cx. tarsalis*-wild bird cycle involves *Ae. melanimon* and the blacktail jackrabbit (*Lepus californicus*). Humans, equids, squirrels, and a few other wild mammal species become infected tangentially with the virus but do not contribute significantly to WEE virus amplification (111).

The 1930 epizootic of WEE in the San Joaquin Valley in California involved about 6,000 horses, with a case fatality rate of about 50% (160). Subsequent severe outbreaks occurred there from 1931 to 1934 and, by 1935, had extended to western Canada (191); a 1933 epizootic in Utah involved 3,958 horses (239). Major epizootics occurred in the United States in 1937, 1938, 1941, 1944, and 1947. During the 1937 to

1938 epizootic, more than 300,000 horses and mules were affected in the United States, and in Saskatchewan, Canada, 52,500 horses were stricken and 15,000 of these died. In 1941, 2,242 human WEE cases were reported in the United States, mostly in North Dakota, South Dakota, Nebraska, and Minnesota, and 1,094 human cases were recorded in western Canada, mostly in Manitoba and Saskatchewan. Human attack rates in the 1941 outbreaks ranged from 22.9 to 171.5/100,000 population, and case fatality rates ranged from 8 to 15%. During the 1952 epidemic (191), attack rates were 50/100,000 humans and 1,120/100,000 equids. Risk factors for human WEE include rural residence (attack rates of 340/100,000 and 61/100,000 in rural and urban areas of residence, respectively, in the 1952 epidemic in Kern County, Calif.); age (highest incidence and most severe disease in members of the youngest age groups, particularly in infants <1 year old [20% of cases occur in this age group and 50% occur in children <10 years old]); length of residence (the longer in residence in an area endemic for WEE virus, the greater the probability of acquiring antibody and the less likely one will develop clinical WEE); and sex (higher proportion of males, possibly due to greater outdoor exposure to infected mosquitoes or possibly due to more effective immune responses by females). Other possible risk factors are occupational exposures, outdoor activities, and proximity to vector breeding sites. Most infections with WEE virus are asymptomatic; the inapparent/apparent infection ratio is age dependent and ranges from about 1:1 in infants <1 year old to 58:1 in children 1 to 4 years old and 1,150:1 in persons >14 years old.

An annual average of 34 (range, 0 to 172) confirmed human cases of WEE occurred from 1955 to 1984 in the United States. States reporting a cumulative total of more than 50 cases in the past 35 years have included California, Colorado, Kansas, Minnesota, North Dakota, Texas, and Utah and Manitoba and Saskatchewan in Canada (7). The most extensive and well-documented WEE epidemic occurred in California in 1952, when 375 confirmed cases and nine deaths were reported (191). In the period 1964 to 1992, a total of 635 human cases of WEE (annual average of 22, a clear decline from the 1955 to 1984 average) were reported to the U.S. Centers for Disease Control and Prevention (CDC), but only 2 cases have been reported since the beginning of 1988, 1 each in Colorado and Kansas (174). The distribution of cases is dependent on the seasonal distribution of the mosquito vector populations, so that most human infections are acquired during June through August, although there is a direct relationship between latitude and onset of epidemics, i.e., the more northerly the site, the later epidemics begin. Human cases of WEE are preceded by equine cases, which provide an early warning system to supplement ongoing virus surveillance programs. Notwithstanding other factors, for epizootic WEE virus transmission to occur, a large vector population is a necessity, although it is not an adequate predictor of outbreaks of human and equid disease.

EEE Virus

As noted above, a single virus makes up the EEE complex of alphaviruses, and only a single subtype, but two varieties of this subtype, is internationally recognized (50). More recently, a second antigenic (39) and genetic (248) subtype of EEE virus was isolated from the cerebrospinal fluid (CSF) of a 6-year-old male with fatal aseptic meningitis. Several standard serologic tests that use polyclonal antibody and indirect immunofluorescence and hemagglutination inhibition tests that use monoclonal antibody were used to determine that this isolate indeed is an antigenic subtype of prototype North American EEE virus. Subsequent genetic studies revealed that two amino acid substitutions in the E2 envelope glycoprotein probably contribute to the antigenic difference between this isolate and other EEE virus isolates with which it was compared.

Casals comprehensively analyzed EEE virus isolates from throughout the Americas, using kinetic hemagglutination tests (54), and concluded that North American and Caribbean isolates are more antigenically homogeneous than are South or Central American isolates but that EEE virus populations in the two areas are distinguishable. EEE virus isolates from birds migrating into the United States at the delta of the Mississippi River in Louisiana showed that the rare EEE virus isolates from northbound birds migrating into the United States were South or Central American varieties (43). It was speculated that viruses incoming to the United States in the early spring do not become established because they arrive when mosquito populations are low.

The taxonomic separation of North and South American varieties of EEE virus has been supported by genetic relationships determined from nucleotide sequences, which indicate that EEE virus in North America persists and evolves as a single lineage (248). Analysis of the recently isolated subtype provided a basis for the antigenic variation but did not provide evidence for the presence of a second lineage in North America.

North and South or Central American varieties of EEE virus exist in relatively discrete ecosystems. In Argentina, an equine epizootic of EEE occurred in irrigated areas of four counties in the province of Santiago del Estero (199). The overall incidence of equine encephalitis was estimated to be 17%, the case fatality rate was 61%, and the inapparent/apparent infection ratio was ≤2.9:1; no human infections were detected. This is fairly typical of the situation with regard to EEE virus in Latin America: isolated equid cases, epizootics at irregular intervals, and few or no human illnesses. *Culex* species mosquitoes have been implicated as epizootic vectors of South American strains of EEE virus.

An exceptional review of the epidemiology of EEE virus has been published by Scott and Weaver (208). Our understanding of the epidemiology of this virus begins with the observation that EEE virus activity is ecologically focal (153) but that outbreaks are related epidemiologically (season, weather, level of vertebrate host population immunity, and mosquito populations). Annual or frequent recurrence of EEE virus in enzootic habitats is the rule, and extension to other areas is the exception. No evidence for a plausible overwintering mechanism has been put forth. Moreover, Watts and coworkers accumulated ecological evidence that mitigates against vertical transmission of EEE virus in mosquitoes at their study site (244). In well-studied areas of New York, New Jersey, and Massachusetts, it has been shown that *Cs. melanura* mosquitoes are the enzootic vectors, transmitting EEE virus between passerine birds, rarely from birds to equids or humans. Equids and humans may be "dead-end" hosts, but the level of viremia in some horses exceeds the minimal titer considered necessary to infect mosquitoes subsequently feeding on them. Epizootics begin in swampy habitats and move outward when viremic birds move; locally migrating viremic birds may move virus to adjacent areas, and distantly migrating viremic birds may disperse virus to areas farther away.

A pivotal phase of the natural cycle of EEE virus is the transfer of virus from enzootic to epizootic vectors and,

from that point, epidemic extension. Considerable field evidence indicates that *Cs. melanura* mosquitoes, which are found in all enzootic foci in North America and which feed principally on birds, do not serve as epidemic vectors. This role is played by mosquitoes such as *Coquillettidia perturbans*, *Aedes vexans*, *Aedes canadensis*, and *Aedes sollicitans*, which more commonly feed on mammals than do *Cs. melanura* mosquitoes. Recent studies in New Jersey have thrown considerable light on the dynamics of transfer of EEE virus from coastal habitats, such as freshwater swamps and adjacent salt marshes, to more inland areas (68). Using banding, radiotelemetry, and color marking, Crans and his coworkers have accumulated evidence indicating that glossy ibis (*Plegadis falcinellus*), which feed in small flocks in fresh- or saltwater marshes, often are found in proximity to mosquitoes carrying EEE virus. These birds roost in trees in freshwater swamps where *Cs. melanura* mosquitoes are abundant and spend the days feeding in salt marsh areas where the potential epidemic vector *Ae. sollicitans* (69) is abundant. The veterinary literature suggests that EEE has existed in New Jersey for at least 90 years but was not recognized in humans until about 1959, when the glossy ibis was introduced there and when New Jersey experienced its first epidemic. It may be that EEE virus existed in enzootic cycles before the introduction of the glossy ibis but that changes in the ecology and the fauna in New Jersey have brought about the current situation.

Other introduced species also may be having an impact on the natural cycles and prevalence of EEE virus. *Aedes albopictus* mosquitoes, originally introduced in 1985 as eggs in used automobile tires from Asia (72), have infested Florida and many other states (172) and may be replacing indigenous mosquito species. In Florida (214), *Ae. albopictus* mosquitoes appear to have become involved in the transmission cycle of EEE virus (162) but likely do not transmit that virus transovarially (204). The isolates of EEE virus recently obtained from *Ae. albopictus* mosquitoes collected in Florida are the first of an arbovirus of proven public health and veterinary importance from naturally infected *Ae. albopictus* in the United States. The widespread distribution of these mosquitoes in Florida and in other areas of the United States where EEE virus is enzootic raises concern that this species may become an epizootic and epidemic vector of EEE virus. The threat indeed is clear. The Florida Department of Health and Rehabilitative Services has confirmed five human cases of EEE in elderly residents of northern Florida where *Ae. albopictus* is known to have become established (57). Although occasional human cases of EEE occurred in this part of Florida before the introduction of this mosquito, there is the distinct possibility that *Ae. albopictus* may become a more effective vector than its predecessors.

Dates of onset of these human illnesses were in mid-June and early July. One patient partially recovered with residual neurologic deficits, two patients were comatose at the time of this report, and two other patients died (57). Efforts thus far to control *Ae. albopictus* have been minimal and unsuccessful. In addition, the exotic mosquito *Aedes bahamensis* is now well established in south Florida, where it is widely distributed in both urban and rural areas east of the Everglades (182). The significance of this development is still unknown.

As might be expected, there is a direct relationship between the amount of information reported about EEE virus and the recognized prevalence of human and equine disease due to this virus. In the United States and Canada, the incidence of illness due to EEE virus in humans since 1955 has ranged from 0 to 36 cases (average, 7) per year. No human cases have been recognized in Canada to date (7). The inapparent/apparent infection ratio in a 1959 outbreak in New Jersey was 23:1, with a clinical attack rate of 1/1,000 population. There may be as few as 17 systemic or asymptomatic infections in children for each encephalitis case; this ratio may be 40:1 in adults, suggesting that EEE virus is more neuroinvasive in the immature central nervous system (CNS). In the period 1964 to 1992, 141 human cases of EEE (annual average, 4.9; as with WEE, a clear decline from the 1955 to 1984 average) were reported to the CDC (174).

Both sexes are equally susceptible to EEE virus infection, albeit there are more clinical infections of males than of females; inapparent infections occur at the same rate for all age groups and both sexes. Risk factors for humans to acquire EEE include age (children <15 years old and adults >55 years old constitute 70 to 90% of cases during outbreaks) and residence or extensive time spent in rural areas, particularly wooded areas near swamps and marshes that serve as vector breeding sites. Certainly, as human populations increase and coincidentally impinge on natural habitats of mosquitoes and their associates, and as subdivisions are built on or abutting swamps where the virus occurs, an increased risk of infection becomes more likely.

SLE Virus

Biological and genetic differences between isolates of SLE virus from North America and South America have been recognized, and the sophistication of epidemiologic studies has thus been enhanced (26, 217, 232). There is a general agreement among results of oligonucleotide patterns, production of viremia in house sparrows, and neuroinvasiveness for weanling mice. Four genetic lineages of SLE virus are found in the United States: one, from the eastern central and Atlantic states including the Mississippi flyway, is most often isolated from *Cx. pipiens-Cx. quinquefasciatus* mosquitoes; two (epidemic and enzootic strains) occur in Florida, where they are transmitted by *Culex nigripalpus* mosquitoes; and a fourth occurs in the western United States and is transmitted by *Cx. tarsalis* mosquitoes. The complexities of these genetic interrelationships have been summarized (232). Partial sequencing of the genome of SLE virus demonstrated homology with others of the Japanese encephalitis complex (231) and provides a means of determining small but significant alterations in genotypes. These techniques can be used to classify SLE virus strains by their geographic origins and are a powerful tool for determining the source of outbreaks. Strains from within a geographically restricted area have been shown to have arisen by genetic drift and to have been introduced from other geographic areas. Such methods have been used to confirm the epidemiologic hypothesis of Luby (151) that epidemic SLE in Dallas, Tex., in 1966 had been caused by virus introduced from the rural cycle involving *Cx. tarsalis*.

That SLE virus persists in certain geographic areas year after year, or nearly so, is evidenced by antibody in resident young birds and mammals and repeated virus isolations from mosquitoes and vertebrates. Because certain species of *Culex* mosquitoes hibernate over winter as adults, considerable effort, particularly in California (189), has been made to obtain evidence that SLE virus overwinters in mosquitoes of this genus. Rare winter isolations of SLE virus have been obtained from *Cx. pipiens* mosquitoes in Maryland (13) and from *Cx. tarsalis* mosquitoes in California (189), and SLE

virus has been isolated from Mexican freetail bats (*Tadarida brasiliensis mexicana*) in Texas (221). However, it is far from clear that any of the above isolations represent an actual overwintering mechanism for SLE virus, or, even if they did, that they would be sufficient to perpetuate the virus perennially. Nevertheless, it is incontrovertible that SLE virus recurs, and it must have some means of doing so. For further details of these studies, the reader is referred to a comprehensive review by Reeves (189). Although rare instances of transovarial transmission have been documented for SLE virus, dengue viruses, and Japanese encephalitis virus, this mechanism has not been shown to be important in their persistence. However, if transovarial transmission is shown for these viruses, it may apply to all other mosquito-borne flaviviruses as well (198).

National surveillance for SLE began in 1955. Since that time, more than 4,000 cases of SLE have been reported to the CDC. The disease occurs in epidemic form at about 10-year intervals. In 1975, an epidemic of SLE affected 1,815 people in the United States and 68 people in Canada (7), but the mean number of cases reported annually in the United States, excluding those occurring in 1975, has been 86 between 1964 and 1992 (range, 4 to 470) (174). Clinical attack rates during epidemics have ranged from 1 to 800/100,000 population. There are several hundred inapparent infections for every apparent infection with SLE virus.

The incidence of SLE in areas of the western United States where SLE is endemic is greater in younger people than in older people, but the opposite is true for SLE in Florida and the eastern central and Atlantic states. This likely is due to cumulative herd immunity in endemic but not epizootic areas rather than to different basic susceptibilities in the two human populations. SLE virus activity can be monitored through surveillance programs that use wild birds (149) or sentinel birds, mosquito collection and testing, and constant monitoring of changing ecologic and environmental circumstances (189). Risk factors associated with SLE are both biological attributes of the human host and increased exposure to virus-infected mosquitoes. Case fatality rates during outbreaks have been estimated at 5 to 20%, but in persons older than 75 years they have approached 70%. In Manitoba, Canada, one human SLE case was reported in 1975 and two cases were reported in 1977 (7). An excellent updated review of the epidemiology and ecology of SLE virus has recently been published (171).

LAC Virus

LAC virus is the most important arboviral cause of pediatric encephalitis in the United States (233). It is transmitted mainly by *Ae. triseriatus* mosquitoes, which breed in tree holes in woodlands and in discarded automobile tires. Its principal vertebrate hosts are eastern chipmunks (*Tamias striatus*), gray squirrels (*Sciurus carolinensis*), and red foxes (*Vulpes fulva*). However, its natural cycle is far more complex than might appear, and this complexity influences the epidemiology of clinical LAC virus infections. Because bunyaviruses have a tripartite genome (three segments of RNA; see above), segment reassortment can and does occur. Genotypic differences are expressed in trivial ways, but such differences can be used to map the distribution of various genotypes (135) and to study the evolution of these genotypes. Further, transovarial (245) and venereal (228) transmissions can occur in the principal arthropod vector of LAC virus, *Ae. triseriatus*, and the virus can overwinter in its eggs (246). The infection of the mosquito's ovarian cells and oocytes results in infection of both sexes of offspring. This not only bypasses the need for a viremic vertebrate host for this generation but provides a mechanism for venereal transmission as well. However, continuous propagation of LAC virus solely by transovarial and venereal transmission, without an occasional or intervening virus meal, has not yet been proven.

The usual vertebrate hosts in the "natural" cycle (chipmunks and squirrels) are readily accessible to the vector mosquito. As already stated, *Ae. triseriatus* is principally a woodlands mosquito that breeds mainly in root cavities of larger hardwood trees. Unfortunately, these preferred breeding sites are not restricted to sparsely inhabited woodlands but may also be abundant in more densely populated suburban areas, literally bringing the virus into one's backyard. *Ae. triseriatus* will also breed in discarded tires and other artificial containers, which increases risk of human infection even more.

Although LAC virus was first isolated, has been best studied, and is most common in the upper midwestern United States, it causes disease in children throughout its entire range. Cases have been recorded in Arkansas, Florida, Georgia, Illinois, Indiana, Iowa, Kentucky, Louisiana, Michigan, Minnesota, Mississippi, Missouri, New York, New Jersey, North Carolina, Ohio, Pennsylvania, Tennessee, West Virginia, and Wisconsin. Most infections occur during the period July to September. From 1963 to 1981, a total of 1,348 (annual average, 71) cases of "California encephalitis" were reported to the CDC (128), and there is reason to believe that this disease is greatly underreported (128). The official term California encephalitis is a poor one, as it is used to designate illnesses that do not necessarily involve the CNS and could be caused by any of the CAL serogroup viruses. From the geographic locations of these cases and the distributions of LAC virus and *Ae. triseriatus* mosquitoes, it is probable that all but a scant few of these 1,348 illnesses were due to infections with LAC virus.

Of the 1,348 cases, Ohio reported 463 (34.3%), Wisconsin reported 329 (24.4%), Minnesota reported 218 (16.1%), and Illinois reported 112 (8.3%), giving a total for these four states of 1,122 cases, or 83.2%. Whereas these numbers undoubtedly indicate the general epidemiologic situation, they probably also reflect, to some extent, the relative effort expended in the states with greater LAC awareness and supportive ongoing programs.

In the period 1964 to 1992, a total of 2,032 cases of California encephalitis were reported to the CDC (174). These numbers do not include three unconfirmed cases reported from states where LAC virus has not yet been isolated. Assuming that essentially all of these infections were caused by LAC virus (and not by Jamestown Canyon or snowshoe hare virus, other CAL serogroup viruses that rarely cause human illness), there has been little change in morbidity over the years. However, credible information on attack rates is lacking. Again, Ohio, Wisconsin, Minnesota, and Illinois reported most of the cases (1,564; 76.9%). The annual number of cases has remained relatively constant at an average of about 70 cases since 1963, when California encephalitis first was made a reportable disease. This pattern of disease differs from those of WEE, EEE, and SLE, in which epidemics occur at regular or irregular intervals but, in some years, virus activity is not evident at all or is barely detected. Such endemicity indicates the stability of LAC virus cycles in nature.

Human LAC virus infections are not distributed uniformly in states where they occur. Rather, they are limited to

particular ecologic areas favorable to the virus-vector-host interactions. Undoubtedly, these areas sometimes straddle state lines, although reports of LAC virus infections may appear to indicate that state boundaries are true delimiting factors, obviously an artifact of local emphasis on testing and reporting. Overt LAC virus infections are more common in individuals of <15 years of age; more than 90% of patients are in this age group (128). In 1981, it was shown that about 64% of illnesses due to LAC virus occurred in males, presumably a reflection of greater exposure to mosquitoes while camping, hiking, and participating in other outdoor activities such as playing in tree houses (128); whether this trend has continued is not known.

The inapparent/apparent infection ratio for LAC virus infection has not been clearly established. On the basis of high antibody prevalence and relatively low disease incidence, one would assume that this ratio is high. It has been estimated that there may be as many as 15,000 infections annually (inapparent and apparent combined) in Indiana alone and 300,000 in the several midwestern states. For children <16 years of age, there may be more than 1,000 infections per reported case (102). On the basis of cases of encephalitis alone, however, excluding inapparent and milder infections, a morbidity/fatality ratio of <26:1 has been estimated for this same clinically susceptible age group (128). Overall, taking all clinical cases of all ages into account, the fatality rate is about 0.3%.

It is clear that Jamestown Canyon (76), snowshoe hare (87), California encephalitis (189), and other CAL serogroup viruses in the United States and Canada infect humans, although rarely. In contrast to the pattern with LAC virus infection, illnesses associated with Jamestown Canyon virus infections have been detected in people >18 years old, the single exception thus far being an 8 year old with a concomitant herpesvirus infection. Snowshoe hare virus infections, on the other hand, have been reported to occur in young people, as with LAC; human clinical illnesses with California encephalitis virus have been too few for age assessment. There are insufficient data on infections by these or by two other possible pathogens of the CAL serogroup, trivittatus and Keystone viruses, to draw conclusions about risk factors or rates. Nevertheless, these viruses occur in discrete cycles (10, 105, 189). Jamestown Canyon virus is transmitted to large mammals by *Culiseta inornata* in the western United States and to deer by *Aedes communis* group and *Aedes stimulans* mosquitoes elsewhere; snowshoe hare virus is transmitted to snowshoe hares (*Lepus americanus*) and ground squirrels (*Spermophilus franklinii* and others) by *Ae. communis* and other aedine mosquitoes; California encephalitis virus is transmitted to blacktail jackrabbits by *Ae. melanimon* and *Cs. inornata* mosquitoes; trivittatus virus is transmitted to cottontail rabbits (*Sylvilagus auduboni*) by *Ae. trivittatus* mosquitoes; and Keystone virus is transmitted to gray squirrels (*Sciurus carolinensis*) and cottontail rabbits (also cotton rats [*Sigmodon hispidus*] in Florida) by *Aedes atlanticus* (possibly also *Aedes infirmatus* in Florida).

The main risk factors for acquiring infections with LAC and other CAL serogroup viruses are the extent of exposure to infected vector mosquitoes and the age group of the person exposed. Generally, a high population density of the vector species with a high infection rate is a determining element.

Alaska is a special case with regard to arboviruses. Although one would assume such a harsh landscape would be a restricted environment, one not conducive to the persistence of arboviruses, the reality is different. No evidence for the presence of WEE, EEE, SLE, or LAC viruses in Alaska has been produced to date; however, many other arboviruses, including snowshoe hare and Jamestown Canyon viruses, occur there, and some have been shown to infect humans without evidence of illness (218). As elsewhere, factors most significantly correlated with presence of antibodies to Jamestown Canyon and snowshoe hare viruses were the amount and duration of fieldwork and a history of travel in certain remote or wilderness areas during the warm season.

POW Virus

Many small mammals serve as hosts for ixodid ticks. Those implicated in the natural cycle of POW virus include marmots (woodchuck [*Marmota monax*]) and snowshoe hares, which assist in amplification of both tick and virus populations. POW virus has been isolated on two occasions from naturally infected foxes (*Vulpes fulva*), but both isolates came from sick animals, an indication that they are unlikely natural hosts. Other isolates of POW virus have been obtained from a red squirrel (*Tamiasciurus hudsonicus*), a white-footed mouse (*Peromyscus leucopus*), and a spotted skunk (*Spilogale putorius*). Their significance in the natural cycle of the virus is unknown; these isolates may reflect only the catholic feeding habits of the tick vectors.

The incidence of human disease caused by POW virus is low, as attested to by 19 symptomatic infections recognized in Canada and the United States before 1981 and 3 (1 fatal) infections since then (6, 7, 90, 122). Thousands of serum samples from humans in Ontario, Canada, and the state of New York, where most of the cases have been recognized, have been tested for antibody to this virus, but only a few have been positive. McLean et al. determined the prevalence of antibody to be 0.5 to 3.3% in northern Ontario and that prevalence rates varied between communities (156). The prevalence of antibody to POW virus is extremely focal, probably reflecting the focality of the virus and, possibly, the focality of the vector tick. As with other tick-borne flaviviruses, POW virus can be transmitted in goat milk. Although no human infections with POW virus have yet been attributed to this route, it would seem unwise to drink unpasteurized milk from goats in areas where this virus has been reported.

The slow spread of the vector ticks, principally *I. cookei* and other *Ixodes* ticks in North America, probably contributes to the focality of POW virus. Focality, however, does not indicate inefficiency of transmission but simply the improbability of human contact with infected ticks that can serve as vectors and which are not detected before they have an opportunity to take a blood meal and infect the unfortunate hiker, woodsman, child, or other person participating in outdoor activities. Infected ticks, brought into the home by cats and dogs, may be a significant mode of epidemiologic spread of POW virus to humans (75, 227).

Of the 19 North American clinical cases (12 males and 7 females) reportedly caused by POW virus, 6 patients were under 6 years old, 10 were 6 to 15 years old, and 1 each were 19, 57, and 82 years old. These statistics indicate that, while individuals of all ages may be susceptible, those in the younger age groups are much more so; children spend much less time in the woods but have a much greater attack rate. Identified risk factors are season of the year (May to December, with peak period of June to September), the amount of time spent in rural areas, and contact with infected ticks.

CTF Virus

CTF virus is transovarially (adult female→egg) and transstadially (larva→nymph→adult) transmitted in *D. andersoni* ticks (94). In a detailed study of CTF virus and CTF in Rocky Mountain National Park, Colo. (summarized in references 25 and 159), increased CTF virus activity was detected at sites with south-facing slopes, open stands of ponderosa pine, and shrubs on dry, rocky surfaces; these landscape characteristics were determined to be fundamental in maintaining the virus. These specific ecologic characteristics were vital to tick populations as they constituted specific habitats for the small mammals on which the ticks depend for both blood meals and shelter. Mammals involved in the natural cycle of CTF virus include the golden mantled ground squirrel (*Spermophilus lateralis*), porcupine (*Erithizon dorsatum*), least chipmunk (*Eutamias minimus*), deer mouse (*Peromyscus maniculatus*), and bushy-tailed woodrat (*Neotoma cinerea*). An excellent review of the ecology of CTF has been published by Emmons (84).

An undoubtedly underreported total of 441 cases of CTF occurred between 1985 and 1989 in the states that had active CTF surveillance programs: Colorado, Montana, Utah, Wyoming, California, and Idaho. An analysis of 606 CTF cases by age and sex (22) revealed that 11.6% (7.3% males and 4.3% females) were 0 to 9 years of age; 16.4% (11.9 and 4.5%) were 10 to 19 years; 23% (18.2 and 4.8%) were 20 to 29 years; 12.7% (10.2 and 2.5%) were 30 to 39 years; 12.8% (8.7 and 4.1%) were 40 to 49 years; 9.6% (6.1 and 3.5%) were 50 to 59 years; 10.9% (7.1 and 3.8%) were 60 to 69 years; and 3.0% (2.0 and 1%) were >70 years of age. In all, 71.5% of cases were in males and 28.5% were in females. Whether the excess cases in males and people aged 20 to 29 years is indicative of more time spent in outdoor activities has not been determined. The primary risk factor for acquiring CTF is exposure to infected *D. andersoni* ticks in areas where CTF virus is endemic. Other factors are being male and aged 10 to 50 years, having an outdoor occupation, having frequent or lengthy outdoor recreational activities, and being exposed to ticks in mountain areas where CTF virus is endemic in the period April through June. Receipt of blood by transfusion from donors living in or traveling through an area of endemicity and accidental exposure to the virus by laboratory workers are minor factors that should also be considered (25).

VECTORS, VERTEBRATE HOSTS, AND TRANSMISSION

For arboviruses to be transmitted, a complex series of interactions among virus, arthropod, and vertebrate host and a range of ecologic conditions must be in place. At each step in the process, biochemical triggers and molecular sequences facilitate reactions and provide the backbones upon which new molecules are built. From afar, we see grasses, drainage ditches, rocky outcroppings, salt marshes, woodlots, and the occasional mammal or bird, amphibian or reptile, equine, or bovine. Observing more closely, we see mosquitoes and ticks and midges and sand flies, and we recognize that these communities have ecosystems of their own. Most of the time we cannot detect the comings and goings of life forms. It is when viruses within the most microscopic aspects of such ecosystems kill or otherwise affect humans, livestock, or the wild birds and mammals we favor that we realize that "something is happening," and, if the vertebrate is sufficiently significant (someone's child, grandparent, or working horse), we might take action.

A biological transmission cycle of an arbovirus can be conceived of as beginning with an uninfected, susceptible, and competent hematophagous arthropod taking a blood meal from an infected vertebrate. If there is no "mesenteronal escape barrier" (a not yet understood mechanism that prevents virus from moving from infected gut tissue to other tissues), ingested virus then replicates in the arthropod's posterior midgut and is disseminated to other tissues (e.g., WEE alphavirus [118, 141]). A mesenteronal escape barrier exists for epizootic, but not enzootic, Central American VEE virus strains (249), indicating how specific and how complicated these mechanisms are. Ludwig et al. (152) contended that because arboviruses are exposed to two notably different environments, vertebrate cells and arthropod cells, processing by host enzymes might influence the capacity of these viruses to adjust to such disparate conditions. They then showed that exposure of LAC bunyavirus to proteolytic enzymes, such as those in the mosquito midgut, increased virus affinity for mosquito cells. The enzymes they used removed the G1 glycoprotein but left the G2 glycoprotein intact. They concluded that processing of LAC virus glycoproteins in the mosquito midgut "may be necessary to expose attachment proteins on the virion surface before attachment to, and infection of, midgut cells can occur" (152) and suggested that this paradigm may answer questions about the molecular basis for midgut infection barriers and susceptibility to arbovirus infections.

Irrespective of the mechanism by which virus reaches the hemocoel of the insect, it is then transported in the hemolymph or along nerves to various organs and tissues, including the salivary gland, where it replicates. Then, within a few days of taking its blood meal and if there is no "salivary gland escape barrier," the arthropod can transmit virus to the vertebrate host on which it subsequently feeds. Under certain circumstances, e.g., when arbovirus populations are amplified during an outbreak, in areas ecologically unusual for a particular virus, or during drought or other conditions incompatible with long-term persistence of an arbovirus, an atypical arthropod species may be infected, as evidenced by WEE virus isolates from *Aedes dorsalis*, *Aedes campestris*, and *Culex erythrothorax* (64, 124). However, such arthropods may be incompetent transmitters and only peripherally involved in viral transmission (189). Tabachnick (222) has made an excellent case for continuing evolutionary studies of arboviruses and their vectors, suggesting that such studies may provide methods for understanding vector-host interactions.

Transstadial, transovarial, and venereal transmission also can take place. Obviously, as indicated earlier, many variables of physiology and biology of the arthropod and physical and genetic characteristics of the viruses must converge for transmission to occur, and it is likely that there are more factors that we do not know than that we do know. The reader is referred to other more extensive and explicit reviews and other publications for further details (80, 112, 161, 213, 214, 216). However, it can be stated unequivocally that an arthropod's susceptibility to arboviruses and its ability to transmit them are under the control of vector genes (140, 223).

A number of factors influence the feeding preferences of successful arthropod vector species; these include the attractiveness of the susceptible vertebrate species to the arthropod, the degree of tolerance of the vertebrate for arthropod feeding, the distribution and abundance (availabil-

ity) of the vertebrates, and the characteristics of the climate and local habitat (202). Once an appropriate vertebrate is fed on by an infected arthropod and is infected, it becomes viremic and is stimulated to produce antibody. The antibody serves to quench the viremia but also may serve to modify the virus population by suppressing some phenotypes but not others. Because phenotypes are simply the expressions of genotypes, genetic selection can be the result. Obviously, this does not happen often enough to affect entire virus populations (arbovirus populations are remarkably stable genetically), but when it does occur, changes conferring selective advantages on those populations can be the result.

The principal (but not exclusive) vertebrate hosts of WEE, EEE, and SLE viruses are birds. Episodes involving endangered whooping cranes (*Grus americana*) and exotic emus (*Dromaius novaehollandiae*) (235) indicate that birds of many species are susceptible, though not necessarily useful hosts to the virus. Seven of 39 captive whooping cranes died from EEE virus infections in Maryland; none of 240 sandhill cranes (*Grus canadensis*) housed with the whooping cranes either died or appeared sick (77). Clinical EEE virus infections were diagnosed in individuals of a flock of emus in southeastern Louisiana. The outbreak was concurrent with an equid outbreak of EEE in the area. No nervous system lesions were observed, but the attack rate was 76% and the case fatality rate was 87%. Isolates of EEE virus have been obtained from two sick emus in 1993; clinical signs included bloody diarrhea, and pathology was reported as hemorrhagic enteritis (4). Recently, WEE virus was isolated from brain tissue of two emus in California (4).

Surveillance of arbovirus activity at local and state levels in some states entails periodic testing of serum samples from sentinel chickens and wild birds to assess antibody prevalences (149, 158, 189). Wild birds can be monitored for antibody during interepidemic and interepizootic periods (85, 157). Also, the relative involvement of a particular bird species in urban and rural epidemics can be estimated by examining two variables: antibody prevalence in the given species, and the proportion of the total bird population represented by that species (150). Although birds are useful as sentinels for surveillance, results must be interpreted with care. It has been shown that detectable neutralizing antibody in some native birds is ephemeral, whereas in others it is extremely long lasting.

Because the natural cycles of LAC, POW, and CTF viruses involve mammals and not birds, different skills and techniques are used for field study of these viruses than for the viruses of WEE, EEE, and SLE, but the study plans are essentially the same. Whereas wild birds are generally netted, a relatively simple process, the principal vertebrate hosts of LAC, POW, and CTF viruses (respectively, eastern chipmunks and gray squirrels; marmots and snowshoe hares; and golden mantled ground squirrels, porcupines, least chipmunks, deer mice, and bushy-tailed woodrats) must be trapped in a variety of ways, some of which are tedious and expensive.

An apparently novel mechanism has been recently discovered for transmission of Thogoto virus, an apparent orthomyxovirus isolated from ticks in Africa, Europe, and Asia (125, 126). Uninfected *Rhipicephalus appendiculatus* ticks acquired virus when they were co-fed with infected *R. appendiculatus* on normal (uninfected, apparently healthy) guinea pigs, which are refractory to infection with this virus. This tick-to-tick transmission was potentiated by factors associated with the salivary glands of ticks (saliva-activated transmission). Thus, a vertebrate apparently refractory to infection by this arbovirus can still play an important role in the epidemiology of the virus. Further, adult *R. appendiculatus* ticks are more efficient than adult but not nymphal *Amblyomma variegatum* ticks in mediating this nonviremic transmission of Thogoto virus. Whether there is a similar mechanism for POW and CTF viruses has not been studied. However, should such be determined to occur in nature, a redefinition of the term "principal vertebrate host" will be in order.

Dispersal of arboviruses from enzootic foci or newly established foci is a function of the arthropod vectors and vertebrate hosts involved in their natural cycles. In a mark-recapture study at an EEE virus focus in central New York State, *Cs. melanura* mosquitoes (a known vector species of EEE virus) were found up to 9.8 km from a release site (119), indicating significant spread by vector flight alone. Infection pressure may also stimulate virus spread beyond its ordinary boundaries. Significant increases in arthropod and vertebrate host populations, generally accepted as predictors of peak viral activity, the movements of foraging mammals and birds, the hatch and birth of susceptible clutches and litters, and increased availability of foods may stimulate virus amplification and produce such pressure.

Winds may play some role in dispersing infected arthropods from enzootic and epizootic areas. Although attempts have been made to demonstrate the importance of this means, evidence is lacking for a general importance of passive dispersal. Sellers and Maarouf analyzed wind velocities, trajectories, temperatures, rainfall, barometric readings, and other meteorologic factors and concluded that sources of EEE virus in outbreaks between 1980 and 1985 in Maryland, New Jersey, New York, and Michigan could have been wind-blown mosquitoes from more southerly points in the United States (211). They hypothesized that EEE virus could have been brought to Quebec in 1972 "by infected mosquitoes carried on surface winds from Connecticut, on the night of August 22–23, 1972" (209), and that WEE virus could have been brought to Minnesota, North Dakota, and Manitoba by infected mosquitoes riding winds moving from areas further south (210).

Whether global climate changes or other, as yet unrecognized, alterations in the environment will significantly affect persistence and transmission of arboviruses is not known. Shope has suggested that global climate changes could impact negatively on the ability of certain viruses, such as LAC virus, to persist if these changes significantly alter their environments and the ecosystems on which they so closely depend (213). However, the occurrence of greater opportunities for natural selection by RNA viruses that already have high rates of mutation certainly cannot be harbingers of good news (see section on epidemiology). Shope also proposes that additional ecological studies be done to establish the baselines that will be necessary to assess the effects of global warming, and he suggests the need for new and more effective methods for control of vectors in the future. In addition, studies of virus, vector, and vertebrate host genes and studies of the control of interactions between them, as well as the actions of genes under different environmental circumstances, are needed for forecasting disease development.

PATHOGENESIS AND CLINICAL DIAGNOSIS

WEE

Illnesses caused by WEE virus range from mild fevers with headache to aseptic meningitis and encephalitis (70, 186, 191). Onset is sudden and accompanied by a constella-

tion of fever, headache, chills, nausea, vomiting, or, occasionally, respiratory symptoms. Symptoms and signs indicative of CNS infection follow within several days and may include lethargy, drowsiness, nuchal rigidity, photophobia, and vertigo. The patient drifts into stupor or coma in severe cases. In particular, infants <2 months old are irritable and have focal or generalized convulsions, upper motor neuron deficits, and tremors. These signs appear in 90% of patients <1 year old and decreasingly in patients of greater age. Confirmed or probable in utero infections were observed in five infants infected with WEE virus near term. They survived acute encephalitic illnesses, but three had severe neurologic sequelae.

Also seen are various pathological reflexes, which vary from patient to patient and appear to depend on the stage of illness. Weakness and hyporeflexia are common, and children often display muscular rigidity, involuntary movements, and paralysis. CSF pressure may be mildly elevated, and CSF contains normal glucose, normal or mildly elevated protein, and elevated leukocyte counts (but generally $\leq 500/mm^3$). Early in infection, polymorphonuclear cells predominate, but mononuclear cells predominate later. After about 10 days, patients begin gradual convalescence.

Patients who die usually do so within 1 week after onset of illness. At autopsy, the significant primary findings are in the CNS. The brain usually is edematous. Multiple necrotic foci, often without cellular infiltrate, are found in striatum, globus pallidus, substantia nigra, cerebral cortex, thalamus, and pons. Widespread perivascular cuffing and meningeal reaction often are observed. Pathologic findings in a 75-year-old woman with focal neurologic signs due to WEE virus encephalitis were perivascular infiltration and multifocal necrosis in the deep gray matter. Similar findings in the basal ganglia and spinal cord corresponded to the focal clinical signs (1).

Sequelae of WEE encephalitis include fatigue, irritability, headache, and tremors for as long as 2 years. The overall case fatality rate is 3 to 4%, but adults usually recover completely. A minority display motor damage, intellectual impairment, emotional lability, or seizures. Of infants and children with CNS involvement, 56% of those <1 month old, 16% of those <2 months old, and 11% of those 2 to 3 months old have severe motor damage or intellectual deficits and often require permanent institutionalization or comprehensive home care. Only occasionally are serious sequelae seen in pediatric patients >1 year old, but 25 to 33% of children with convulsions during the acute illness will have subsequent seizures, some beginning 18 to 24 months after the acute phase.

Virus is rarely isolated from blood or CSF at the time of onset of illness but can be isolated from postmortem brain and other tissues; WEE virus has been isolated from the biopsied frontal lobe from a child (86). The incubation period is 5 to 10 days, likely explaining the presence of antibody at the time of onset.

EEE

The course of infection with EEE virus can be either systemic or encephalitic, depending on the age of the patient and other factors (70, 176, 186). Human infection is characterized by virus replication in peripheral sites, followed by viremia. Systemic infection has an abrupt onset, with chills and fever followed by malaise, arthralgia, and myalgia. Maximum temperatures can be 37.8 to 40°C. The illness lasts 1 to 2 weeks, and recovery is complete when there is no clinical CNS involvement; most systemic infections are subclinical.

CNS involvement may be due to virus dissemination from EEE virus-damaged CNS vessels. EEE virus encephalitis in infants is characterized by abrupt onset, but in older children and adults encephalitis is manifested after a few days of systemic illness. Signs and symptoms in encephalitic patients are fever of 38.9 to 41.3°C, headache, irritability, restlessness, drowsiness, anorexia, vomiting, diarrhea, cyanosis, convulsions, and coma. Children present with edema (either generalized, facial, or periorbital); when it occurs, paralysis develops during the acute phase of illness. Tremors and muscular twitching usually are accompanied by continuous nuchal rigidity. CSF pressure is usually increased; 200 to 2,000 cells per mm^3, 60 to 90% of which are neutrophils, may be present. Death usually occurs 2 to 10 days after onset of symptoms but rarely occurs much later. Death is due to encephalitis, sometimes with evidence of myocardial insufficiency and impairment of pulmonary function due, in turn, to impairment of autonomic functions. Brain lesions are typical of encephalomyelitis and include neuronal destruction and vasculitis, which is perivascular and parenchymous at the forebrain, basal ganglia, cortex, midbrain, and brain stem. Electron microscopy of brain tissue taken at autopsy has shown virions in the oligodendroglia. Electron microscopy of brain tissue taken by biopsy from an 8-month-old patient with acute EEE virus encephalitis revealed virions in extracellular spaces and not intracellularly (132), suggesting that the specimen was obtained relatively late in the encephalitic phase of the illness. There is minimal involvement of the cerebellum and spinal cord.

EEE encephalitis is considered the most severe arthropod-borne encephalitis in North America. Mortality during epidemics has been estimated variously as 50 to 75%, and most of those who recover are left with disabling and progressive mental and physical sequelae, which include minimal brain dysfunction to severe intellectual impairment, personality disorders, seizures, spastic paralysis, and specific cranial nerve dysfunction. Many patients with severe sequelae die within a few years. A study of late outcomes showed that an initial mortality rate of 74% had progressed, 9 years after infection, to total mortality (associated with EEE sequelae) of 90%, with only 3% recovering (12).

EEE virus rarely is isolated from blood or CSF at the time of onset of illness but can be isolated from postmortem brain and other tissues. Antibody is present at the time of onset of clinical illness. If the situation in humans is similar to that in equids, this likely is due to an extended incubation period, during which virus replicates, antibody synthesis begins, and symptoms follow, usually 5 to 7 days after infection.

SLE

Humans infected with SLE virus may have any of an extensive array of clinical manifestations, but most persons are asymptomatic. In 40% of the young adults and children who do become sick, fever and headache or aseptic meningitis may be the only signs. Detailed definitions of the terms encephalitis, aseptic meningitis, and febrile headache, as they relate to SLE virus infections, have been proposed (27). In the eastern United States and Canada, increasing severity of illness is related to increasing age. Almost 90% of elderly SLE patients develop encephalitis. The risk of fatal disease also increases with age. Onset of serious illness generally is sudden, with headache, fever, dizziness, nausea, and malaise, but onset can also be less abrupt, with symptoms

evolving slowly. Signs and symptoms increase for up to 1 week, after which time there is defervescence and general resolution of the illness in some patients. In others, CNS symptoms develop and progress, with nuchal rigidity, disorientation, tremors, and unsteady gait commonly observed. A normal mental status may be apparent, but apathy, confusion, and disorientation developing to coma may occur in severe cases. Incontinence, frequency and urgency of urination, and retention of urine may occur in about 25% of SLE patients. The pathologic basis for urinary tract disorders is not known, but inappropriate secretion of antidiuretic hormone has been documented (256) and may account for hyponatremia observed in some SLE cases. The hypothalamic-pituitary-adrenal axis appears to be functionally intact even in severely affected patients (79), but the cerebral metabolic rate may be depressed, while cerebral blood flow remains normal (212). Cranial nerve palsies occur in about 20% of cases.

Clinical laboratory findings usually are not distinctive. Inappropriate secretion of antidiuretic hormone in patients with hyponatremia may imply further problems. Early in the disease process, CSF may show a preponderance of polymorphonuclear cells, and a shift toward lymphocytic pleocytosis is usual. Cells in CSF usually amount to fewer than 200/mm^3. Protein in CSF usually is only slightly elevated, i.e., 1.5 to 2.0 times normal. Computer-assisted tomographic scans of the head do not provide useful information, and electroencephalogram tracings show generalized slowing without focal activity.

A review of the pathogenesis of SLE virus and a mechanism for its entry to the CNS via olfactory bulbs has been published (168). By whatever means it arrives in the CNS, SLE virus produces brain lesions. Postmortem examinations (99) show that the brain and spinal cord are of normal weight and appearance except for diffuse mild to moderate leptomeningeal and parenchymal congestion. The brain and meninges are congested and hyperemic; microscopic analysis reveals diffuse mild to moderate meningitis, with leptomeningeal infiltrates most prominent at the base of the brain stem and around the cerebellum. These infiltrates are composed of small lymphocytes, scattered plasma cells, macrophages, and a few polymorphonuclear leukocytes. Perivascular inflammatory cells are found in the Virchow-Robbin spaces of penetrating blood vessels, particularly in the midbrain and pons. Neurons are found in various stages of degeneration, including in phagocytosis, and SLE viral antigen can be detected in these degenerating cells. Cytoplasmic swelling with eccentric displacement of nuclei and other cytopathologic changes have been observed. Cellular nodules, composed of monocytes, lymphocytes, and microglial cells in the parenchymal white matter, are seldom associated with tissue destruction. In the gray matter, the substantia nigra of the midbrain and thalamic nuclei are the most severely affected regions. Perivascular infiltrates, cellular nodules, and occasional neuronophagia are observed in the thalamic nuclei. Cerebellum, pontine tegmentum, medulla oblongata, striatum, and anterior and posterior horns of the spinal cord are among other areas involved, although the frequency of lesions in these areas is low.

Protracted convalescence characterizes patient recovery in 30 to 50% of SLE cases involving the CNS. Asthenia, irritability, tremors, sleeplessness, depression, memory loss, and headaches usually last no longer than 3 years, but in approximately 20% of these patients, and apparently depending on the age of the patient and severity of the acute illness, symptoms persist for much longer periods. These symptoms include gait and speech disturbances, sensorimotor impairment, psychoneurotic complaints, and tremors (89, 166).

SLE virus rarely is isolated from blood or CSF of patients in the acute phase of their illness but can be isolated from postmortem brain and other tissues; antibody usually is present at the time of onset of illness, likely due to the relatively long (4- to 21-day) incubation period.

POW

Because so few cases of clinically apparent POW virus infection have been recorded, few definitive and generally applicable statements can be made about infection with this virus. In the few human infections described, onset is sudden, with headache and fever to 40°C and convulsions. Prodromal symptoms include sore throat, sleepiness, headache, and disorientation. Encephalitic cases are characterized by vomiting, prolonged fever or fever of variable length, respiratory distress, lethargy, and other nonspecific symptoms throughout the acute phase. Patients may become semicomatose with some paralytic manifestations, but general neurologic signs of meningeal irritation presage encephalitis, which is often severe. Most diagnosed cases display evidence of focal lesions, but one patient had major involvement of the right temporal lobe, more typical of herpes encephalitis (6). Laboratory findings for the CSF are normal glucose, elevated protein, and an initial polymorphonuclear pleocytosis shifting to lymphocytic pleocytosis later in the illness. Human infections with POW virus have been reported from Far East Asia (146). Russian workers, analyzing 14 cases of POW encephalitis, reported seven patients (one death) with signs of meningoencephalitic lesions, two with meningeal manifestations, and five with inapparent or uncomplicated febrile infections. POW encephalitis was characterized by signs of cerebellovestibular lesions, which differ from signs of tick-borne encephalitis.

Pathologic findings at autopsy of the index case (155) were inflammatory process in all areas of the brain, with the spinal cord and cerebellum less affected; the inflammatory process involved infiltration of perivascular areas, mainly by lymphocytes and monocytes and confined to the perivascular spaces, and focal parenchymatous infiltration. Focal infiltrations usually are in the gray matter, with macrophages or microglial cells predominating but with occasional polymorphonuclear cells.

Sequelae have been reported in 8 of 17 individuals who survived the acute infection. The most common indication of neurologic damage was hemiplegia, but recurrent severe headaches and damage to the upper cervical cord resulting in paralysis and atrophy of shoulder muscles, a common feature of Russian spring-summer and Central European encephalitides, also have been reported. Neuromuscular manifestations, specifically hypotonia, have been documented in a middle-aged male patient from Ontario, Canada (122). Spasticity can persist for weeks after the initial illness in patients who eventually improve.

The incubation period is at least 1 week after being fed on by an infected tick. Virus may be isolated from basal ganglia, cortex, and cerebellum of fresh postmortem brain tissue from fatal cases. Neutralizing antibody is usually detectable at the time of onset of illness, but other antibodies may not be detectable until weeks later.

LAC

LAC virus may be the best-studied virus of the family *Bunyaviridae* with respect to virus ecology, disease etiology, and pathogenesis (52). Extensive reviews of clinical and pathologic aspects of LAC virus infections, including detailed results of neurologic manifestations and sequelae, have been published (52, 137, 187).

As noted above, most LAC virus infections are subclinical, but when illness occurs, onset is abrupt. Patients present with fever, chills, and headache with or without photophobia; abdominal pain and upper respiratory symptoms with or without sore throat and cough may occur. Progression to more serious illnesses usually is predicted or defined by one or more of the following symptoms: vomiting, nuchal rigidity, lethargy, and coma (106). Most patients have fevers >37°C, and 40% have fevers >39°C. Gundersen and Brown (106) have divided the 178 patients they studied into three groups: (I) no seizures during the acute illness (58%), (II) seizures occurring only during the acute illness (33%), and (III) seizures occurring after the acute illness (9%). Seventy-two (40%) patients had acute seizures, with 14 (19.4%) of these having recurrent seizures. Of the 104 patients in group I, about the same proportion had subsequent seizure disorders as is true for the general population. The authors concluded that LAC virus infection in their study area (the upper midwestern United States) is highly epileptogenic. Most patients develop meningitis with polymorphonuclear leukocytes and mononuclear cells; CSF protein is elevated in about 20% of patients.

LAC virus produces an acute encephalitis that begins as a mild febrile illness that lasts for 1 to 3 days and sometimes for 1 week or more (61). In CNS infections, subsequent signs and symptoms usually subside after a few more days. Paresis, learning disabilities, and other cognitive deficits occur in no more than 2% of those with CNS infections due to LAC virus. Learning efficiency and behavior of most recovered patients do not differ from those of control groups in the same communities.

In the two fatal human cases studied, neuronal and glial damage, perivascular cuffing of capillaries and venules, and cerebral edema were noted on pathologic examination (127). While these findings are not quantitatively different from pathologic findings in other viral encephalitides, the distribution of the pathologic changes sometimes is distinctive, being most marked in cortical gray matter of frontal, temporal, and parietal lobes, basal nuclei, midbrain, and pons, with other regions spared.

Neither LAC nor any other CAL serogroup virus has been isolated from human blood or CSF; LAC virus has been isolated from humans twice, both times from postmortem brain tissues. Again, the length of the incubation period (estimated at about 1 week for LAC virus) provides sufficient time for initiation of antibody production and consequent quenching of the relatively brief viremia (estimated duration, 1 to 3 days). In one study, 94% of the patients had immunoglobulin M (IgM) antibody on the day of onset of illness (47).

In summarizing clinical findings in 12 patients ill after infection with the less common Jamestown Canyon virus, Deibel et al. (76) reported that most had nonspecific, mild prodromata (fever, chills, abdominal pains, cough and other upper respiratory symptoms, headache, and photophobia), but 8 patients developed meningitic or encephalitic syndromes; all but one of these patients were adults, in conspicuous contrast to LAC virus disease. One of these eight patients had seizures, one died, and one reported mild seizures 2 to 4 months after her illness.

Clinical findings in ten humans (two females and eight males; mean age, 14.6 years old; range, 3 to 59 years old) infected with snowshoe hare virus have been summarized by Artsob (5). Five patients had encephalitis, four had meningitis, and one had meningoencephalitis.

CTF

Classically, symptoms of CTF appear abruptly, with initial features of high fever, chills, joint and muscle pains, severe headache, ocular pain, conjunctival injection, nausea, and occasional vomiting. Fever, headache, lumbar pains, aching in the extremities, and anorexia may continue for a few days more; the spleen and/or liver may be palpable. A transitory petechial or maculopapular rash is seen in a minority of patients. The biphasic character of CTF is exemplified by defervescence for a few days followed by a relapse of 2 to 3 days. A small proportion of patients complain of more severe illness including extended prostration, anorexia, continuing fatigue, and convalescence for several or more weeks. A more severe picture occasionally is seen in children, who may have hemorrhagic manifestations ranging from more pronounced rash to disseminated intravascular coagulopathy and gastrointestinal bleeding. CNS involvement, including aseptic meningitis and encephalitis, has been seen in severely affected children. Rarely, adult orchitis, pericarditis, hepatitis, and symptoms mimicking myocardial infarction have been reported (25, 81, 104, 136).

Clinical abnormalities in laboratory mice are consistent with features of human disease, but little information on human pathologic changes is directly available (136). Leukopenia and, in some instances, thrombocytopenia have been observed, as has toxic granulation of neutrophils; immature granulocytes may appear in the peripheral blood. Granulocytes do not mature and megakaryocytes are depleted in bone marrow. High levels of circulating alpha interferon have been detected in 78% of CTF patients during the first 10 days of illness, and interferon levels correlate with fever but not with the frequency or severity of symptoms. Few fatalities have been recorded to be due to CTF. In one, a 4 year old with encephalitis and hemorrhagic diathesis, pathologic findings included skin purpura and petechiae, swollen endothelial cells of capillaries in lymph nodes, and prominent hyaline membranes in pulmonary alveoli. In a second, a 10 year old with renal failure, hemorrhage, and disseminated intravascular coagulopathy, pathologic studies revealed focal necrosis of liver, myocardium, spleen, intestine, and brain. Nevertheless, the vast majority of cases are the uncomplicated, mild to severe illnesses described above.

The incubation period is <1 to 19 days (average, about 4 days), possibly dependent on the dose of CTF virus the patient receives from the infecting tick. In contrast to WEE, EEE, SLE, POW, and LAC virus infections, antibody to CTF virus is not detected until 1 or 2 weeks after the onset of illness (46). One remarkable finding in both humans and experimental animals is prolonged viremia, which may last for several months (184), a phenomenon advantageous for diagnosis. Early in the disease, virus can be isolated from both serum and blood clots with about the same frequency, but later it is more easily isolated from blood clots and from erythrocytes.

DIFFERENTIAL DIAGNOSIS

Tsai has made a thorough case for the continued relevance of arboviruses as disease problems of humans, arguing that "secular trends in human behavior and modifications of the environment may have profound influences on the epidemiology of vector-borne infections" (233), of which arboviral infections are only one segment (192). Because there are no pathognomonic profiles, no specific symptom or array of signs and symptoms, and no physical findings, laboratory abnormality, or radiographic or electroencephalographic features that can be used to define any of the diseases discussed here, a differential diagnosis on clinical grounds can be very difficult. However, a thorough knowledge of both the illnesses arboviruses cause and the epidemiologic aspects of the natural cycles of these viruses should enable an attentive clinician to make a rational differential diagnosis.

If the patient is not an infant, nonherpetic encephalitis is usually a self-limited disease. Most patients recover without significant sequelae and require only supportive therapy during the acute illness. Identification of the infecting agent has prognostic value that can complement clinical measurements of disease severity. The critical initial task of the clinician is to eliminate the possibility of a treatable illness when one of the possibilities is a presumptive viral encephalitis. Albeit nonspecific, leukopenia can be a first and simple tool to provide initial information in forming a clinical impression. Slow-wave background activity by electroencephalogram and a mild lymphocytic pleocytosis in the CSF are indicators of encephalitis, rather than the less worrisome, but still serious, aseptic meningitis.

WEE, EEE, and SLE viruses should be suspected as possible etiologic agents in febrile illnesses with CNS involvement in a geographic (ecologic) area where these viruses are known or suspected to occur. Symptoms in common with those caused by enteroviruses, leptospirosis, and bacterial meningitis may confound the initial clinical impression. Whereas WEE and SLE and EEE and SLE may be sympatric, it is only in areas where the geographic distributions of WEE and EEE viruses might overlap, in the general area of midwestern Canada and the United States, that both WEE and EEE virus infections must be excluded from the diagnosis. Other than an increasing likelihood of SLE and a decreasing likelihood of either WEE or EEE with increasing age of the patient, only good laboratory diagnostics can be helpful. Nevertheless, patient age, seasonality of the disease, location of exposure (if not in immediate area of residence), occurrence of similar cases in the community, and other epidemiologic features all must be taken into consideration. The possibility of strokes, brain tumors, and other noninfectious CNS disorders also must be excluded, as must herpes, mumps, influenza, adenovirus, respiratory syncytial, lymphocytic choriomeningitis, encephalomyocarditis, and hepatitis viral encephalitides, Lyme disease, AIDS encephalopathy, Creutzfeldt-Jakob disease, Alzheimer's disease, long-term alcohol abuse, other dementias with other etiologies, Reyes syndrome, and treatable bacterial, mycobacterial, and fungal infections in otherwise healthy and in immunocompromised individuals. SLE often is characterized by confusion, a slow evolution of disease, absence of focal findings and seizures, and generalized weakness and tremor. Taken together, these constitute a constellation that may be distinctive to the experienced clinician and which may differentiate SLE from herpes and other viral encephalitides (234). Brinker and Monath have reviewed the differential diagnosis of SLE in North America and devised a comprehensive list of conditions that might allow misdiagnosis of a condition requiring urgent therapeutic intervention (27). In addition, Gardner and Reyes have compared the microscopic pathology of WEE, EEE, and SLE in older children and in adults and attempted to conceive a set of clinicopathologic correlates (99).

Absence of a history of recent travel, knowledge of vaccinations, and information as to recent exposure to animals are pieces of epidemiologic information useful in making an initial differential diagnosis. Information that the patient has travelled may broaden the possibilities. As one example of the latter, a child in Ohio presented with ataxia, head tilt, irritability, headache, fever, anorexia, and myalgia. Upon additional questioning, it was related that she had had fever, headache, anorexia, myalgia, and tick bite while on a family vacation to Hungary. The patient's biphasic illness eventually was identified as Central European encephalitis (73). Many more such anecdotal reports are in the literature of many countries; inclusion here is intended to emphasize the need to obtain a complete history on admission.

One cannot definitively attribute the presence of antibody to present or previous infection with WEE, EEE, SLE, POW, LAC, and CTF viruses, as one may do with other viruses. Numerous serologic surveys have demonstrated that human populations in areas of endemicity have low, moderate, or high prevalence of antibody to certain arboviruses. Further, in many geographic areas some of these viruses occur sympatrically: WEE and SLE and CAL viruses, EEE and SLE and CAL viruses, or WEE (or EEE) and SLE and POW viruses. Finding antibody to any of them cannot be taken as absolute evidence of an association of the current illness with the particular virus to which antibody is detected or, except for antibody to CTF virus, even with a member of that serogroup, for example, antibody to the apparent human nonpathogen Highlands J alphavirus in the eastern United States and Canada. Occasionally, single serological determinations may be taken to be provisionally diagnostic, such as in rabies (when the patient has not received immune prophylaxis), EEE, and AIDS, because seropositivity is unusual and strongly associated with symptomatic illness.

The differential diagnosis of LAC virus infection, particularly LAC encephalitis, usually is based on the constellation of signs and symptoms manifested, the season of the year, the patient's age, and the geographic area of residence or travel. Acute viral meningoencephalitides that must be excluded are those caused by SLE, herpes, measles, mumps, and enteroviruses, as well as acute CNS infections caused by bacteria and other infectious agents; other confounding possibilities, as noted above, must be considered.

A history of tick exposure, knowledge of a patient being fed on by ticks (90% of patients recall having had an attached tick or having seen a tick crawling on their body or clothing [104]), or early clinical signs and symptoms may provide preliminary evidence that the patient has CTF or Rocky Mountain spotted fever. However, the characteristics of the rash and the progression of the illness, as well as the presence of leukocytosis, distinguish Rocky Mountain spotted fever from CTF. Other diseases with which CTF might be confused are tick-borne tularemia, relapsing fever, and acute rheumatic fever. Differential diagnosis from these infections is important because CTF is not treatable with antibiotics. An algorithm for the differential diagnosis and management of CTF has been presented (71).

CONTROL AND TREATMENT

Over the past 50 years, active groups of innovative and insightful researchers at the University of California, Rockefeller Foundation, Yale University, CDC, U.S. Army, U.S. Department of Agriculture, University of Notre Dame, University of Wisconsin, Rutgers University, and other institutions have succeeded in enhancing the development of our understanding of arboviral diseases, the virus vectors, and the ecosystems in which these viruses and diseases are found (163, 165, 189, 190). It is impossible to summarize here even a small portion of the available literature on the natural history of the mosquito and tick vectors of arboviruses in North America. As summarized by Eldridge (83), strategies for surveillance, prevention, and control are now available and being applied, but methods of prediction of outbreaks are still imprecise. Well-organized, well-funded, routine mosquito abatement remains the most effective method of preventing human infections caused by mosquito-borne viruses, although emergency methods are employed when outbreaks are imminent or in progress. Eldridge encourages developing better information management systems, applying newer sampling theory, and continuing research on vector-host interactions to improve accuracy of outbreak prediction. Recommendations recently published by the CDC may provide guidelines for uniform arbovirus surveillance systems in the United States (173).

In California, better irrigation management has considerably reduced vector mosquito populations. Also in California, a correlation has been shown between increased use of air-conditioning and television viewing during peak *Cx. tarsalis* activity periods and decreased attack rates of WEE and SLE (98). This correlation also seems to be true in Florida, where *Cx. nigripalpus* is the SLE virus vector. People are staying indoors with windows closed during summer evenings, instead of sitting outdoors being bitten by mosquitoes. Surely, expanded education programs can promote other changes in behavior and help protect people from vector-borne diseases and complement existing vector control programs. In the case of LAC virus, removing or punching holes in discarded automobile tires and filling tree holes with cement have been shown to reduce *Ae. triseriatus* populations by limiting breeding sites (185).

Vector control measures include spraying or fogging insecticides (larvicides and adulticides) from the air or by ground equipment and reducing or altering habitat (source reduction). In many areas these measures are being supplemented by surveillance programs intended to provide early warning of virus activity. For WEE, EEE, and SLE, blood samples are collected from penned or caged sentinel birds (usually young chickens) or wild birds and tested for antibody; mosquitoes are collected and tested for virus. Increasing prevalence of antibody in birds before and during months of expected virus activity is taken to indicate a buildup of infection and the need for increased vector control. Sentinel studies in New Jersey, however, indicate that, in the case of EEE, chickens alone may not suffice because the virus may "leap" the supposed sentinel in favor of other birds (67; see above, Epidemiology). Clearly, no single surveillance system may be adequate between ecologic zones or for different viruses.

When prevention efforts fail and human infections occur, it is up to the clinician to make the proper differential diagnoses and symptomatically treat the patients. At present, there is no specific antiviral therapy for any of the arboviruses covered in this review. Therapeutic efforts (70) are directed towards managing symptoms, such as reducing fever, maintaining hydration and electrolyte balance, assuring adequate respiratory function, administering anticonvulsants (phenobarbital or diazepam), giving osmotic diuretics to decrease intracranial pressure, and providing physical therapy as may be indicated. Because person-to-person transmission does not occur with these viruses, containment barriers are not necessary, but care should be taken in handling tissues and body fluids because they may contain virus and laboratory infections can happen. Patients with neurologic sequelae should be evaluated for rehabilitation programs or follow-up care.

Although corticosteroids and similar compounds have immunosuppressive capacities, uncontrolled trials of combined steroid, barbiturate, and hyperventilation have provided promising results; such combinations require further trials. Amantadine, rimantidine, chloroquine, selanazofurin, tiazofurin, and many other compounds have been shown to have antialphaviral and antiflaviviral activities and to interfere with the replication of other viruses in vitro or in laboratory animals. However, none of these drugs has undergone trials in humans, and none is licensed for human use.

Patients with mild cases of LAC virus infection can be treated symptomatically to reduce fever, headache, nausea, and vomiting (74). Treatment may include use of intravenous fluids in slightly reduced amounts to minimize the risk of symptomatic cerebral edema. This feature of treatment of LAC virus underscores the need for appropriate differential diagnosis. For the agitated or delirious child, sedation may be appropriate. Notwithstanding this recommendation, it is also important to observe the child's neurologic status; minimizing stimulation of the patient may be as useful as sedation. Major therapeutic concerns in cases of LAC encephalitis are management of seizures and increased intracranial pressure. Measurements of blood glucose, calcium, and blood urea nitrogen are important in determining secondary causes of seizures. Management of seizures includes maintenance of adequate ventilation and blood pressure. Phenobarbital and phenytoin have been used to reduce relatively moderate seizures. When patients have prolonged convulsions (longer than 30 min) or status epilepticus (generalized convulsive seizure lasting more than an hour or repetitive convulsive seizures between which the patient does not regain consciousness), a more aggressive approach has been recommended (74). This includes simultaneous administration of diazepam and phenytoin.

Education is the best defense. People should be aware of the arthropod-borne diseases in their home areas and in the areas they visit and learn to avoid contact with suspected vectors during the transmission season. Mosquitoes generally have preferred feeding periods; avoid their habitats at such times. Control mosquitoes in the immediate vicinity and use repellents. To avoid ticks, stay out of woods where infected ticks are common and stay on recommended paths; wear adequate clothing to minimize exposed skin and trousers with close-fitting cuffs tucked into socks; treat clothes with insecticides or acaricides; and use repellents and insecticides as sprays, lotions, or solid bars according to the manufacturer's directions.

Vaccines for preexposure prophylaxis are available for WEE, EEE, and VEE viruses but not for SLE, POW, LAC, or CTF virus. WEE and EEE vaccines have been used successfully for both humans and equids. EEE vaccine has also been used to protect pen-raised, ring-necked pheasants along the eastern seaboard and lately for whooping cranes

(65). However, none of these vaccines is available for general human use; they are accessible only through the U.S. Army to laboratory workers and others at particular risk. In view of the low attack rate for EEE, and low morbidity rate of WEE, their use for the general public would be impractical. Annual revaccinations are required for those given WEE and EEE virus vaccines (made with inactivated virus), but a single dose of live-attenuated VEE TC-83 vaccine is sufficient to protect most humans and horses for life. However, as shown by essentially anecdotal studies of a few humans and by laboratory studies in mice (88), vaccination with strain TC-83 does not elicit antibody to heterologous VEE virus subtypes and varieties and does not cross-protect against accidental infection with other VEE viruses or against challenge with other VEE viruses. It is known from both field observations and experimental studies that preexisting antibody to WEE, EEE, or both may interfere with VEE TC-83 vaccine efficacy in equids and with challenge by equine-virulent VEE virus in equids (48, 242); whether this is true in humans has not been adequately evaluated. Because an unacceptable 15 to 30% of people vaccinated with VEE TC-83 become viremic, shed virus from the pharynx, and have mild to severe febrile responses (241), an inactivated VEE virus vaccine which appears to have fewer side effects but may not protect against aerosol exposure to virulent VEE virus has been developed for humans (123). A recombinant vaccine for both humans and equids is also being developed (133), but it, too, does not appear to protect against accidental aerosol exposure to VEE viruses.

LABORATORY DIAGNOSIS

Specific, or at least provisional, diagnosis of an arboviral infection is important for the community as well as for the patient. Whole blood, serum, or tissue samples taken for virus isolation attempts should be processed immediately or placed on dry ice (−70°C) or otherwise suitably frozen until they can be tested. Although for antigen detection this may not be a critical issue, it is reasonable to ship and store specimens for this purpose at low temperatures to prevent further degradation of proteins and RNAs. When serum samples are to be tested for antibody only, they can be shipped and stored at ambient temperatures, unless they are contaminated with microorganisms or will be in transit for long periods.

Serologic conversion from a negative or low titer to a positive or high titer is most often used for confirmatory diagnosis. Because a person can be infected and seroconvert to a virus without becoming ill, identifying a virus isolated from the patient is a more dependable basis for laboratory confirmation of a specific infection. However, viremias in arbovirus infections usually are brief, being quenched by antibody at or before the time of onset of illness, and the probability of obtaining a virus isolate from patient blood is not high, with the exception of CTF virus. Because WEE, EEE, SLE, POW, and LAC viruses are rarely or never obtained from blood, isolation of these viruses is not attempted as an integral part of routine diagnosis; postmortem brain may be a source of isolates of WEE, EEE, SLE, POW, and LAC viruses. If an isolate is obtained, various methods are available for its identification. It should be noted that special safety precautions are recommended for arboviruses; work with specimens that might contain these viruses is best left to those who have relevant experience and suitable, secure working facilities (2, 129).

Electron microscopy can be used at an early stage of virus identification, allowing placement of the isolate into a particular virus family and thus greatly facilitating or making unnecessary subsequent characterization (determination of size, species of nucleic acid, ether or sodium deoxycholate sensitivity, pH stability, and spectrum of animal or cell culture sensitivity). Detection of viral antigen by immunofluorescence or enzyme-linked immunosorbent assay (ELISA) (9, 115, 116, 203, 205–207) and detection and identification of viral RNA by nucleic acid hybridization or PCR have replaced the classical methods of hemagglutination inhibition (66) and complement fixation (56) in many laboratories and are used for both diagnosis (in humans and equids) and surveillance (virus detection in field-collected arthropods). Virus antigen or nucleic acid is detected either directly in the infected specimen or after amplification in laboratory animals, vertebrate or mosquito cell cultures, or live mosquitoes (197) inoculated with material suspected to contain virus. Various cell cultures from vertebrates (Vero, LLCMK2, BHK-21, baby hamster kidney, chicken embryo, duck embryo) and invertebrates (C6/36, AP-61, and TR-248 cells from *Ae. albopictus*, *Aedes pseudoscutellaris*, and *Toxorhynchites amboinensis* mosquitoes, respectively) are susceptible to arboviruses; POW and CTF viruses do not replicate in mosquito cells.

Direct immunofluorescence, with a battery of polyclonal or monoclonal antibodies, has been used to detect viral antigen in thin sections of patient tissue; indirect immunofluorescence also uses polyclonal or monoclonal antibodies but requires the additional use of anti-species antibody. Nevertheless, the latter is a more widely used test because of its flexibility (169). ELISA depends on reactions between viral antigens and antibodies to them (for example, those prepared in mice) and then uses an enzyme-labelled reporter antibody (continuing example, anti-mouse) to demonstrate those reactions. Antigen capture ELISA, employing highly avid and specific antibody, is used routinely in some laboratories. The use of ELISA for detecting antigen in clinical specimens requires construction of a sensitive and specific immunoassay utilizing the most appropriate combination of capture and detecting antibodies, enzyme, and substrate and optimal conditions of incubation. Nucleic acid hybridization can be used to detect viral nucleic acid in situ, the results providing information about the localization and distribution of viral sequences (17). A synthetic oligonucleotide corresponding to a region of a consensus nucleotide sequence is prepared. Extracted viral RNA or patient or arthropod tissue suspected to contain viral nucleic acid is denatured, fixed to nylon membranes, baked, and hybridized with the probe. Although not yet in routine use, numerous probes are being developed for nucleic acid hybridization, but this technique still is experimental. Its diagnostic efficacy remains to be determined, but indications are that it will become a preferred technique in the near future.

The intent of PCR assays is to select a segment of the viral gene sequence, reverse transcribe it with reverse transcriptase, amplify the resulting cDNA, and detect the amplified product. Provided that at least part of the gene sequence of the virus is known, PCR can be used to detect the RNA of that virus (143). PCR is exquisitely specific and sensitive. It detects fewer than 100 viral genome equivalents, can be adapted to specific experimental ends, and can be used to detect arboviral RNA in human serum and tissues as well as in mosquitoes, ticks, and other arthropods even when infectious virus is not present or cannot be isolated. Furthermore, it can be designed to analyze viral genomes

and, thereby, provide information about the molecular epidemiology and evolution of arboviruses isolated during epidemics or by routine surveillance (60, 251), and it does not require radioisotopes. Its high sensitivity allows rapid (1-day) detection of minute amounts of virus genome; specificity depends on that of the primers used to detect the amplified product. Significant progress is being made in converting PCR from an experimental tool to a routine diagnostic one. As would be expected with such a sensitive system, PCR must be done with great care to avoid contamination that might give false-positive results; appropriate reagent, equipment, and space controls also are critical.

No matter whether immunofluorescence, nucleic acid hybridization, PCR, or any of a number of other sophisticated techniques is used for detecting viruses, specific identifications still are done by using neutralization tests. Such tests can be virus or serum dilution (148) neutralization, in cell cultures when possible (51) or in laboratory animals, usually suckling mice (16). Specific identifications of viruses are useful not only for identification of an etiologic agent but also for better understanding of molecular epidemiology and virus evolution.

Type-specific antigens (polypeptides) and antibodies (reactant against glycoproteins and nucleocapsids) can be used as reagents for identifying arboviruses. For rapidly determining virus identity, group-specific and complex-specific monoclonal antibodies to arboviruses are available from arbovirus reference centers, and type-specific and variant-specific monoclonal antibodies to many individual arboviruses, i.e., WEE, North and South American varieties of EEE, vaccine-type and wild-type VEE, and LAC viruses, are available from various research laboratories (103, 121, 193, 194, 257). For final identification, an antiserum also is prepared against the isolate and cross-tested against reference antigens and viruses of the serogroup to which the isolate belongs. Ultimate definitive determination of genotypic variation can only be done by genomic analysis (oligonucleotide fingerprinting, gene sequencing, and protein sequencing).

Infection with WEE, EEE, SLE, POW, LAC, and CTF viruses leads to production of IgM, IgG, and, probably, IgA antibodies. These can be detected by hemagglutination inhibition (66), complement fixation (56), neutralization (148), or any of a great variety of other assays, including dot ELISA (22, 183), gel diffusion, immunoelectrophoresis, radioimmunoassay, hemadsorption-immunosorbence, and many other techniques too numerous to list here. Some have been used to advantage in limited studies but have not yet been applied to a large number of the arboviruses and therefore are not discussed further.

As detected by IgM antibody capture ELISA (MACELISA), IgM antibody to each of these viruses cross-reacts with heterologous viruses of the same group but is most reactive with other viruses of the same antigenic complex (36, 37, 44, 47, 170, 196). For example, IgM antibody from patients with LAC virus infection has highest titers to LAC virus itself, but it also reacts to a lesser extent with snowshoe hare virus and to a still lesser extent with Jamestown Canyon virus. Of course, it does not react at all with WEE, EEE, SLE, POW, or CTF virus (35).

IgM antibody in infections caused by WEE, EEE, SLE, and LAC viruses is almost always present on the day of onset of illness, an interesting observation and a critical diagnostic issue. Thereafter, IgM antibody peaks 2 to 3 weeks after onset but persists at high levels for at least 2 months. IgM antibody to CTF virus, on the other hand, is not detected until 1 or 2 weeks after the onset of illness (46). IgG antibody to CTF virus also appears relatively soon after onset but, unlike IgM, persists for many months or years after the illness or may persist for life.

Various tests simply detect IgM and IgG antibodies in different configurations. Because of the persisting nature of IgG antibody, detecting IgG antibody to a given virus in a serum sample from an acutely ill, a convalescing, or a recovered patient cannot alone be used as evidence of current infection with that virus. Indeed, because IgG antibody is so highly cross-reactive (and could be due to immunization with WEE and EEE vaccines), such a finding cannot even be used as concrete evidence of infection with that virus at any time in the past. Alternatively, because of the ephemeral nature of IgM antibody, detecting IgM antibody to a given virus in a serum sample from an acutely ill, a convalescing, or a recovered patient is at least provisional evidence of infection with that virus.

Other insights into arboviral infections also can be gathered from studies of IgM antibody presence or absence and persistence. For example, Burke et al. (30), in a study of fatal outcomes with Japanese encephalitis flavivirus, have shown a constellation of findings that correlated with a fatal outcome: low levels of Japanese encephalitis virus-specific IgM and IgG in both CSF and serum, infectious virus in CSF, and a severely depressed sensorium. Neither age, sex, days ill before admission, distance from home to the hospital, past medical history, CSF protein content, nor CSF leukocyte count was a significant risk factor. These workers concluded that, among patients hospitalized for acute Japanese encephalitis, a vigorous virus-specific IgM response, both systemically and locally within the CNS, is a good marker for survival and may be an inherently important factor in recovery from illness.

At present, and for the foreseeable future, the MACELISA is the serodiagnostic test of choice for determining recent human, equine (42), or avian (28, 38) infections with arboviruses (if confirmed by neutralization tests) and, under the pressures of an epidemic, may be applied to single serum or CSF samples for making provisional serodiagnoses. MACELISA has the distinct advantage of being able to provide clinicians with relevant results within a matter of hours, rather than days or weeks as with most other techniques. Nevertheless, whereas it might be acceptable to consider MACELISA-positive sera as presumptive evidence of recent infection during such a situation, in all such instances, supportive epidemiological information must be obtained and attempts must be made to obtain additional serum specimens.

A serologically confirmed infection with a virus requires demonstration of a significant (fourfold or greater) increase or decrease in antibody titer between paired acute-phase and convalescent-phase serum samples collected days to weeks apart. A serologically presumptive infection is one in which only an acute-phase serum is available, but that serum contains IgM antibody to the virus in question and it is negative or shows only very low titer to this virus by other assays, including those for IgG antibody; often, collecting and testing another serum sample later in the illness reveal antibody of sufficient titer to allow shifting the "presumptive" designation to "confirmed." A definition of inconclusive is a temporizing one; only an acute-phase sample is available, and that serum contains no IgM antibody and it is negative or has a very low titer to this virus by other methods. Any other results should be considered negative.

Whereas IgM antibody usually begins to decline a few

weeks or a few months after onset of illness, a minority of patients have prolonged IgM antibody responses, limiting somewhat the value of these assays as a measure of very recent infection. For example, Beaty et al. (18) demonstrated the persistence of IgM antibody in patients who had been diagnosed up to 7 years previously with LAC encephalitis. Thus, the presence of IgM class antibody in a patient with an illness clinically compatible with that of one of the arboviruses is, in itself, not confirmatory of such an infection. As mentioned above, certain arboviruses may occur sympatrically in North America. Antibody to one of these viruses does not provide clear-cut evidence for infection with that virus but may reflect infection with a related virus. Obviously, this is not the case with antibody to CTF virus. When antibody to one virus is detected by screening only with that antigen, additional tests are necessary.

Antibody reagents, assistance in preparing such antibodies, and additional information can be obtained from any of the World Health Organization Centers for Arbovirus Reference and Research. The World Reference Center is the Yale Arbovirus Research Unit, Yale University, New Haven, Conn.; the reference center for the Americas is at the CDC, Fort Collins, Colo.

Exotic arbovirus infections have been diagnosed on a number of occasions in travelers returning to the United States or Canada. Some are mentioned here to alert clinicians who might encounter them. In 1990, 102 cases of imported dengue were reported from 24 states in the United States and the District of Columbia; of these, 24 were serologically or virologically confirmed as dengue. In 1991, 82 cases (25 laboratory diagnosed) of imported dengue were reported in 27 states of the United States and the District of Columbia (3). In 84 overseas travelers from six provinces of Canada (8), diagnostic seroconversion was documented in 1 to chikungunya virus and in 83 to "flavivirus," most of which were due to a dengue virus. Also in recent years, an American serviceman returned from Asia with Japanese encephalitis, another travelled to Germany infected with CTF virus, cockpit personnel of an international airline flying from Polynesia may have introduced dengue-3 virus to the Caribbean, and Jamaicans returning from West Africa brought back dengue-1 virus. Introduction of the last virus to this hemisphere may have set off the chain of events that led to the first, and devastating, occurrence of dengue hemorrhagic fever-dengue shock syndrome in the Western Hemisphere (139). Following an epidemic of dengue-1 in Jamaica, an island-wide epidemic of this virus occurred in Cuba in 1977. Then, in 1981, Cuba experienced an epidemic of dengue-2 (probably brought from Viet Nam), and immune enhancement brought about by prior infection with dengue-1 virus likely elicited hemorrhagic fever with shock. The epidemiology of this experience has not been well documented, but the political repercussions were severe.

Many more viruses, including yellow fever virus, have the potential for such introduction, and many, including yellow fever virus, could arrive from South America or Africa. It is incumbent on clinicians in travel clinics, public and private hospitals in large urban centers, and private practices to recognize the possibility of attending such a patient and to take a complete travel history.

A final consideration in a similar vein is the following: in both the United States and Canada, scores of different viruses have been isolated but are not known to be human pathogens or are known to cause the rare case of febrile illness, meningitis, or encephalitis. Perhaps further study will show that some of them are of medical importance. For instance, Cache Valley virus, a mosquito-borne arbovirus of the United States and Canada and once thought to be insignificant, has recently been implicated in an outbreak of congenital malformations in sheep in Texas in 1987 (62) and preliminarily associated with congenital defects in humans (49). It seems that the arbovirus story in North America has not yet unfolded.

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