CHANGING PATTERNS IN MOSQUITO-BORNE ARBOVIRUSES

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ABSTRACT. Research leading to the current state of knowledge on the epidemiology of La Crosse virus (LACV), Jamestown Canyon virus (JCV) and dengue (DEN) viruses is summarized in relation to the generally recognized criteria for incriminating vectors. The importance of vector biology and local ecological conditions is emphasized as is the necessity of a good balance between laboratory and field-based studies. The influence of human activity in shaping the epidemiological patterns of all three of these arboviruses is readily apparent.

INTRODUCTION

Four basic criteria must be met in incriminating the mosquito vector(s) of an arbovirus: 1) isolation of virus from naturally infected mosquitoes; 2) laboratory demonstration of the ability of the mosquitoes to become infected by feeding on a viremic host; 3) laboratory demonstration of the ability of these infected mosquitoes to transmit the virus during bloodfeeding, and; 4) evidence of bloodfeeding contact between the suspected mosquito vector and suspected vertebrate hosts under natural conditions. In a companion review (DeFoliart et al. 1987), the focus was on the importance of a host of variables that must be considered in attempting to satisfy these basic criteria and to assess true vector competence. It was emphasized that a good balance of field- and laboratory-based studies must be maintained to ascertain the unique combination of ecological and behavioral features essential to survival of a given arbovirus in a given situation. With the input of field-based studies, it becomes apparent as stated by the above authors that "there is no aspect of vector ecology or behavior that may not be found to require clarification during the epidemiological investigation of an arbovirus."

The implications of the preceding statements can best be illustrated by the changing questions that arise at progressive stages in the investigation of a specific arbovirus. As examples, we discuss here the 3 viruses with which we have had our most extensive research experience, La Crosse (LACV), Jamestown Canyon (JCV) and dengue (DEN). Each virus has unique characteristics that make its inclusion appropriate. LaCrosse virus is the most recently recognized of the major mosquito-borne arboviruses in North America, and, as such, the suddenly voluminous research on this virus and its major vector, *Aedes triseriatus* (Say), is relatively condensed in time compared to any other arbovirus. It illustrates the types of questions raised in investigating a virus known to be transovarially transmitted in nature. Jamestown Canyon virus appears to be the most rapidly emerging "new" arboviral problem of public health importance in North America. Although serologically close to LACV, its ecological support system (i.e., vectors, vertebrate hosts, landscape) is completely different. Despite this, these two viruses interface in intricate ways that carry potentially important public health implications. Current questions relative to JCV typify the formulation of hypotheses necessary during the early phases of vector incrimination. Dengue viruses, a group of 4 closely related serotypes (DEN-1,2,3,4), warrant inclusion because dengue has greater world-wide public health importance than any other mosquito-borne arboviral entity. Regarded until recently as an anthroponosis (i.e., transmitted by vectors solely from human to human), it is probably a zoonosis with an ecological variety of transmission cycles including sylvan, rural, and urban. The DEN viruses vividly portray the difficulties encountered with a public health problem intimately adapted to the cultural and socio-economic environment of human populations in the tropics.

LA CROSSE VIRUS

La Crosse virus is a Bunyavirus belonging to the California (CAL) serogroup which contains 14 recognized virus types, subtypes and varieties (Calisher 1983). At least 6 of the viruses cause human illness. La Crosse virus was originally isolated from the brain of a child who died of encephalitis in 1960 in La Crosse, Wisconsin (Thompson et al. 1965). The majority of virus isolations and human clinical cases have occurred in Ohio, Wisconsin, Minnesota, Iowa, Illinois, Indiana and New York, but there have been serologically confirmed human infections in Nebraska and the majority of states contiguous with or east of the Mississippi

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River (Calisher 1983, Calisher and Thompson 1983).

It required 7 years after the identification of LACV before the basic criteria for vector incrimination had been satisfied. Chipmunks and gray squirrels were suspected as vertebrate hosts based on their high antibody prevalence in nature (Moulton and Thompson 1971). *Aedes triseriatus*, a tree hole breeding mosquito, became suspected as the primary vector on the basis of virus isolations from naturally infected mosquitoes (Thompson et al. 1972), and demonstration under simulated field conditions of bloodfeeding contact with the suspected vertebrate amplifying hosts (Wright and DeFoliart 1970). Also, seasonal and geographic compatibility was shown between peak populations of *Ae. triseriatus* (Loor and DeFoliart 1970) and human clinical cases (Thompson and Inhorn 1967). This early era of vector incrimination was capped by the laboratory demonstration of high transmission rates (93% or higher) by *Ae. triseriatus* orally infected on virus dosages as low as 1.8 median tissue culture infective dose ($TCID_{50}$)/0.1 ml (Watts et al. 1972), and demonstration that chipmunks and gray squirrels developed viremias sufficient to infect *Ae. triseriatus* (Pantuwatana et al. 1972). Other mosquito species tested were relatively inefficient oral transmitters of the virus, indicating there were no important secondary vectors in the upper Midwest (Watts et al. 1973a). Additionally, the early conclusion that chipmunks and squirrels were amplifying hosts, originally somewhat tentative as it was based on presence of antibody to undifferentiated CAL serogroup virus, was substantiated by isolations of LACV from both of these mammalian species (Gauld et al. 1975, Ksiazek and Yuill 1977), and by evidence of high LACV transmission activity in areas with good chipmunk habitat (Gauld et al. 1974). Subsequent research (discussed below) showed 3 additional types of transmission and additional vertebrate and potential vector species. Also, it became evident that, to explain the transmission dynamics of LACV, information was needed on almost every aspect of vector biology and behavior.

In the early 1970s, Watts et al. (1973b) experimentally demonstrated transovarial transmission of LACV by *Ae. triseriatus* and this breakthrough was followed quickly by the finding that the virus overwinters in the diapaused eggs of the mosquito (Watts et al. 1974). In endemic areas of Wisconsin, overwintering virus prevalence was found to be about 0.4 to 0.6% in larvae and adults from overwintered eggs (Beaty and Thompson 1975, Lisitza et al. 1977), and Miller et al. (1977) demonstrated maternal vertical transmission rates (MVTRs) of up to 70% (the MVTR is the product of the percentage of infected females passing virus to at least some of their progeny and the filial infection rate [FIR], i.e., the mean percentage of infected progeny from transovarially-transmitting females). These results led to the view that LACV maintenance relies primarily on this vertical transmission component with horizontal amplification via vertebrates viewed as a process for recruiting enough new vertical transmitters to maintain a relatively stable virus prevalence in the vector population. Increasing evidence, however, that LACV undergoes high attrition at several points during vertebrate amplification has raised a question as to whether this horizontal process can fully negate the erosion of virus prevalence that occurs during maternal vertical transmission (DeFoliart 1983). The question was first raised by Miller et al. (1979) who reported that orally infected *Ae. triseriatus* did not become vertical transmitters until the second oviposition after the infectious blood meal. Female daily life expectancies of 80–95% have been reported for *Ae. triseriatus* (Beier et al. 1982, Haramis and Foster 1983, Sinsko and Craig 1979), and it can be calculated that the orally infected cohort probably experiences a mortality rate of about 90% prior to deposition of infected eggs. This assumption was strengthened by the finding at one endemic site that only 7.1% of bloodseeking *Ae. triseriatus* females were biparous (Porter and DeFoliart 1985).

Overall, there appear to be at least 4 sequential points and 7 or 8 factors during the vertebrate amplification process that will, or may under certain conditions, result in high virus attrition:

1) Virus attrition resulting from bloodfeeding on non-amplifier species of vertebrates: *Ae. triseriatus* is catholic in its feeding behavior (Burkot and DeFoliart 1982, Nasci 1982a, 1985; Wright and DeFoliart 1970). White-tailed deer are non-amplifiers of LACV (Issel et al. 1972a, 1972b), but a 3-year study of blood meal sources at an endemic site in Wisconsin revealed that 65% of *Ae. triseriatus* blood meals were from this vertebrate while only 24% were from chipmunks and gray squirrels (Burkot and DeFoliart 1982). A blood meal ratio such as this from deer and amplifier species results in a nearly 16-fold decrease in amplification potential, because only about 25% of the potentially infective bites are delivered to amplifier species and only 25% of the potentially infectable vector population feeds on amplifier species. Although such dispersion of bloodfeeding among host species might not be important with a high population density

mosquito, *Ae. triseriatus* generally is a low density species.

A second factor of similar magnitude may also be operative at this point. Preliminary data (Patrican et al. 1985b) indicated that *Ae. triseriatus* became infected after ingesting white-tailed deer blood-LACV mixtures containing JCV neutralizing antibody, but failed to transmit LACV. If this inhibitory effect occurs in nature among LACV-infected mosquitoes that imbibe JCV antibody, it could explain the focal distribution of LACV because of the high JCV antibody prevalence in many midwestern deer populations. It appears that LACV could eventually become largely restricted to urban/suburban localities where deer abundance does not present a barrier to virus dispersion.

2) Virus attrition resulting from bloodfeeding on the immune portion of amplifier populations. In active transmission areas, chipmunks and squirrels sometimes exhibit LACV neutralizing antibody prevalences exceeding 40–50% (Gauld et al. 1974, Moulton and Thompson 1971). Infective bites delivered to these animals are "wasted" insofar as virus amplification is concerned. Once again, a second attritional factor may be important, as there is preliminary evidence of reduced transmission of LACV by *Ae. triseriatus* that have imbibed chipmunk blood-LACV mixtures containing LACV neutralizing antibody (Patrican et al. 1985b). It remains to be seen whether this phenomenon occurs in previously infected mosquitoes; however, reduced transmission has also been observed in *Ae. triseriatus* that engorged on chipmunks with LACV antibody 5–11 days prior to venereal infection (Thompson 1983).

3) Virus attrition resulting from inefficient transfer from infected mosquito to susceptible amplifier animal to susceptible mosquito. Not all susceptible amplifier hosts fed upon by infected *Ae. triseriatus* develop high enough viremia to infect other feeding mosquitoes. Patrican et al. (1985a,b) found that the virus titer required to insure high transmission rates (i.e., 90% or higher) by feeding mosquitoes was approximately 3.4 $\log_{10}$ median suckling mouse intracerebral lethal dose ($SMICLD_{50}$)/0.025 ml and that only 52% of chipmunks exposed to the bite of a single transovarially-infected *Ae. triseriatus* developed a viremia of this level or higher. The mean duration of "effective viremia" was only 1 day per infective bite delivered to the susceptible portion of the chipmunk population. In addition, *Ae. triseriatus* that feed on chipmunks and squirrels have reduced reproductive capacity compared to those feeding on deer, placing orally-acquired virus at a competitive disadvantage insofar as vertical transmission is concerned (Mather and DeFoliart 1983).

4) Virus attrition resulting from high vector mortality between the infectious blood meal and the second post-infection oviposition. The findings of Miller et al. (1979) were discussed above.

Possibly contradictory to the above evidence for a reduced role for vertebrate amplifiers was failure to isolate LACV from 22,479 *Ae. triseriatus* complex mosquitoes collected on the Delmarva Peninsula (and near absence of neutralizing antibody in mammalian sera assayed) where chipmunks are scarce (Clark et al. 1986). Gray squirrels are present in the area, however, making it likely that factors other than scarcity of chipmunks will be found to explain the apparent absence or scarcity of LACV infections.

One aspect of feeding behavior, probing without complete engorgement, could be important in partially negating the attritional factors discussed above, but "probing" is not well understood despite several recent papers. A single probe is sufficient for transmission of LACV (Grimstad et al. 1980). Such probing appears to be inherent in *Ae. triseriatus* behavior even when the mosquito is attempting to obtain a blood meal from a non-defensive host. Walker and Edman (1985a) found that approximately 55% of nulliparous *Ae. triseriatus* (6–16 days old) probed only once before beginning to feed on anesthetized chipmunks and gray squirrels, while 45% probed 2 to 6 times. Multiple probing may be more common in nature than indicated by these results, however, as the mosquitoes used were in the F_{14} generation of colonization, and long-colonized *Ae. triseriatus* evolve toward single-probe behavior (Grimstad et al. 1977). In a study of *Ae. triseriatus* orally infected with LACV, Grimstad et al. (1980) found evidence for increased transmission by multiple-probing mosquitoes of strains with "low" transmission capability, and somewhat reduced ability by infected mosquitoes to feed to repletion. Patrican et al. (1985a, 1985b) found no significant difference, however, between transovarially infected and uninfected *Ae. triseriatus* in the proportion of single versus multiple probes during refeeding. Multiple-probers were more successful in transmitting LACV and the majority (both transovarially-infected and uninfected) were successful in obtaining a complete blood meal while the majority of single-probers were not successful. Probing activity was found to be highest among transovarially-infected and uninfected mosquitoes after 15 days of age, indicating a positive correlation between age

and probing, but the sample of less-than-15-day-old mosquitoes was too small for statistical confidence.

Clearly, probing behavior is complicated and may be influenced by age, length of colonization, and infection status, particularly in the case of orally acquired virus. Additional investigation of this exceedingly important parameter is needed. The above data were obtained on immobilized vertebrates. Studies have been made of the persistence of attack by *Ae. triseriatus* against defensive hosts (Walker and Edman 1985b), but at present the number of "infective contacts" per ovarian cycle completed by mosquitoes in the field cannot be estimated with any confidence.

Thompson and Beaty (1977, 1978) demonstrated paternal venereal transmission (PVT) of LACV from transovarially-infected *Ae. triseriatus* males to uninfected females during copulation. Paternal venereal transmission has appeared to be the most likely alternative or supplement to vertebrate amplification in negating virus erosion during maternal vertical transmission, but, again, the quantitative contribution of paternal venereal transmission has not been determined. Paternal transmission rates, as measured by the venereal infection rate of females mated with infected males, were less than 5% in early experiments but rates up to 49% were obtained when *Ae. triseriatus* were provided a blood meal 6 to 8 days prior to mating with transovarially-infected males (Kramer and Thompson 1983, Thompson 1979). Little is known about *Ae. triseriatus* mating behavior but some mating occurs on or in the vicinity of the vertebrate host (Loor and DeFoliart 1970) and once young females have obtained a blood meal, they are immediately willing to accept mates (Mather and DeFoliart 1984b). Thus, it appears that in nature blood engorgement seldom precedes mating by the 6 to 8 days discussed above.

In Thompson's experiments (1979), venereally-infected *Ae. triseriatus* females exhibited oral transmission rates to suckling mice of 5% and 32%, 4–7 and 8–17 days, respectively, postmating. No transovarion transmission (0/23) by venereally-infected females occurred during the first ovarian cycle and only 40% transovarian transmission (4/10) in the second ovarian cycle with a FIR of 64% in progeny of the 4 transovarially-transmitting females, an MVTR of only 26%. L.A. Patrician (unpublished data) found venereal infection rates averaging 46% in F_2 females receiving a blood meal 6–8 hours before their first opportunity to mate, and 45% in females given their first blood meal 7 days after their first opportunity to mate, indicating that the timing of the blood meal relative to mating had no effect on the venereal infection rate. In one cohort of those that fed only a few hours before mating, 60% and 94% of the venereally-infected females transmitted virus to progeny of their second and third ovarian cycles, respectively. Filial infection rates were 46% and 65%, respectively, but, as in Thompson's experiments, infection was not detected in progeny of the first ovarian cycle. Venereal infection rates were lower when F_4 and F_7 females were used.

In general, this information suggests that unless PVT is more efficient in the production of infected progeny than currently shown, it makes only a modest compensation for even relatively low virus erosion rates during maternal vertical transmission. As 81% of the oviposition by *Ae. triseriatus* in the field appears to be by females completing the first oviposition (S.V. Landry, unpublished data), absence of infection in first ovarian cycle progeny of venereally infected females has the same quantitative difficulty as posed by absence of infection in first ovarian cycle progeny of orally infected females. As virus dissemination to ovaries occurs within 3 to 5 days after mating, however (Kramer and Thompson 1983, Thompson and Beaty 1978), investigators should be alert to the possible presence of infection in first ovarian cycle progeny of venereally infected females.

Recently discovered vertebrate hosts of LACV include red and gray fox (Amundson and Yuill 1981) and woodchuck (Amundson et al. 1985). The finding that free-ranging foxes develop viremias sufficient to orally infect *Ae. triseriatus* (Amundson and Yuill 1981, Amundson et al. 1985) suggests this mammal may act as a vehicle for transporting and reintroducing LACV from one woodlot to another. Of interest is the recent finding (T. E. Amundson, personal communication) that the red fox can become infected after ingesting infected chipmunks and suckling mice and develop viremias similar to those of foxes bitten by infected mosquitoes. This represents a previously unknown mode of arbovirus transmission. Surprisingly, in view of the findings on foxes, dogs apparently are less susceptible to LACV (M.S. Godsey, personal communication) as only 1 of 88 dog sera from the endemic region of Wisconsin had detectable LACV neutralizing antibody. Of 6 dogs inoculated intramuscularly and subcutaneously, none developed detectable viremia although 3 exhibited a low neutralizing antibody response.

As happens with many important vector species, *Ae. triseriatus* has benefitted enormously from its interaction with humans.

Although originally a tree hole breeding species, research on its vector biology and control has centered increasingly on its adaptation to breed in small man-made water containers, especially discarded automobile and truck tires. Tires are estimated to be increasing in the United States at the rate of 200 million per year (Beier et al. 1983, Deese et al. 1981), and Craig (1983) has emphasized the need for technology and legislation for disposing of the steel-belted tire. The association of tires and other small water-containers with human cases of La Crosse encephalitis is well documented (Hedberg et al. 1985). When in close proximity to human residences or other areas of high human activity, these containers become particularly dangerous and persistent foci of infection because of the vertical transmission capability of *Ae. triseriatus*. Such containers are readily colonized by *Ae. triseriatus* from wooded areas or from other concentrations of such containers. Although basically a woodland mosquito, *Ae. triseriatus* is now known to disperse readily along fencerow "corridors" (Mather and DeFoliart 1984a, Nasci 1982b) and across such open terrain as corn and alfalfa fields (Mather and DeFoliart 1984a). In a survey of South Bend, Indiana, Leiser (1981) found *Ae. triseriatus* present throughout the city, except for an industrialized section where trees and other vegetation were sparse. Haramis (1984) has shown that, because water in tires is relatively warm, peak pupation of *Ae. triseriatus* occurs 2 to 3 weeks earlier than in tree holes, and, thus, TO-infected females from tires may initiate LACV transmission earlier in the season. Means et al. (1977) found females from tires in New York to be more aggressive biters than females from tree holes.

Larvae in tires are dependent on the autumn accumulation of leaf litter as a substrate for the saprophytic fungi upon which they feed (Fish and Carpenter 1982). Adults emerging in late summer from larvae in tires are smaller and fewer, probably because of increased intraspecific competition resulting from seasonal depletion of nutrients (Haramis 1984). Similarly small females can be produced in tree holes. "Small" females have higher oral and TOT rates than "large" females (Grimstad and Haramis 1984, Patrican et al. 1985b), although this may be offset by lowered fecundity in the smaller females (Patrican et al. 1985b). Also, larger females have higher parity rates, indicating greater success in bloodfeeding, increased longevity, or both (Haramis 1983).

A thorough, ongoing program involving the removal or insecticidal treatment of the small-container and tree hole *Ae. triseriatus* breeding sites near human habitations in high-risk areas will presumably reduce the number of clinical cases of La Crosse encephalitis. In fact, this appears to have been the result of such a program initiated in 1979 in La Crosse County, Wisconsin (Parry 1983). In an 11-county area of westcentral Wisconsin, the number of serologically confirmed cases of La Crosse encephalitis in La Crosse County declined from 22% of the total cases in 1978 and 1979 to only 6% of the total during the ensuing 3-year period, 1980 through 1982. This program and reduced number of cases is still being maintained (J.E. Parry, personal communication).

Oligonucleotide mapping of LACV apparently allows some clarification of the long uncertain vector status of *Aedes canadensis* (Theobald). Over the years, there have been 41 isolates of LACV from *Ae. canadensis* in Ohio, far more than from any other species in that state although the field infection rate of 0.5/1000 is lower than the 1.1/1000 recorded from *Ae. triseriatus* (Berry et al. 1986). Early experiments with *Ae. canadensis* from Wisconsin and a Wisconsin isolate of LACV from *Ae. triseriatus* indicated that *Ae. canadensis* supported virus replication poorly (Watts et al. 1973a). Klimas et al. (1981), however, described 3 geographic variants of LACV with Types A and B occurring in the upper midwestern states, while a third type, C, was found in New York and several southern states. While LACV isolates from *Ae. triseriatus* in Ohio are similar to virus strains in Wisconsin, an Ohio LACV isolate (CDC No. R-15804) from *Ae. canadensis* was related to LACV strains from New York (Type C) (Klimas et al. 1981). Of the 41 isolates from Ohio *Ae. canadensis*, 39 were from mosquitoes collected in the northeastern corner of the state, in close proximity to New York. Laboratory experiments with strain R-15804 showed an infection rate of 75%; 54% of these transmitted virus to suckling mice, a population transmission rate of 40%.

Aedes hendersoni Cockerell, a treehole breeding species that is sympatric with *Ae. triseriatus* throughout most of the eastern United States, has been shown to have low LACV transmission capability, with population rates of only 0–14% (Grimstad et al. 1985, Watts et al. 1975). Recent findings by Nasci (1985), however, of heavy utilization of tree squirrels as a blood meal source by *Ae. hendersoni*, and that this species occurred at more than twice the density of *Ae. triseriatus* in an Illinois enzootic area dictate caution in assuming that this species has no vector role. To date, however, field isolates of LACV have not been obtained from this species, and data from Illinois (Clark et al. 1985), Indiana (Novak et al. 1981), Wisconsin

(Burkot and DeFoliart 1982, Loor and DeFoliart 1970), and the Delmarva Peninsula (Clark and Craig 1985) indicate that *Ae. hendersoni* poses little direct risk to humans as it is either of low population density or is infrequently encountered at ground level. As is often the situation in vector complexes, *Ae. hendersoni* creates a problem in field studies on the vector biology of *Ae. triseriatus* because adult females of the 2 sibling species can be distinguished reliably only by electrophoresis (Munstermann et al. 1982, Saul et al. 1977).

The laboratory finding that *Aedes atropalpus* (Coquillett) from Indiana is capable of horizontal and vertical transmission of LACV (Freier and Beier 1984) adds a potentially important new dimension to the ecology and distribution of the disease. A rock hole breeder in the north and east, *Ae. atropalpus* has rapidly extended its range southwestward and, since 1979, been found breeding in tires from New York to Indiana. These tire-adapted populations, true *Ae. atropalpus* on the basis of morphology and enzyme phenotype (Munstermann 1980), are autogenous for the first ovarian cycle, but similar to the southwestern and closely related *Aedes epactius* Dyar and Knab in the avidity of their biting. Whereas *Ae. triseriatus* larvae occur more abundantly in shaded tires, *Ae. atropalpus* prefers tires in the open (Beier et al. 1983, Berry and Craig 1984), thus potentially further increasing the importance of tires as an ecological determinant in the incidence and spread of LACV infections. Because of its oviposition behavior, *Ae. atropalpus* is also a more rapid colonizer than *Ae. triseriatus* (Berry and Craig 1984). On the negative side relative to a potential vector role, although *Ae. atropalpus* feeds readily on humans and "rodents" in the laboratory (Freier and Beier 1984), limited blood meal analyses have shown engorgement only on deer, humans and canines (W. J. Berry, cited in Freier and Beier 1984) and parity analysis at the one site for which data are available, indicated that only a low percentage were successful in obtaining a blood meal (Berry and Craig 1984). In addition, while oral infection and transmission rates were high and comparable to the Walton (Indiana) and Kerrville (Texas) strains of *Ae. triseriatus*, and the vertical FIR was comparable to the Walton strain, the FIR of the latter (10.7%) was well below that found in Wisconsin populations of *Ae. triseriatus* (50% or higher). Thus, a more precise definition of the vector role of tire-adapted *Ae. atropalpur* awaits more information on the nature and degree of vector-vertebrate host contact, the testing of additional populations for their vertical transmission capability, and the initial isolation of LACV from this species in the field.

From research to date, it becomes obvious that the urban-suburban environment readily meets the transmission requisites of LACV, which, evolutionarily, was a disease of the deciduous forests of the upper Midwest. As shown by Burkot and DeFoliart (1982), where deer are numerous, they minimize vector-amplifying host contact, and if JCV antibody prevalence is high, may exert further inhibitory effects on LACV transmission (Patrican et al. 1985b). On the other hand, as shown by Nasci (1982a), in the urban-suburban environment and some rural environments where deer are absent or scarce, contact between vector and amplifying hosts is greatly increased. Thus, risk of LACV infection in many endemic localities is directly proportional to the density of breeding sites available to the vector, and the importance of source reduction becomes obvious.

JAMESTOWN CANYON VIRUS

Jamestown Canyon virus, a subtype of Melao virus of the CAL serogroup, was first isolated from *Culiseta inornata* (Williston) mosquitoes in Colorado in 1961 (Hammon and Sather 1966). For the next 2 decades, JCV was considered of minimal public health significance. A 1963 Wisconsin study identified 3 young men who experienced mild febrile illness that was attributed to a "California virus" infection (Thompson and Evans 1965) later determined to have been caused by JCV (Thompson and Gunderson 1983). These 3 cases constituted the only published evidence that JCV infection might cause human disease until 1982, when Grimstad et al. (1982) reported a case of severe primary encephalitis that resulted from a JCV infection in a young Michigan girl. Subsequent studies documented over 40 clinical cases of central nervous system illness and other syndromes caused by JCV in residents of Ontario (Deibel et al. 1983) and the Northwest Territories in Canada (H. Artsob, personal communication), and in residents of Illinois, New York (R. Deibel, personal communication), and Ohio in the United States (Deibel et al. 1983, Grimstad et al. 1986). Grimstad et al. (1986) have recently shown that human infection with JCV is widespread in the upper Midwest, (e.g., the statewide neutralizing antibody prevalence rate in Michigan residents was 28%). The geographic pattern of antibody prevalence was similar to that of the population distribution of the white-tailed deer, the primary vertebrate host of JCV. Similarly, in Indiana, the geographic pattern of human antibody prevalence follows that of the distribution pattern of deer density (Grimstad 1987).

The lack of human case detection in the light of such widespread infection is not surprising given that only neutralization tests in cell culture with JCV as test antigen lead to recognition of current or past infection (Grimstad et al. 1986). Virtually all state diagnostic laboratories that test for CAL group antibody in clinical central nervous system cases use LACV in complement fixation or hemoglobin inhibition tests (Calisher and Bailey 1981). Unfortunately, these 2 procedures detect less than 1.5% of JCV infections (Grimstad et al. 1986). The critical need for a rapid sensitive diagnostic procedure for JCV is obvious, especially since its range extends throughout temperate North America (Calisher 1983, Grimstad 1987).

Extensive studies in the East and Midwest have demonstrated that the white-tailed deer is the primary vertebrate host in the enzootic cycle (Issel 1973, Issel et al. 1972a, 1972b; Watts et al. 1979, 1982). The sole isolation of JCV from a vertebrate was from a sentinel white-tailed deer in Wisconsin (Issel 1973). Unlike other CAL serogroup viruses in the Midwest, JCV apparently does not produce a detectable viremia in rabbits or squirrels (Seawright et al. 1974, Watts et al. 1979); however, squirrels do seroconvert following JCV infection (Watts et al. 1979). Several recent studies detected JCV antibody in domestic livestock (Grimstad 1987). Grimstad (unpublished data) found neutralizing antibody prevalence rates of 40–60% in bovine and equine herds in Indiana and Michigan; however, these species do not develop a viremia following experimental exposure to JCV (M. Godsey, personal communication). Thus, neither small forest-dwelling mammals nor large domestic mammals seem likely to serve as a source for arthropod infection (Grimstad 1987).

Critical to the maintenance of an arbovirus cycle is the seasonal recruitment of susceptible vertebrate hosts. Studies in Michigan and Wisconsin have demonstrated a May through June period of JCV circulation in older deer (Grimstad et al. 1987, Issel et al. 1972a, 1972b). Maternal antibody in the colostrum of nursing does protect fawns from a primary infection with JCV for 8 to 23 weeks (mean of 19 weeks) following their birth in April through June (Issel 1974). Since most deer seroconversion to JCV presumably occurs by late June in the midwestern and northeastern states, this lengthy period of protection by maternal antibody provides a large number of susceptible vertebrate hosts (i.e., the year-old deer) the following season. Serologic surveys of deer taken during the fall hunting seasons in Indiana and Wisconsin have demonstrated the significantly lower prevalence of JCV neutralizing antibody in deer fawns compared to yearling (1.5-yr-old) deer (Boromisa and Grimstad 1987, Issel et al. 1972b). At the Houghton Lake (Michigan) Wildlife Research Area, a known JCV focus (Grimstad and Mandracchia 1985), fawns born in 1983 had lost maternal antibody by midwinter; however, all subsequently seroconverted during a 6-week period in 1984 (late May to early July). All newborn fawns bled there as early as 4 days after birth in 1984 had maternal antibody to JCV; by late fall all were seronegative. Seroconversion of the 1984 cohort to JCV occurred the following spring within a 10-week period (Grimstad et al. 1987). Seroconversion of year-old deer at Houghton Lake has primarily occurred coincident with spring emergence of snow-melt *Aedes* mosquitoes; seroconversion in deer at Houghton Lake, Kingsbury State Fish and Wildlife Area in northern Indiana, and elsewhere has not been associated with blackfly, tabanid or tick biting activity (Boromisa and Grimstad 1986).

Three tabanid species and numerous mosquito species have yielded JCV isolates throughout North America (Grimstad 1983). In New York, JCV has been isolated from 13 mosquito species, 12 of which were *Aedes* (Grayson et al. 1983). Midwestern workers have shown the marked preference of woodland *Aedes* for deer (Nasci 1982a, 1984; Wright and DeFoliart 1970). Turell and LeDuc (1983) stated that *Culiseta inornata* was the primary vector of JCV. While that may be true in the western United States, *Aedes* species, notably *Aedes stimulans* (Walker) (Boromisa and Grimstad 1986), and perhaps *Ae. communis* (DeGeer) group members, are probably the primary regional vectors in the eastern United States and Canada (Grimstad 1987). In the 2 upper Midwestern JCV foci (Kingsbury [Boromisa and Grimstad 1986] and Houghton Lake), *Cs. inornata* is quite rare; however, antibody prevalence to JCV ranges from 70 to 100% in yearling and adult deer (Boromisa and Grimstad 1987, Grimstad et al. 1987).

Evidence is emerging linking multiple species to the JCV transmission cycle. In northern Indiana, JCV is apparently transovarially transmitted by *Ae. stimulans*; isolations have been made from separate pools of teneral males and females in late May prior to any bloodfeeding (Boromisa and Grimstad 1986). At Kingsbury a minimum field infection rate (MFIR) of 1:1424 for male and 1:591 for female *Ae. stimulans* was noted in May 1983; the seasonal MFIR for females was 1:1260 (Boromisa and Grimstad 1986). Bloodmeal precipitin tests indicated that free-ranging deer were essentially the only blood source for *Ae. stimulans* at this JCV site

(Boromisa and Grimstad 1986). Laboratory transmission trials with field-collected membrane-fed *Ae. stimulans* resulted in a 44% population infection rate and a 12% population transmission rate. All *Ae. stimulans* females with disseminated infections (detected by head-squash immunofluorescence) transmitted JCV to suckling mice (Boromisa and Grimstad 1986). These data suggest that a midgut barrier to JCV exists in *Ae. stimulans,* but there is apparently no salivary gland barrier. Unfortunately, *Ae. stimulans* has not been colonized; one difficulty in rearing this species is the obligate 180-day egg incubation at 4°C necessary to break diapause (Horsfall 1974).

Isolations of JCV have been obtained from *Aedes vexans* (Meigen) in Connecticut, New York and Wisconsin (Grimstad 1983). In New York, Grayson et al. (1983) reported a 1:160,811 MFIR for JCV in *Ae. vexans.* No JCV isolates were obtained at Kingsbury in Indiana from more than 83,000 *Ae. vexans* collected over 3 years; spring and early summer collections were made at the same time that isolates were obtained from *Ae. stimulans* (Boromisa and Grimstad 1986). In the laboratory, *Ae. vexans* showed a 35% population infection rate; however, infection remained localized in the midgut, thus precluding virus transmission to suckling mice (Boromisa and Grimstad 1986). The dose of virus ingested was equivalent to peak titers (3.5 $\log_{10}$/0.025ml) demonstrated in experimentally viremic deer (Issel 1972a).

Berry et al. (1977) isolated JCV from adult *Ae. triseriatus* reared from field-collected eggs in Ohio and subsequent field studies have led to additional JCV isolates from eggs of this species (R. L. Berry, personal communication). These multiple isolations from overwintering eggs strongly suggest a regional vector role for *Ae. triseriatus.* However, laboratory transmission trials with 8 geographic strains of *Ae. triseriatus* failed to demonstrate oral transmission to suckling mice (P. R. Grimstad, unpublished data). Thus, the vector status of this species remains uncertain until additional populations can be tested for transmission capability.

Human infection with JCV occurs bimodally in the upper Midwest and New York State, with onset of clinical cases in 2 distinct periods: May–June and August–September (in contrast to LACV clinical cases which show a single peak in August–September) (Grimstad 1987). Clinical cases documented to date in New York have had onset from May 14 to October 3 (R. Deibel, personal communication); one clinical encephalitis case in a south Chicago youth had onset of September 30, 1983 (Grimstad et al. 1986). Of the 39 serologically confirmed JCV cases documented through 1984, 62% (24/39) had onset during August to October, and 31% (13/39) in September–October (Grimstad 1987). Since transmission to deer in this area occurs in the spring, and univoltine *Aedes* do not persist much beyond July, other mosquito vectors must be primarily responsible for late summer human infections.

Evidence gained through the deer and human epidemiologic studies support a working hypothesis (P. R. Grimstad) that anopheline mosquitoes, notably *Anopheles punctipennis* (Say) and/or *Anopheles quadrimaculatus* Say, are potential vector candidates. This "anopheline vector" hypothesis is based on 3 main considerations. *First,* isolations of JCV have been made in Ohio from *An. punctipennis* in July and September (MFIRs of 1:128 and 1:733 in different years and localities; R. L. Berry, personal communication) and from *An. quadrimaculatus* in July (MFIR of 1:274) (Berry et al. 1983; R. L. Berry, personal communication), and in New York from *An. punctipennis* during the summer (Grayson et al. 1983). *Second,* in 1983 in the upper Midwest (including the Chicago area) a drought summer drastically reduced mosquito populations to record low levels in most areas. However, populations of *An. punctipennis* and *An. quadrimaculatus* were above average during late August and September; the only potential vectors that might have transmitted JCV in late September to the south Chicago youth mentioned above were *An. punctipennis* or *An. quadrimaculatus*—no *Ae. triseriatus* females were collected that month (K. K. Liem, personal communication). *Third,* at Houghton Lake, one-third of the adult does showed serologic evidence of very recent JCV infection in late March and early April of 1984 and 1985, as did a 1-year-old doe in 1985; one other adult doe (of 3 bled biweekly in 1984) showed evidence of reinfection in late September 1984. All other deer showed anamnestic responses or seroconverted coincident with the emergence of snow-melt *Aedes* (Grimstad et al. 1987). These early spring seroconversions and anamnestic responses were detected 1 to 3 weeks after a warm period each year during which conservation workers reported mosquito biting activity; in 1985, workers noted biting by "spotted-winged" mosquitoes, presumably *An. punctipennis* as no other potential vectors were present. At Houghton Lake, collections of biting mosquitoes in late March, 1986, were all *An. punctipennis.* If JCV is transmitted by anophelines in the early spring, it must persist overwinter in bloodfed adult females.

It must be emphasized that anopheline involvement is a working hypothesis. It appears to offer the best explanation, however, for the persistence of JCV in the field from the time

transmission to deer ceases and snow-melt *Aedes* females die, until human cases increase in August-September. *Anopheles punctipennis* has not been colonized *per se* and difficulties abound in maintaining and infecting field-collected specimens and in stimulating an overwintering cycle to test the hypothesis. Preliminary laboratory trials with a long-established colony of *An. quadrimaculatus* indicate that this strain is readily infected with JCV (P. R. Grimstad, unpublished data).

Maintenance of JCV in the field in the upper Midwest thus appears to be by at least two mechanisms. Given a) the probable involvement of a number of species (representing several genera of mosquitoes) in multiple JCV transmission cycles throughout temperate North America, and b) the widespread infection of humans, domestic livestock, and white-tailed deer, JCV is the most epidemiologically complex of the North American CAL group viruses. At the turn of the century, deer populations had been extirpated in many midwestern and eastern states; herds were rebuilt in the 1920–40 era and rapidly expanded only in the past 3 decades (see references cited in Grimstad et al. 1986). Jamestown Canyon virus encephalitis is thus an "emerging" disease, probably expanding with the exploding deer populations in many regions of North America.

DENGUE VIRUSES

Dengue viruses consist of an antigenic subgroup of 4 closely related, yet antigenically distinct viruses, DEN-1, 2, 3, 4, within the genus *Flavivirus*, family Flaviviridae (Westaway et al. 1985). All produce human disease, ranging from the relatively mild dengue fever, a self-limiting acute febrile illness, to the severe dengue hemorrhagic fever (DHF) characterized by hemorrhaging with or without a fatal shock syndrome (Halstead 1984). Dengue hemorrhagic fever is a major cause of morbidity and mortality among children in many countries of southeast Asia and has recently assumed considerable public health importance in the Pacific islands (Rosen 1984) and the Americas (Guzman et al. 1984). The growing importance of DHF and the recent resurgence of dengue epidemics (Gratz 1985), particularly in the Americas, have led to the recognition of DEN viruses as the most important arboviruses transmitted to man.

Although dengue was recognized as a human disease much earlier, the 4 virus types were originally isolated from both man and mosquitoes between 1943 and 1960 (Johnson et al. 1967). The domestic mosquito, *Aedes aegypti* (Linnaeus), was incriminated as the primary vector, and only man and subhuman primates were recognized as natural hosts. Primary infection of man following the bite of an infected *Ae. aegypti* produces a viremia lasting 4 to 5 days during the acute phase, beginning a few days after exposure. After ingesting an infectious bloodmeal from a viremic human, *Ae. aegypti* attains a lifelong vector status following a suitable extrinsic incubation period. Transmission occurs during refeeding on a susceptible human, thus allowing for the endemic persistence of these viruses in urban foci.

An enormous amount of historical and scientific literature provides the basis for our knowledge of the DEN viruses, and only the more recent publications, primarily those since 1983, will be alluded to here. The picture that has emerged of *Ae. aegypti* over the past 4 decades of dengue research is that of an ideal urban vector (Halstead 1984). Where urban transmission occurs, *Ae. aegypti* breeds predominantly in a variety of artificial containers such as household water containers, discarded automobile tires, tin cans, and plastic pails inside of or in close proximity to human dwellings. It feeds predominantly on man, primarily inside houses, and commonly takes multiple bloodmeals during a single gonotrophic cycle (Klowden and Lea 1978, Watts et al. 1985, Yasuno and Tonn 1970).

Although DEN virus transmission in urban areas is continuous, the rate varies, normally peaking during the wet seasons and decreasing markedly with onset of the dry season and its accompanying reduced temperature (Halstead 1984). The determinants of this reduction in DEN transmission are not well understood, but it has been attributed primarily to fluctuations in *Ae. aegypti* population density. However, temperature was implicated as a strong determinant in a recent study in Bangkok, Thailand (Watts et al. 1986), where *Ae. aegypti* breeding is primarily in stored water and slight seasonal changes in mosquito density are independent of the wet season (Sheppard et al. 1969). Biting rates peaked during the hot and rainy seasons (Yasuno and Tonn 1970) when the extrinsic incubation period for DEN-2 virus was less than 7 days. During the cool seasons, however, biting rates showed a marked decline and the extrinsic incubation period exceeded 25 days.

Vertical transmission may contribute to the maintenance of DEN viruses in urban areas, but the low rates so far observed seem to preclude a significant role. Dengue-2 virus was transmitted vertically by 4 of 5 experimentally infected strains of *Ae. aegypti*, but filial infection rates (FIR) were only 0.3 to 1.2% (Jousset

1981). A comparable rate was noted for DEN-1 transmitted vertically by 1 of 5 strains of *Ae. aegypti* (Rosen et al. 1983), but attempts to demonstrate vertical transmission of DEN-2, 3, and 4 by these 5 strains were unsuccessful. In Rangoon, Burma, DEN-2 was obtained from field-collected larvae at MFIRs of 1:2067 for larvae, 1:3865 for males, and 0:8528 for females (Khin and Than 1983). In Trinidad, DEN-4 was obtained from a mixed pool of male and female *Ae. aegypti* reared from eggs collected on the premises of residences with recent human DEN virus infections (Hull et al. 1984). The MFIR was 1:1855. By contrast, DEN viruses were not isolated from 5,766 *Ae. aegypti* larvae collected in houses in Bangkok, Thailand, in which one or more persons had recent DEN virus infection, despite DEN-2 isolations from 14 of 268 female *Ae. aegypti* collected from some of the same houses where negative larvae were obtained (Watts et al. 1985).

The peridomestic mosquito, *Aedes albopictus* (Skuse), indigenous to tropical Asia but with a range extending from Africa to the Pacific islands, fills a vector role secondary to that of *Ae. aegypti*. Except for the recent isolations of DEN-4 and DEN-2 from *Ae. albopictus* in the absence of *Ae. aegypti* during massive epidemics in southern China (Qui et al. 1981) and the Seychelles Islands (Metselaar et al. 1980), respectively, prior evidence for *Ae. albopictus* as a vector has been limited to intermittent epidemics in which *Ae. aegypti* was identified as the principal vector. The secondary vector role of *Ae. albopictus* is dictated by its breeding habitat and behavior (Wisseman and Sweet 1961) which allow less intense interaction with humans than for the highly domesticated *Ae. aegypti*. *Aedes albopictus* breeds in urban, rural and forested areas in water retained by artificial containers and natural vegetation, but it is exophilic and prefers to breed outside human dwellings. It feeds on both man and domestic animals with relative utilization governed by host availability (Sullivan et al. 1971). Diversion to non-human vertebrate blood sources and the fact that its adult activity is generally interrupted by the dry seasons may reduce its role as an endemic vector and largely preclude the likelihood of a continuous cycle involving this species and man. Experimental studies have demonstrated that all DEN virus types are transmitted vertically by *Ae. albopictus* (Rosen et al. 1983), but there have been few attempts to obtain isolations that would demonstrate this route of transmission in nature.

Other peridomestic *Aedes (Stegomyia)* species, including *Ae. scutellaris* (Walker), *Ae. polynesiensis* Marks, *Ae. pseudoscutellaris* (Theobald) and *Ae. rotumae* Belkin (Suizuki and Hirshman 1977) have been suspected of transmitting DEN viruses to man in some of the South Pacific islands. Experimental studies have supported a vector role for most of these species, but field evidence is based solely on their temporal and spatial association with human infections (Rosen et al. 1985).

Aedes mediovittatus (Coquillett) a peridomestic man-biting mosquito, may be a potential vector of DEN viruses in Puerto Rico (Gubler et al. 1985). This species breeds in suburban and rural areas and frequently shares larval habitats with *Ae. aegypti*. Laboratory studies revealed that *Ae. mediovittatus* was readily infected with DEN-1 and DEN-2, that both viruses were transmitted horizontally, while DEN-1 was transmitted vertically. The latter route of transmission was suggested as a possible mechanism for maintenance of DEN viruses in rural areas where human population densities are insufficient to sustain these viruses via an *Ae. aegypti*-man cycle. There is no direct field evidence as yet, however, to support a vector role for *Ae. mediovittatus*.

Although a sylvan maintenance cycle for DEN viruses, involving subhuman primates, has long been suspected, evidence was lacking until recently when extensive ecological studies in the Malaysian Peninsula revealed that all 4 DEN viruses were enzootic in a cycle involving several species of monkeys and *Aedes* mosquitoes (Rudnick 1984). Previous DEN virus infection was demonstrable in a high percentage of 4 species of *Macaca* and *Presbytis* monkeys, but more conclusive was isolation of DEN-1, 2 and 4, and serological evidence of recent infections by DEN-1, 2 and 3. These data were obtained in studies using monkeys as virus sentinels in the forest canopy; in contrast, DEN virus infections were not demonstrated in *Macaca nemestrina* used as sentinels on the forest floor. DEN-4 was isolated from an *Aedes (Finlaya) niveus* (Ludlow) group species collected in a monkey-baited forest canopy trap. It is not known which of the 11 species in the *Ae. niveus* group yielded the isolate, but the most common species of the group in the area were *Aedes pseudoniveus* (Theobald) and *Aedes subniveus* Edwards. Monkeys were considered the preferred hosts of these species. At ground level, *Ae. albopictus* was prevalent but more common at the forest edge and in adjacent agricultural areas and villages. Virus transmission to humans apparently occurred mainly in rubber tree-forest transitional areas where both *Ae. albopictus* and *Ae. niveus* group mosquitoes were abundant, suggesting spillover from the sylvan cycle. Further studies will be necessary to fully incriminate the species that serve as vectors, but *Ae. aegypti* was not observed in the study

villages, thus demonstrating that DEN viruses were being maintained and transmitted to man in the absence of this mosquito.

Recent field studies in forested areas of West Africa have also provided evidence of a sylvan cycle for DEN-2 involving *Aedes* mosquitoes and monkeys. Mosquitoes collected in 1981 and 1982 in forests of the Upper Volta, Senegal and the Ivory Coast yielded more than 200 strains of DEN-2 virus isolated from 5 mosquito species (Cordellier et al. 1983, Roche et al. 1983). These included *Aedes (Stegomyia) opok* Corbet and Van Someren, *Ae. (Stg.) luteocephalus* (Newstead), *Ae. (Stg.) africanus* (Theobald), *Ae. (Diceromyia) furcifer* (Edwards), and *Ae. (Dic.) taylori* Edwards, all associated with the forest canopy. In addition, an isolate of DEN-2 was obtained from a pool of male *Ae. taylori* and *Ae. furcifer* collected in a forested area of the Ivory Coast and from male *Ae. taylori* in Senegal. Monkeys were implicated as hosts on the basis of an isolation of DEN-2 from *Erythrocebus patas,* and serological evidence of recent infections in a high percentage of wild monkeys (Cornet et al. 1984). These observations provide convincing evidence in support of the emerging concept that DEN viruses are maintained in sylvan cycles analogous to those of jungle yellow fever virus. While a horizontal cycle involving mosquitoes and monkeys may not allow indefinite persistence of DEN viruses because of relatively short viremia in monkeys and slow population turnover which would not sustain an adequate number of non-immune hosts (Rosen 1984), the isolation of DEN-2 from male mosquitoes implies that vertical transmission may serve as an enzootic maintenance mechanism.

Although the public health importance of dengue and DHF has long been recognized, efforts to combat these diseases have been largely unsuccessful. Vaccines have been developed, but none is currently considered satisfactory for general use (Halstead 1984). Vector surveillance and control, or attempted eradication, have provided the most effective strategies (Halstead 1984), but, except where enforced such as in Singapore and, to a lesser extent, Cuba, these approaches have not been successful in permanently interrupting DEN virus transmission to man. Despite an enormous amount of investigation of the ecology and epidemiology of DEN viruses, their endemic persistence remains poorly understood, as are the implications of enzootic cycles such as those recently discovered in Malaysia and Africa. Recent emphasis on laboratory studies, particularly on the potential vector and reservoir role of mosquitoes, has not been paralleled with critically needed multidisciplinary field studies. Finally, critical epidemic precursors such as cultural and socio-economic factors that permit the perpetuation of *Ae. aegypti* remain underemphasized, at least by most public health funding sources.

THE IMPACT OF HUMAN ACTIVITY

The utilization of small water containers of all types, including drinking-water containers inside houses by *Ae. aegypti,* and the adaptation of *Ae. triseriatus* to old tires which are accumulating in the Untied States at the rate of 200 million per year, show how pervasively vector species can root themselves within the cultural fabric of their human hosts. Jamestown Canyon virus, in a less obvious manner, is also subject to the influence of human activity. Wildlife management programs designed to increase deer populations, while making conditions less favorable for LACV, can improve conditions for JCV, as well as for Lyme disease, the spirochaete of which is transmitted by ticks that utilize deer as hosts.

Mitchell (1977), Smith (1975), Surtees (1971), Ward (1977) and others have listed human cultural factors that influence patterns of disease. Some factors of greatest relevance to specific arbovirus vectors include the development of irrigation agriculture, e.g., utilization of rice fields and irrigation waste water by *Culex tarsalis* Coquillett and *Culex tritaeniorhynchus* Giles; the proliferation of small man-made water containers, e.g., *Ae. aegypti* and *Ae. triseriatus;* and inadequate disposal of polluted water resulting from burgeoning urbanization and industrialization, e.g., *Culex quinquefasciatus* Say. Other factors of importance, some of them interacting, include the development of faster transportation (increasing the frequency of introduction of new vector species, infected vectors and infected persons); human population movements; changes in human behavior; modification of vertebrate faunas; deforestation; wars and political instability; insecticides; vector resistance to insecticides (often resulting from agricultural insect control rather than from vector control); development of vaccines, introduction of vectors to unexploited habitats, etc. Some influencing factors are quite subtle. Gahlinger et al. (1986) found that encephalitis cases in California were negatively associated with television ownership. It was found that television and air conditioner utilization times corresponded closely to the feeding period of *Cx. tarsalis,* the vector of St. Louis encephalitis (SLE) and western

equine encephalomyelitis (WEE) viruses in California.

A factor of special importance in today's fast-paced and problem-plagued world, and which is no doubt of great benefit to vector species, is public complacency resulting from competing societal concerns and priorities. Competing priorities and complacency result in vector control budgets that are inadequate for the task. Based on data by Challet and Keller (1981), Beams (1985) stated that, while the annual expenditures of 106 U.S. mosquito control agencies increased an average of only 5.4% per year during the 5-year period from 1976 through 1980, the consumer price index (a measure of inflation) increased an average of 8.9% per year. Vector control budgets in developing countries are frequently even more hard pressed (Knudsen 1983 and others). From 193 responses to a questionnaire mailed to U.S. mosquito control agencies, Beams (1985) found that although 50% of the respondents considered public education to be "as important as chemical, biological and physical control" and 10% considered it "more important," 97% of the agencies allocated 10% or less of their budget to education and the average was only 1.7%.

The sheer increase of activity in a more populous world and increased technological complexity in all fields of endeavor exacerbates problems in vector control and surveillance. Chadee (1984), for example, reported the results of inspecting 46,892 boats entering the harbor of Port-of-Spain, Trinidad, W.I. from 1972 to 1982. The yearly average derivable from this total, 4,689, compares to 727 boats inspected by port health officers in 1934. And the boats were smaller in 1934. Reiter and Darsie (1984) discussed two modern innovations in shipping, containerization and the Lighter Aboard Ship (LASH) system, that shorten considerably cargo delivery time and involve dockside handling methods that make inspection of the cargo difficult. They noted that, relative to shipping volume, exports to the United States from the South-East Asian Trading Nations (ASEAN) increased by more than 1,200% in the past decade.

No other mosquito vector species has intertwined its destiny so intimately with human society as has *Ae. aegypti.* McClelland (1973) and Rodhain (1983) have traced the sequence of human socio-economic developments, from the beginnings of primitive agriculture to the rise of modern urbanization, that encouraged evolution of the intimate association of this species with man. As the vector of dengue and yellow fever viruses, no other mosquito species illustrates so well the impact of human activity on patterns of mosquito-borne disease. Dengue, with the close association of its vector with man, seems made to order for effective control based largely on source reduction and public education. But dengue, more than any other mosquito-borne virus disease appears to benefit from competing societal priorities. There seems to be no other explanation that the disease flourishes and appears to have a very bright future. Consider the scenario that presently exists in the Western Hemisphere:

1) Following the near eradication of its vector during the early 1960s, hundreds of thousands of cases of dengue have occurred in the Caribbean Basin in the past 2 decades (Gratz 1985, Tonn et al. 1982).

2) Its vector, *Ae. aegypti,* not only thrives under the socio-economic conditions that exist in much of the region (Waterman et al. 1985), but it moves freely within the region, having now reclaimed much of its former territory (Chadee 1984, Cookman and LeBrun 1986, Knudsen 1983, LeMaitre and Chadee 1983, Mayers 1983, Tonn et al. 1982, Wallis et al. 1984). According to the most recent information (for the years 1982 and 1983) (Anonymous 1985, Knudsen 1983), only 4 Caribbean countries (Barbados, Cuba, Grenada and Guyana) had larval house indices under 5, the infestation level generally considered by the Centers for Disease Control (CDC) (1979) and others to be that below which epidemic transmission is unlikely to occur.

3) Based on the 1977 Puerto Rico epidemic, there is a minimum lagtime of 20–35 days between the onset of epidemic activity and the implementation of control measures even in localities with a good health system, high awareness of dengue and efficient arbovirus response capability (Morens et al. 1986). In general in the Caribbean, however, few countries have sufficient insecticide reserves on hand to mount an immediate wide-scale response to a dengue or yellow fever outbreak; few have relevant health education programs, which are essential for enlisting support in source reduction at the community level; only about half of the countries have adequate vehicles for an emergency campaign; and in some countries inadequate government support reduces program efficiency (Knudsen 1983).

4) The costs of an epidemic are high. Von Allmen et al. (1979) estimated the cost of the 1977 Puerto Rico epidemic at between $6.0 and $15.6 million including direct costs (medical care and epidemic control measures) and indirect costs (lost production of ill workers and of parents of ill children). These estimates did not include costs of school absenteeism nor

losses of tourism revenue which, although potentially important, may not have been an important factor in this epidemic.

5) Although some vector breeding occurs in sylvan habitats, well over 95% occurs in or in close proximity to human dwellings, and mostly in manmade containers (Chadee et al. 1984, Knudsen 1983, Moore 1983, Moore et al. 1978, Tonn et al. 1982).

6) Despite efforts of national governments and the Panamerican Health Organization (PAHO), dengue not only flourishes south of the United States but threatens increasingly to spread northward. Several dozen cases are imported to the U.S.A. annually, mostly travelers returning from the Caribbean region (Knudsen 1983, Malison and Waterman 1983), and after an absence of more than 30 years, the virus has again been transmitted endemically within the United States (Texas) (Hafkin et al. 1982). Mass movements of people pose an additional avenue for entry of dengue, such as the thousands of Vietnamese refugees entering Florida in 1975 and the thousands of Haitians and Cubans entering the state in 1980 (Breeland and Mulrennan 1983).

7) *Aedes aegypti* is again widespread in the cities and rural areas of the southern United States (Fochs et al. 1981, Tabachnick 1982, Welch and Long 1984). Fochs et al. (1981) reported a house index of 35 in a substandard residential area of New Orleans.

8) *Aedes albopictus,* indigenous to tropical Asia, and an important secondary vector of dengue viruses, has recently been introduced to the United States and is now known from Texas (Sprenger and Wuithiranyagool 1986), all of the Gulf Coast states eastward to Florida and Georgia (Centers for Disease Control [CDC] 1986), Tennessee (Reiter and Darsie 1984), Ohio (M.A. Parsons, personal communication), Indiana, Illinois and Missouri (C. G. Moore, personal communication). (It has also been found in Rio de Janeiro and two neighboring states in Brazil [CDC 1986]). In Harris County, Texas, *Ae. albopictus* was the most abundant species found breeding in used tires and other water-filled containers (Sprenger and Wuithiranyagool 1986) and it was abundant and aggressively biting humans in many other Gulf states sites (CDC 1986). According to the CDC report, the presence of *Ae. aegypti* has not appeared to interfere with establishment of *Ae. albopictus,* and, in fact, the latter has replaced *Ae. aegypti* in many localities. The Harris County, Texas population has been shown to resemble populations from northern Asia that are capable of diapause, indicating that the species will be capable of surviving northern winters on this continent. In addition, it has been found capable of oral transmission of LACV to suckling mice at a rate of 40–50% on days 14 and 21 following the infective blood meal (P.R. Grimstad, unpublished data). Oral transmission of JCV to suckling mice was less than 10% on postinfection days 14 and 21.

9) *Aedes triseriatus* has been found to be a capable oral transmitter of DEN-1 virus (Freier and Grimstad 1983). Two good research questions are: Can *Ae. triseriatus* transmit DEN viruses transovarially, and, if so, can the virus survive northern winters in the egg of the mosquito?

10) In addition to other man-made containers, all 3 of these species readily utilize auto tires for breeding, and as stated earlier, used auto tires are accumulating at the rate of 200 million per year in the United States (Deese et al. 1981).

Research will yield no magic bullet solutions to the problem of dengue. Experienced workers are increasingly emphasizing that effective control of dengue must be based largely on source reduction, with strong public education and participation, and sufficient resources in the form of trained personnel to tailor management programs to individual situations (Beams 1985, Gratz 1985, Halstead 1984, Morens et al. 1986). Increasingly, there is a call for the imposition of reasonable penalties against citizens who fail or refuse to eliminate breeding sites on their own property. These 4 factors, source reduction, public education, trained personnel and legal sanctions were strong ingredients in the intensive campaign launched during the Cuban epidemic of 1981 (Tonn et al. 1982). They were also given prominence on a more general policy statement adopted in 1979 by the American Mosquito Control Association (R. A. Hart, cited by Beams 1985). Knudsen (1983) summarized the outlook for dengue as follows:

> "Thus, one can predict *Ae. aegypti* will continue to live in close association with man in the Caribbean as long as traditional water storage habits persist, lip service is given to control programs, source reduction is ignored, community participation is not applied and research is treated superficially. As a result, we will continue to be faced with the consequences of *Ae. aegypti*-borne diseases."

As discussed in this paper and by DeFoliart et al. (1987), "changing patterns" of mosquito-borne arboviruses result both from the steady accumulation of new research findings and from continuing change in the ecology of the vectors and their human and non-human vertebrate hosts. Unexpected happenings such as the 1984 occurrence of 25 cases of St. Louis

encephalitis in southern California remind us that our knowledge is quite incomplete and much is yet to be learned even about arboviruses that have been extensively studied. As stated by Reeves (1986) in reference to the Los Angeles area epidemic, "the heart of research is the reexamination of accepted concepts on the basis of new facts." The current situation with dengue reemphasizes the fact that research alone does not alleviate public health problems and is a reminder to investigators, funding agencies and governments that research findings are useless until they are applied.

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References Cited

Amundson, T. E. and T. M. Yuill. 1981. Natural La Crosse virus infection in the red fox (*Vulpes fulva*), gray fox (*Urocyon cinereoargenteus*), raccoon (*Procyon lotor*), and opossum (*Didelphis virginiana*). Am. J. Trop. Med. Hyg. 30:706–714.

Amundson, T. E., T. M. Yuill and G. R. DeFoliart. 1985. Experimental La Crosse virus infection of red fox (*Vulpes fulva*), raccoon (*Procyon lotor*), opossum (*Didelphis virginiana*), and woodchuck (*Marmota monax*). Am. J. Trop. Med. Hyg. 34:586–595.

Anonymous. 1985. Communicable diseases in the Caribbean, 1983. Weekly Epidemiol. Rec. 60:229–232.

Beams, B. F. 1985. Analysis of mosquito control agency public education programs in the United States. J. Am. Mosq. Control Assoc. 1:212–219.

Beaty, B. J. and W. H. Thompson. 1975. Emergence of La Crosse virus from endemic foci: Fluorescent antibody studies of overwintered *Aedes triseriatus*. Am. J. Trop. Med. Hyg. 24:685–691.

Beier, J. C., W. J. Berry and G. B. Craig, Jr. 1982. Horizontal distribution of adult *Aedes triseriatus* (Diptera: Culicidae) in relation to habitat structure, oviposition, and other mosquito species. J. Med. Entomol. 19:239–247.

Beier, J. C., M. Travis, C. Patricoski and J. Kranzfelder. 1983. Habitat segregation among larval mosquitoes (Diptera: Culicidae) in tire yards in Indiana, USA. J. Med. Entomol. 20:76–80.

Berry, W. J. and G. B. Craig, Jr. 1984. Bionomics of *Aedes atropalpus* breeding in scrap tires in northern Indiana. Mosq. News 44:476–484.

Berry, R. L., B. J. LaLonde-Weigert, C. H. Calisher, M. A. Parsons and G. T. Bear. 1977. Evidence for transovarial transmission of Jamestown Canyon virus in Ohio. Mosq. News 37:494–496.

Berry, R. L., M. A. Parsons, B. J. LaLonde-Weigert, J. Lebio, H. Stegmiller, and G. T. Bear. 1986. *Aedes canadensis*, a vector of La Crosse virus (California serogroup) in Ohio. J. Am. Mosq. Control Assoc. 2:73–78.

Berry, R. L., M. A. Parsons, R. A. Restifo, E. D. Peterson, S. W. Gordon, M. R. Reed, C. H. Calisher, G. T. Bear and T. J. Halpin. 1983. California serogroup virus infections in Ohio: An 18-year retrospective survey, pp. 215–223. *In*: C. H. Calisher and W. H. Thompson (eds.). California serogroup viruses. A. R. Liss, New York.

Boromisa, R. D. and P. R. Grimstad. 1986. Virus-vector-host relationships of *Aedes stimulans* and Jamestown Canyon virus in a northern Indiana focus. Am. J. Trop. Med. Hyg. 35:1285–1295

Boromisa, R. D. and P. R. Grimstad. 1987. Seroconversion rates to Jamestown Canyon virus among six Indiana white-tailed deer (*Odocoileus virginianus*) populations. J. Wildl. Dis. 23:(in press).

Breeland, S. G. and J. A. Mulrennan, Jr. 1983. An overview of organized mosquito control in Florida. Mosq. News 43:276–280.

Burkot, T. R. and G. R. DeFoliart. 1982. Bloodmeal sources of *Aedes triseriatus* and *Aedes vexans* in a southern Wisconsin forest endemic for La Crosse encephalitis virus. Am. J. Trop. Med. Hyg. 31:376–381.

Calisher, C. H. 1983. Taxonomy, classification, and geographic distribution of California serogroup bunyaviruses, pp. 1–16. *In*: C. H. Calisher and W. H. Thompson (eds.) California serogroup viruses. A. R. Liss, New York.

Calisher, C. H. and R. E. Bailey. 1981. Serodiagnosis of La Crosse virus infections in humans. J. Clin. Microbiol. 13:344–50.

Calisher, C. H. and W. H. Thompson, (eds.) California serogroup viruses. A. R. Liss, New York. 399 pp.

Centers for Disease Control. 1986. *Aedes albopictus* infestation—United States, Brazil. Morb. Mort. Wkly. Rep. 35:493–495.

Centers for Disease Control, Bureau of Tropical Diseases, Vector Biology and Control Division. 1979. Vector Topics No. 4. Biology and control of *Aedes aegypti*. CDC, PHS, DHEW, Atlanta, GA., 68 pp.

Chadee, D. D. 1984. *Aedes aegypti* aboard boats at Port-of-Spain, Trinidad, West Indies (1972–82). Mosq. News 44:1–3.

Chadee, D. D., N. K. Connel, A. Le Maitre and S. B. Ferreira. 1984. Surveillance for *Aedes aegypti* in Tobago, West Indies (1980–82). Mosq. News 44:490–492.

Challet, G. L. and J. Keller (eds.) 1981. Directory of mosquito control agencies in the United States and Canada. Am. Mosq. Control Assoc., Fresno, CA.

Clark, G. G. and G. B. Craig, Jr. 1985. Oviposition behavior of *Aedes triseriatus* and *Aedes hendersoni* on

the Delmarva Peninsula. J. Am. Mosq. Control Assoc. 1:526–528.

Clark, G. G., C. L. Crabbs and B. T. Elias. 1986. Absence of La Crosse virus in the presence of *Aedes triseriatus* on the Delmarva Peninsula. J. Am. Mosq. Control Assoc. 2:33–37.

Clark, G. G., W. H. Rohrer and D. N. Robbins. 1985. Diurnal biting activity of *Aedes triseriatus* complex (Diptera: Culicidae) in a focus of La Crosse virus transmission. J. Med. Entomol. 22:684–686.

Cookman, J. E. and R. A. LeBrun. 1986. *Aedes aegypti* larvae in Portsmouth, Rhode Island. J. Am. Mosq. Control Assoc. 2:96–97.

Cordellier, R., B. Bouchiteé, J. C. Roche, N. Monteny, B. Diaco and P. Akoliba. 1983. Sylvatic circulation of the dengue 2 virus in 1980, in the sub-sudanian savannas of the Ivory Coast. Cahiers O.R.S.T.O.M. Ser. Entomol. Med. Parasitol. 21:165–179. (In French)

Cornet, M., J. F. Saluzzo, J. P. Hervy, J. P. Digoutte, M. Germain. M. F. Chauvancy, M. Eyraud, L. Ferrara, G. Hème and F. Legros. 1984. Dengue 2 au Senegal oriental: Une poussee epizootique en milieu selvatique; Isolements du virus a partir de moustiques en d'un singe et considerations epidemiologiques. Cah. O.R.S.T.O.M. Ser. Entomol. Med. Parasitol. 22:313–323.

Craig, G. B., Jr. 1983. Biology of *Aedes triseriatus*: Some factors affecting control, pp. 329–341. *In*: C. H. Calisher and W. H. Thompson (eds.). California serogroup viruses. A. R. Liss, New York.

Deese, P. L., J. F. Hudson, R. C. Innes and D. F. Funkhouser. 1981. Options for resource recovery and disposal of scrap tires. EPA Mimeo Publ., 144 pp.

DeFoliart, G. R. 1983. *Aedes triseriatus*: Vector biology in relationship to the persistence of La Crosse virus in endemic foci, pp. 89–104. *In*: C. H. Calisher and W. H. Thompson (eds.). California serogroup viruses. A. R. Liss, New York.

DeFoliart, G. R., P. R. Grimstad and D. M. Watts. 1987. Advances in mosquito-borne arbovirus/vector research. Annu. Rev. Entomol. 32:479–505.

Deibel, R., S. Srihongse, M. A. Grayson, P. R. Grimstad, M. S. Mahdy, H. Artsob and C. H. Calisher. 1983. Jamestown Canyon virus: The etiologic agent of an emerging human disease?, pp. 313–325. *In*: C. H. Calisher and W. H. Thompson (eds.). California serogroup viruses. A. R. Liss, New York.

Fish, D. and S. R. Carpenter. 1982. Leaf litter and larval mosquito dynamics in tree-hole ecosystems. Ecology 63:283–288.

Fochs, D. A., S. R. Sackett and D. L. Bailey. 1981. Observations on larval breeding and population density of *Aedes aegypti* (L.) in New Orleans, Louisiana. Am. J. Trop. Med. Hyg. 30:1329–1335.

Freier, J. E. and J. C. Beier. 1984. Oral and transovarial transmission of La Crosse virus by *Aedes atropalpus*. Am. J. Trop. Med. Hyg. 33:708–714.

Freier, J. E. and P. R. Grimstad. 1983. Transmission of dengue virus by orally infected *Aedes triseriatus*. Am. J. Trop. Med. Hyg. 32:1429–1434.

Gahlinger, P. M., W. C. Reeves and M. M. Milby. 1986. Air conditioning and television as protective factors in arboviral encephalitis risk. Am. J. Trop. Med. Hyg. 35:601–610.

Gauld, L. W., R. P. Hanson, W. H. Thompson and S. K. Sinha. 1974. Observations on a natural cycle of La Crosse virus (California group) in southwestern Wisconsin. Am. J. Trop. Med. Hyg. 23:983–992.

Gauld, L. W., T. M. Yuill, R. P. Hanson and S. K. Sinha. 1975. Isolation of La Crosse virus (California encephalitis group) from the chipmunk (*Tamias striatus*), an amplifier host. Am. J. Trop. Med. Hyg. 24:999–1005.

Gratz, N. R. 1985. The future of vector biology and control in the World Health Organization. J. Am. Mosq. Control Assoc. 1:273–278.

Grayson, M. A., S. Srihongse, R. Deibel and C. H. Calisher. 1983. California serogroup viruses in New York State: A retrospective analysis of subtype distribution patterns and their epidemiologic significance, 1965–1981, pp. 257–267. *In*: C. H. Calisher and W. H. Thompson (eds.). California serogroup viruses. A. R. Liss, New York.

Grimstad, P. R. 1983. Mosquitoes and the incidence of encephalitis. Adv. Virus Res. 28:357–438.

Grimstad, P. R. 1987. California group virus disease. *In*: T. P. Monath (ed.). Epidemiology of Arthopod-Borne Viral Diseases. CRC Press, Boca Raton, Florida. (in press)

Grimstad, P. R. and L. D. Haramis. 1984. *Aedes triseriatus* (Diptera: Culicidae) and La Crosse virus. III. Enhanced oral transmission by nutrition-deprived mosquitoes. J. Med. Entomol. 21:249–256.

Grimstad, P. R. and M. J. Mandracchia. 1985. Record of Michigan mosquito species (Diptera: Culicidae) collected in a natural focus of Jamestown Canyon virus in 1984. Great Lakes Entomologist 18:45–49.

Grimstad, P. R., S. L. Paulson and G. B. Craig, Jr. 1985. Vector competence of *Aedes hendersoni* (Diptera: Culicidae) for La Crosse virus and evidence of a salivary-gland escape barrier. J. Med. Entomol. 22:447–453.

Grimstad, P. R., C. H. Calisher, R. N. Harroff and B. B. Wentworth. 1986. Jamestown Canyon virus (California serogroup) is the etiologic agent of widespread human infection in Michigan humans. Am. J. Trop. Med. Hyg. 35:376–386.

Grimstad, P. R., G. B. Craig, Jr., Q. E. Ross and T. M. Yuill. 1977. *Aedes triseriatus* and La Crosse virus: Geographic variation in vector susceptibility and ability to transmit. Am. J. Trop. Med. Hyg. 26:990–996.

Grimstad, P. R., Q. E. Ross and G. B. Craig, Jr. 1980. *Aedes triseriatus* (Diptera: Culicidae) and La Crosse virus. II. Modification of mosquito feeding behavior by virus infection. J. Med. Entomol. 17:1–7.

Grimstad, P. R., D. G. Williams and S. M. Schmitt. 1987. Infection of white-tailed deer (*Odocoileus virginianus*) in Michigan with Jamestown Canyon virus (California serogroup) and the importance of maternal antibody in viral maintenance. J. Wildl. Dis. 23:(in press).

Grimstad, P. R., C. L. Shabino, C. H. Calisher and R. J. Waldman. 1982. A case of encephalitis in a human associated with a serologic rise to Jamestown Canyon virus. Am. J. Trop. Med. Hyg. 31:1238–1244.

Gubler, D. J., R. J. Novak, E. Vergne, N. A. Colon,

M. Velez and J. Fowler. 1985. *Aedes (Gymnometopa) mediovittatus* (Diptera: Culicidae), a potential maintenance vector of dengue viruses in Puerto Rico. J. Med. Entomol. 22:469–475.

Guzman, M. G., G. Kouri, L. Morier, M. Soler and A. Fernandez. 1984. A study of fatal hemorrhagic dengue cases in Cuba, 1981. Bull. PAHO 18:213–220.

Hafkin, B., J. E. Kaplan, C. Reed, B. Elliot, R. Fontaine, G. E. Sather and K. Kappus. 1982. Reintroduction of dengue fever into the continental United States. I. Dengue surveillance in Texas, 1980. Am. J. Trop. Med. Hyg. 31:1222–1228.

Halstead, S. B. 1984. Selective primary health care: Strategies for control of disease in the developing world. XI. Dengue. Rev. Infect. Dis. 6:251–264.

Hammon, W. McD. and G. E. Sather. 1966. History and recent reappearance of viruses of the California encephalitis group. Am. J. Trop. Med. Hyg. 15:199–204.

Haramis, L. D. 1983. Increased adult size correlated with parity in *Aedes triseriatus*. Mosq. News 43:77–79.

Haramis, L. D. 1984. *Aedes triseriatus*: A comparison of density in tree holes vs. discarded tires. Mosq. News 44:485–489.

Haramis, L. D. and W. A. Foster. 1983. Survival and population density of *Aedes triseriatus* (Diptera: Culicidae) in a woodlot in central Ohio, USA. J. Med. Entomol. 20:391–398.

Hedberg, C. W., J. W. Washburn and R. D. Sjogren. 1985. The association of artificial containers and La Crosse encephalitis cases in Minnesota, 1979. J. Am. Mosq. Control Assoc. 1:89–90.

Horsfall, W. R. 1974. Effect of simulated seasonal thermal conditions on hatching of eggs of a univoltine mosquito. Ann. Entomol. Soc. Am. 67:991–992.

Hull, B., E. Tikasingh, M. DeSouza and R. Martinez. 1984. Natural transovarial transmission of a dengue 4 virus in *Aedes aegypti* in Trinidad. Am. J. Trop. Med. Hyg. 33:1248–1250.

Issel, C. J. 1973. Isolation of Jamestown Canyon virus (a California group arbovirus) from a white-tailed deer. Am. J. Trop. Med. Hyg. 22:414–417.

Issel, C. J. 1974. Maternal antibody to Jamestown Canyon virus in white-tailed deer. Am. J. Trop. Med. Hyg. 23:242–245.

Issel, C. J., D. O. Trainer and W. H. Thompson. 1972a. Experimental studies with white-tailed deer and four California group arboviruses (La Crosse, trivittatus, snowshoe hare, and Jamestown Canyon). Am. J. Trop. Med. Hyg. 221:979–984.

Issel, C. J., D. O. Trainer and W. H. Thompson. 1972b. Serologic evidence of infections of white-tailed deer in Wisconsin with three California group arboviruses (La Crosse, trivittatus, and Jamestown Canyon). Am. J. Trop. Med. Hyg. 21:985–988.

Johnson, K. M., S. B. Halstead and S. N. Cohen. 1967. Hemorrhagic fevers of Southeast Asia and South America: A comparative appraisal. Prog. Med. Virol. 9:105–158.

Jousset, F-X. 1981. Geographic *Aedes aegypti* strains and dengue 2 virus: Susceptibility, ability to transmit to vertebrates and transovarial transmission. Ann. Virol. (Inst. Pasteur) 132E:357–370.

Khin, M. M. and K. A. Than. 1983. Transovarial transmission of dengue 2 virus by *Aedes aegypti* in nature. Am. J. Trop. Med. Hyg. 32:590–594.

Klimas, R. A., W. H. Thompson, C. H. Calisher, G. G. Clark, P. R. Grimstad and D. H. L. Bishop. 1981. Genotypic varieties of La Crosse virus isolated from different geographic regions of the continental United States and evidence for a naturally occurring intertypic recombinant La Crosse virus. Am. J. Epidemiol. 114:112–131.

Klowden, M. J. and A. O. Lea. 1978. Blood meal size as a factor affecting continued host-seeking by *Aedes aegypti* (L.). Am. J. Trop. Med. Hyg. 27:827–831.

Knudsen, A. B. 1983. *Aedes aegypti* and dengue in the Caribbean. Mosq. News 43:269–275.

Kramer, L. D. and W. H. Thompson. 1983. Quantitation of La Crosse virus in venereally infected *Aedes triseriatus*. Am. J. Trop. Med. Hyg. 32:1140–1146.

Ksiazek, T. G. and T. M. Yuill. 1977. Viremia and antibody response to La Crosse virus in sentinel gray squirrels (*Sciuris carolinensis*) and chipmunks (*Tamias striatus*). Am. J. Trop. Med. Hyg. 26:815–821.

Leiser, L. 1981. Distribution of *Aedes triseriatus* (Say) in an urban area: Comparison of two survey methods. Proc. Indiana Acad. Sci. 90:248–253.

LeMaitre, A. and D. D. Chadee. 1983. Arthropods collected from aircraft at Piarco International Airport, Trinidad, West Indies. Mosq. News 43:21–23.

Lisitza, M. A., G. R. DeFoliart, T. M. Yuill and M. G. Karandinos. 1977. Prevalence rates of La Crosse virus (California encephalitis group) in larvae from overwintered eggs of *Aedes triseriatus*. Mosq. News 37:745–750.

Loor, K. A. and G. R. DeFoliart. 1970. Field observations on the biology of *Aedes triseriatus*. Mosq. News 30:60–64.

Malison, M. D. and S. H. Waterman. 1983. Dengue fever in the United States. A report of a cluster of imported cases and review of the clinical, epidemiologic, and public health aspects of the disease. J. Am. Med. Assoc. 249:496–500.

Mather, T. N. and G. R. DeFoliart. 1983. Effect of host blood source on the gonotrophic cycle of *Aedes triseriatus*. Am. J. Trop. Med. Hyg. 32:189–193.

Mather, T. N. and G. R. DeFoliart. 1984a. Dispersion of gravid *Aedes triseriatus* (Diptera: Culicidae) from woodlands into open terrain. J. Med. Entomol. 21:384–391.

Mather, T. N. and G. R. DeFoliart. 1984b. Conditions for increased insemination rates in caged *Aedes triseriatus* (Diptera: Culicidae) reared from field-collected eggs. Am. J. Trop. Med. Hyg. 33:731–735.

Mayers, P. J. 1983. Recent introduction of *Aedes aegypti* in Bermuda. Mosq. News 43:361–362.

McClelland, G. A. H. 1973. Some man-made mosquito problems in Africa and prospects for their rational solution. Proc. 5th Tall Timbers Conf. Ecol. Animal Control, Tallahassee, FL., pp. 27–41.

Means, R. G., M. Grayson and E. Blakemore. 1977.

Preliminary studies on two biologically different strains of *Aedes triseriatus* in New York. Mosq. News 37:609–615.

Metselaar, D., C. R. Grainger, K. G. Oei, D. G. Reynolds, M. Pudney, C. J. Leake, R. M. Tukei, R. M. D'Offay and D. I. H. Simpson. 1980. An outbreak of type 2 dengue fever in the Seychelles, probably transmitted by *Aedes albopictus* (Skuse). Bull. W.H.O. 58:937–943.

Miller, B. R., G. R. DeFoliart and T. M. Yuill. 1977. Vertical transmission of La Crosse virus (California encephalitis group): Transovarial and filial infection rates in *Aedes triseriatus* (Diptera: Culicidae). J. Med. Entomol. 14:437–440.

Miller, B. R., G. R. DeFoliart and T. M. Yuill. 1979. *Aedes triseriatus* and La Crosse virus: Lack of infection in eggs from the first ovarian cycle following oral infection of females. Am. J. Trop. Med. Hyg. 28:897–901.

Mitchell, C. J. 1977. Arthropod-borne encephalitis viruses and water resource developments. Cahiers O.R.S.T.O.M. Ser. Entomol. Med. Parasitol. 15:241–249.

Moore, C. G. 1983. Habitat differences among container-breeding mosquitoes in western Puerto Rico (Diptera: Culicidae). Pan-Pacif. Entomol. 59:218–228.

Moore, C. G., B. L. Cline, E. Ruiz-Tibén, D. Lee, H. Romney-Joseph and E. Rivera-Correa. 1978. *Aedes aegypti* in Puerto Rico: Environmental determinants of larval abundance and relation to dengue virus transmission. Am. J. Trop. Med. Hyg. 27:1225–1231.

Morens, D. M., J. G. Rigau-Pérez, R. H. López-Correa, C. G. Moore, E. E. Ruiz-Tibén, G. E. Sather, J. Chiriboga, D. A. Eliason, A. Casta-Veléz, J. P. Woodall and the Dengue Outbreak Investigation Group. 1986. Dengue in Puerto Rico, 1977: Public Health response to characterize and control an epidemic of multiple serotypes. Am. J. Trop. Med. Hyg. 35:197–211.

Moulton, D. W. and W. H. Thompson. 1971. California group virus infections in small, forest-dwelling mammals of Wisconsin. Am. J. Trop. Med. Hyg. 20:474–482.

Munstermann, L. E. 1980. Distinguishing geographic strains of the *Aedes atropalpus* group (Diptera: Culicidae) by analysis of enzyme variation. Ann. Entomol. Soc. Am. 73:699–704.

Munstermann, L. E., D. B. Taylor and T. C. Matthews. 1982. Population genetics and speciation in the *Aedes triseriatus* group, pp. 433–453. *In*: W. W. M. Steiner, W. J. Tabachnick, K. S. Rai and S. Narang (eds.). Recent developments in the genetics of insect disease vectors. Stipes Publ. Co., Champaign, IL.

Nasci, R. S. 1982a. Differences in host choice between the sibling species of treehole mosquitoes *Aedes triseriatus* and *Aedes hendersoni*. Am. J. Trop. Med. Hyg. 31:411–415.

Nasci, R. S. 1982b. Activity of gravid *Aedes triseriatus* in wooded fencerows. Mosq. News 42:408–412.

Nasci, R. S. 1984. Variations in the blood-feeding patterns of *Aedes vexans* and *Aedes trivittatus* (Diptera: Culicidae). J. Med. Entomol. 21:95–99.

Nasci, R. S. 1985. Local variation in blood feeding by *Aedes triseriatus* and *Aedes hendersoni* (Diptera: Culicidae). J. Med. Entomol. 22:619–623.

Novak, R. J., J. Peloquin and W. Rohrer. 1981. Vertical distribution of adult mosquitoes (Diptera: Culicidae) in a northern deciduous forest in Indiana. J. Med. Entomol. 18:116–122.

Pantuwatana, S., W. H. Thompson, D. M. Watts and R. P. Hanson. 1972. Experimental infection of chipmunks and squirrels with La Crosse and trivittatus viruses and biological transmission of La Crosse virus by *Aedes triseriatus*. Am. J. Trop. Med. Hyg. 21:476–481.

Parry, J. E. 1983. Control of *Aedes triseriatus* in La Crosse, Wisconsin, pp. 355–363. *In*: C. H. Calisher and W. H. Thompson (eds.). California serogroup viruses. A. R. Liss, New York.

Patrican, L. A., G. R. DeFoliart and T. M. Yuill. 1985a. La Crosse viremias in juvenile, subadult and adult chipmunks (*Tamias striatus*) following feeding by transovarially-infected *Aedes triseriatus*. Am. J. Trop. Med. Hyg. 34:596–602.

Patrican, L. A., G. R. DeFoliart and T. M. Yuill. 1985b. Oral infection and transmission of La Crosse virus by an enzootic strain of *Aedes triseriatus* feeding on chipmunks with a range of viremia levels. Am. J. Trop. Med. Hyg. 34:992–998.

Porter, C. H. and G. R. DeFoliart. 1985. Gonotrophic age, insemination, and *Ascogregarina* infection in a southern Wisconsin population of *Aedes triseriatus*. J. Am. Mosq. Control Assoc. 1:238–240.

Qiu, F., H. Zhang, L. Shao, X. Li, H. Luo and Y. Yu. 1981. Studies on the rapid detection of dengue virus antigen by immunofluorescence and radioimmunoassay. Chinese Medical J. 94:653–658.

Reeves, W. C. 1986. Perspectives and predictions following the St. Louis encephalitis outbreak in southern California. Proc. Pap. 53rd Annu. Conf. Calif. Mosq. Vector Control Assoc. 54:30–31.

Reiter, P. and R. F. Darsie, Jr. 1984. *Aedes albopictus* in Memphis, Tennessee (USA): An achievement of modern transportation? Mosq. News 44:396–399.

Roche, J. C., R. Cordellier, J. P. Hervy, J. P. Digoutte and N. Monteny. 1983. Isolement de 96 souches de virus dengue 2 a partir de moustiques captures en Cote Divoire et Haute-Volta. Ann. Virol. 134E:233–244.

Rodhain, F. 1983. Maladies transmises par les culiciñes et urbanisation: Un example de coévolution. Bull. Inst. Pasteur 81:33–54.

Rosen, L. 1984. The global importance and epidemiology of dengue infection and disease, pp. 1–6. *In*: T. Pang and R. Pathamanathan (eds.). Proc. Int. Conf. Dengue Hemorrhagic Fever, Kuala Lumpur, Malaysia, 1983.

Rosen, L., L. E. Rozeboom, D. J. Gubler, J. C. Lien and B. N. Chaniotis. 1985. Comparative susceptibility of mosquito species and strains to oral and parenteral infection with dengue and Japanese encephalitis viruses. Am. J. Trop. Med. Hyg. 34:603–615.

Rosen, L., D. A. Shroyer, R. B. Tesh, J. E. Frier and J. C. Lien. 1983. Transovarial transmission of dengue viruses by mosquitoes: *Aedes albopictus* and *Aedes aegypti*. Am. J. Trop. Med. Hyg. 32:1108–1119.

Rudnick, A. 1984. The ecology of the dengue virus complex in peninsular Malaysia, pp. 7–15. *In*: T.

Pang and R. Pathamanathan (eds.). Proc. Int. Conf. Dengue Hemorrhagic Fever, Kuala Lumpur, Malaysia, 1983.

Saul, S. H., M. J. Sinsko, P. R. Grimstad and G. B. Craig, Jr. 1977. Identification of sibling species, *Aedes triseriatus* and *Aedes hendersoni*, by electrophoresis. J. Med. Entomol. 13:705–708.

Seawright, G. L., R. O. Anslow, J. P. Spalatin and R. P. Hanson. 1974. Domestic rabbits as indicators of California-group arbovirus activity in Wisconsin: Experimental studies. Am. J. Vet. Res. 35:1093–1095.

Sheppard, P. M., W. W. MacDonald, R. J. Tonn and B. Grub. 1969. The dynamics of an adult population of *Aedes aegypti* in relation to dengue hemorrhagic fever in Bangkok. J. Anim. Ecol. 38:661–702.

Sinsko, M. J. and G. B. Craig, Jr. 1979. Dynamics of an isolated population of *Aedes triseriatus* (Diptera: Culicidae). I. Population size. J. Med. Entomol. 15:89–98.

Smith, C. E. G. 1975. Changing patterns of disease in the tropics, p. 345–362. *In*: N. F. Stanley and M. P. Alpers (eds.), Man-made lakes and human health. Academic Press, London, New York and San Francisco.

Sprenger, D. and T. Wuithiranyagool. 1986. The discovery and distribution of *Aedes albopictus* in Harris County, Texas. J. Am. Mosq. Control Assoc. 2:217–219.

Sullivan, M. F., D. J. Gould and S. Maneechai. 1971. Observations on the host range and feeding preferences of *Aedes albopictus* (Skuse). J. Med. Entomol. 8:713–716.

Surtees, G. 1971. Urbanization and the epidemiology of mosquito-borne disease. Abstr. Hyg. 46:121–134.

Suzuki, T. and J. H. Hirshman. 1977. Distribution and density of *Aedes aegypti* in the South Pacific. N. Z. Med. J. 85:374–380.

Tabachnick, W. J. 1982. Geographic and temporal patterns of genetic variation of *Aedes aegypti* in New Orleans. Am. J. Trop. Med. Hyg. 31:849–853.

Thompson, W. H. 1979. Higher venereal infection and transmission rates with La Crosse virus in *Aedes triseriatus* engorged before mating. Am. J. Trop. Med. Hyg. 28:890–896.

Thompson, W. H. 1983. Lower rates of oral transmission of La Crosse virus by *Aedes triseriatus* venereally exposed after engorgement on immune chipmunks. Am. J. Trop. Med. Hyg. 32:1416–1421.

Thompson, W. H. and B. J. Beaty. 1977. Venereal transmission of La Crosse (California encephalitis) arbovirus in *Aedes triseriatus* mosquitoes. Science 196:530–531.

Thompson, W. H. and B. J. Beaty. 1978. Venereal transmission of La Crosse virus from male to female *Aedes triseriatus*. Am. J. Trop. Med. Hyg. 27:187–196.

Thompson, W. H. and A. S. Evans. 1965. California encephalitis virus studies in Wisconsin. Am. J. Epidemiol. 81:230–244.

Thompson, W. H. and C. B. Gundersen. 1983. La Crosse encephalitis: Occurrence of disease and control in a suburban area, pp. 225–236. *In*: C. H. Calisher and W. H. Thompson (eds.). California serogroup viruses. A. R. Liss, New York.

Thompson, W. H. and S. L. Inhorn. 1967. Arthropod-borne California viral encephalitis in Wisconsin. Wis. Med. J. 66:250–253.

Thompson, W. H., B. Kalfayan and R. O. Anslow. 1965. Isolation of California encephalitis group virus from a fatal human illness. Am. J. Epidemiol. 81:245–253.

Thompson, W. H., R. O. Anslow, R. P. Hanson and G. R. DeFoliart. 1972. La Crosse virus isolations from mosquitoes in Wisconsin, 1964–68. Am. J. Trop. Med. Hyg. 21:90–96.

Tonn, R. J., R. Figueredo and L. J. Uribe. 1982. *Aedes aegypti*, yellow fever and dengue in the Americas. Mosq. News 42:497–501.

Turell, M. J. and J. W. LeDuc. 1983. The role of mosquitoes in the natural history of California serogroup viruses, pp. 43–55. *In*: C. H. Calisher and W. H. Thompson (eds.). California serogroup viruses. A. R. Liss, New York.

Von Allmen, S. D., R. H. López-Correa, J. P. Woodall, D. M. Morens, J. Chiriboga and A. Casta-Veléz. 1979. Epidemic dengue fever in Puerto Rico, 1977: A cost analysis. Am. J. Trop. Med. Hyg. 28:1040–1044.

Walker, E. D. and J. D. Edman. 1985a. Feeding-site selection and blood-feeding behavior of *Aedes triseriatus* (Diptera: Culicidae) on rodent (Sciuridae) hosts. J. Med. Entomol. 22:287–294.

Walker, E. D. and J. D. Edman. 1985b. The influence of host defensive behavior on mosquito (Diptera: Culicidae) biting persistence. J. Med. Entomol. 22:370–372.

Wallis, G. P., W. J. Tabachnick and J. P. Powell. 1984. Genetic heterogeneity among Caribbean populations of *Aedes aegypti*. Am. J. Trop. Med. Hyg. 33:492–498.

Ward, R. A. 1977. Recent changes in the epidemiology of malaria relating to human ecology. Proc. 15th Int. Congr. Entomol., pp. 523–529.

Waterman, S. H., R. J. Novak, G. E. Sather, R. E. Bailey, I. Rios and D. J. Gubler. 1985. Dengue transmission in two Puerto Rico communities in 1982. Am. J. Trop. Med. Hyg. 34:625–632.

Watts, D. M., D. S. Burke, B. A. Harrison, R. E. Whitmire and A. Nisalak. 1986. Effect of temperature on the vector efficiency of *Aedes aegypti* for dengue 2 virus. Am. J. Trop. Med. Hyg. 35:(in press).

Watts, D. M., P. R. Grimstad, G. R. DeFoliart and T. M. Yuill. 1975. *Aedes hendersoni*: Failure of laboratory-infected mosquitoes to transmit La Crosse virus (California encephalitis group). J. Med. Entomol. 12:451–453.

Watts, D. M., P. R. Grimstad, G. R. DeFoliart, T. M. Yuill and R. P. Hanson. 1973a. Laboratory transmission of La Crosse encephalitis virus by several species of mosquitoes. J. Med. Entomol. 10:583–586.

Watts, D. M., B. A. Harrison, S. Pantuwatana, T. A. Klein and D. S. Burke. 1985. Failure to detect natural transovarial transmission of dengue viruses by *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae). J. Med. Entomol. 22:261–265.

Watts, D. M., J. W. LeDuc, C. L. Bailey, J. M. Dalrymple and T. P. Gargan, II. 1982. Serologic evidence of Jamestown Canyon and Keystone virus

infection in vertebrates of the DelMarVa Peninsula. Am. J. Trop. Med. Hyg. 31:1245–1251.

Watts, D. M., C. D. Morris, R. E. Wright, G. R. DeFoliart and R. P. Hanson. 1972. Transmission of La Crosse virus (California encephalitis group) by the mosquito *Aedes triseriatus*. J. Med. Entomol. 9:125–127.

Watts, D. M., S. Pantuwatana, G. R. DeFoliart, T. M. Yuill and W. H. Thompson. 1973b. Transovarial transmission of La Crosse virus (California encephalitis group) in the mosquito, *Aedes triseriatus*. Science 182:1140–1141.

Watts, D. M., R. F. Tammariello, J. M. Dalrymple, B. F. Eldridge, P. K. Russell and F. H. Top, Jr. 1979. Experimental infection of vertebrates of the Pocomoke Cypress Swamp, Maryland, with Keystone and Jamestown Canyon viruses. Am. J. Trop. Med. Hyg. 28:344–350.

Watts, D. M., W. H. Thompson, T. M. Yuill, G. R. DeFoliart and R. P. Hanson. 1974. Overwintering of La Crosse virus in *Aedes triseriatus*. Am. J. Trop. Med. Hyg. 23:694–700.

Welch, J. B. and J. D. Long. 1984. *Aedes aegypti* collections in rural southeast Texas. Mosq. News 44:544–547.

Westaway, E. G., M. A. Brinton, S. Ya. Gaidamovich, M. C. Horzinek, A. Igarishi, L. Kääriäinen, D. K. Lvov, J. S. Porterfield, P. K. Russell and D. W. Trent. 1985. Flaviviridae. Intervirology 24:183–192.

Wisseman, C. L., Jr. and B. H. Sweet. 1961. The ecology of dengue, pp. 15–39. *In*: J. M. May (ed.). Studies in disease ecology. Macmillan, New York.

Wright, R. E. and G. R. DeFoliart. 1970. Associations of Wisconsin mosquitoes and woodland vertebrate hosts. Ann. Entomol. Soc. Am. 63:777–786.

Yasuno, M. and R. J. Tonn. 1970. A study of biting habits of *Aedes aegypti* in Bangkok, Thailand. Bull. W.H.O. 43:319–325.