Comparative Potential of *Aedes triseriatus*, *Aedes albopictus*, and *Aedes aegypti* (Diptera: Culicidae) to Transovarially Transmit La Crosse Virus

MARK T. HUGHES, JANICE A. GONZALEZ, KRYSTLE L. REAGAN, CAROL D. BLAIR, AND BARRY J. BEATY

Arthropod-Borne and Infectious Diseases Laboratory, Department of Microbiology, Immunology, and Pathology, Colorado State University Fort Collins, CO 80523

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ABSTRACT *Aedes triseriatus* (Say) (Diptera: Culicidae), the major vector of La Crosse (LAC) virus, efficiently transmits LAC virus both horizontally and transovarially. We compared the vector competence and transovarial transmission ability of *Ae. triseriatus*, *Aedes albopictus* Skuse, and *Aedes aegypti* (L.) for LAC virus. *Ae. triseriatus* and *Ae. albopictus* were significantly more susceptible to oral infection with LAC virus than *Ae. aegypti*. The three species also differed in oral and disseminated infection rates (DIRs). Transovarial transmission (TOT) rates and filial infection rates (FIRs) were greater for *Ae. triseriatus* than either *Ae. albopictus* or *Ae. aegypti*. These measures were integrated into a single numerical score, the transmission amplification potential (TAP) for each species. Differences in TAP scores were due mainly to the differences in DIRs and FIRs among these mosquitoes. Although the TAP score for *Ae. albopictus* was lower than that of *Ae. triseriatus*, it was 10-fold greater than that for *Ae. aegypti*.

KEY WORDS mosquito, La Crosse virus, *Aedes* spp., transovarial transmission

La Crosse (LAC) virus (family *Bunyaviridae*, genus *Orthobunyavirus*, LACV) emerged as a significant human pathogen in the 1960s (Thompson et al. 1965) and has remained a significant cause of encephalitis and therefore an important public health problem in the Unites States (Rust et al. 1999, McJunkin et al. 2001). The incidence of LAC encephalitis in endemic areas exceeds that of bacterial meningitis (McJunkin et al. 2001). Historically, most cases of LAC encephalitis have occurred in upper midwestern states. More recently, however, cases have been identified in North Carolina, West Virginia, and Tennessee (Jones et al. 1999). *Aedes triseriatus* (Say) is the major vector of LACV and efficiently transmits LACV both horizontally and transovarially (Watts et al. 1973). Recent isolation of LACV from field-collected larvae and male *Aedes albopictus* Skuse (Diptera: Culicidae) and association of this vector with human cases in Tennessee are causes for public health concern (Gerhardt et al. 2001, Erwin et al. 2002). *Ae. albopictus* is displacing *Aedes aegypti* (L.) from peridomestic breeding sites in many areas in the southern United States, and laboratory studies have demonstrated that *Ae. albopictus* from Hawaii are competent vectors of LACV (Tesh and Gubler 1975). If U.S. strains of *Ae. albopictus* are more permissive to LACV infection, transmission, and maintenance than *Ae. aegypti*, there could be serious epidemiological consequences in this region. Studies were conducted to test the hypothesis that *Ae. albopictus* is a more competent vector of LACV than *Ae. aegypti*. These two species as well as *Ae. triseriatus* were compared in their susceptibility to oral infection with LACV and the subsequent ability to transmit the virus transovarially to their progeny.

Materials and Methods

Cell Culture and Virus Stocks. Baby hamster kidney epithelial (BHK-21) cells were grown in Dulbecco's modified Eagle's medium (Invitrogen, Carlsbad, CA) in 5% CO_2 at 37°C. African green monkey kidney epithelial (Vero) cells were maintained in Liebovitz (L-15) medium containing 10% fetal calf serum at 37°C (no CO_2).

A working stock of LACV (Human/78) was prepared by infecting confluent BHK-21 cultures in 150-cm ^{2} flasks at a multiplicity of infection of 0.001. After ≈72 h, when cytopathic effects (CPEs) reached 100%, virus titers were quantified by 50% tissue culture infectious dose ($TCID_{50}$) endpoint titration on Vero cells. Titers were determined in replicates of four dilutions by the Kärber method (Kärber 1931). Aliquots of the stock virus preparations were used in all infection experiments.

Maintenance of Mosquito Species. *Ae. albopictus* (Lake Charles; Louisiana strain) and *Ae. aegypti* (RexD; Puerto Rico strain) were maintained at 26.5°C and 80% RH under a photoperiod of 12:12 (L:D) h.

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Ae. triseriatus mosquitoes (AIDL; La Crosse, WI, strain) were maintained at 24°C and 70% RH under a photoperiod of 16:8 (L:D) h. Adult mosquitoes were provided sugar cubes and water ad libitum. Forty-eight hours before artificial membrane feeding, sugar cubes were removed, and mosquitoes were allowed only water.

Infection of Mosquitoes by Artificial Bloodmeal. For oral infection of female mosquitoes, 15 ml of newly propagated (unfrozen) infected BHK-21 cell culture supernatants containing $1.58–5.00 \times 10^7$ $TCID_{50}$ of LACV were mixed with an equal volume of mechanically defibrinated sheep blood (Colorado Serum Company, Denver, CO). The infectious bloodmeal was then transferred to a 37°C water-jacketed membrane feeder (Rutledge et al. 1978), and sugar-deprived mosquitoes were allowed to feed for 4 h. After feeding, engorged females were separated and transferred to cartons containing an equal number of males to promote mating. A total of three experiments were performed. In each, the three mosquito species were fed on the same infectious bloodmeal. $TCID_{50}$ titers of these bloodmeals were 5.0×10^7/ml, 1.6×10^7/ml, and 2.8×10^7/ml, respectively.

Oviposition and Larval Rearing. Individual bloodfed mosquitoes were maintained in cartons containing oviposition cups containing oak leaf–water (for *Ae. triseriatus*) or distilled H_2O (dH_2O) (for *Ae. aegypti* and *Ae. albopictus*) and paper strips to enhance egg laying. Ten days after the bloodmeal, oviposition (OP) liners were collected, partially dried, and stored in plastic bags at 24°C for 2 wk to allow embryonation. To induce egg hatching, the OP liners were placed in ≈1 liter of water containing 0.1% brain heart infusion broth. After larval hatching, progeny mosquitoes were given adequate food (Purina Mouse Chow and Tetramin fish food) and raised under conditions identical to that of the parents until pupation. Mosquitoes were then removed at the pupal stage, transferred to cartons, and adults were allowed to emerge.

Midgut Infection Rates. Mosquito infection rates were determined by direct immunofluorescence assay (IFA) by using mouse anti-LACV antibodies conjugated with fluorescein isothiocyanate to detect LACV antigen in midgut tissues following standard protocol with some modifications (Beaty and Thompson 1975). Briefly, engorged females were dissected at 3, 5, 7, and 10 d after a bloodmeal, and midguts were fixed for 2 h at room temperature in 4% paraformaldehyde. Tissues were rinsed in phosphate-buffered saline (PBS) containing 0.1% bovine serum albumin (BSA) and 0.2% Triton X-100 and then washed for 1 h at room temperature in PBS/BSA plus 0.2% Triton X-100. Midguts were stained for 4 h at room temperature with the anti-LACV antibodies (diluted 1:200 in PBS). Antibody was removed, and the midguts were washed once in PBS/BSA + 0.1% Triton X-100 followed by PBS. Stained midguts were then mounted on slides with Vectashield mounting medium (Vector Laboratories, Burlingame, CA). The midgut infection rate (MIR) was calculated as the percentage of females with LACV antigen positive midguts in the total number of females engorged on the infectious bloodmeal.

Disseminated Infection, Transovarial Transmission, and Filial Infection Rates. To determine the disseminated infection rate (DIR), transovarial transmission rate (TOTR), and filial infection rate (FIR) of LACV in each species, the remainder of the females not used for MIR determination were provided two additional noninfectious bloodmeals at 2-wk intervals and allowed to mate and oviposit after each. For the head tissue assays, heads were removed after the third oviposition, squashed on acid-washed glass slides, fixed for 15 min in acetone at −20°C, and air-dried. Tissues were then stained with the anti-LACV antibodies (as described above) for 30 min at 37°C in a humidified chamber. Slides were washed for 10 min in PBS, briefly washed in dH_2O, and mounted in PBS/glycerol (3:1). For both assays, tissues were examined for LACV antigen using fluorescent microscopy (Olympus BH2, Olympus, Melville, NY). The disseminated infection rate (DIR) was determined as the percentage of mosquitoes with viral antigen detectable in head tissues in the total number of females that engorged the infectious bloodmeal. For determination of TOTR and FIR, progeny from the third oviposition were hatched and tested for LACV infection by IFA of head tissues. The TOTR was calculated as the percentage of females with a disseminated LACV infection that transmitted the infection to at least one progeny. The FIR was determined as the percentage of progeny in any one brood with LACV infection from a female that had transovarially transmitted the infection. The transmission amplification potential (TAP) was calculated as $TAP = DIR \times TOTR \times FIR \times 100$.

Results

Midgut Infection Rates. All three mosquito species became infected after ingestion of an infectious bloodmeal, albeit at different rates (Fig. 1A). The midgut infection rates were 94% ($n = 70$ females) for *Ae. triseriatus*, 77% ($n = 61$) for *Ae. albopictus*, and 26% ($n = 53$) for *Ae. aegypti*. The MIRs of *Ae. triseriatus* and *Ae. albopictus* differed statistically ($P \leq 0.01$, $\chi^2 = 8.17$) (Georgetown Linguistics Web χ^2 calculator, http://www.georgetown.edu/faculty/ballc/webtools/web_chi_tut.html). The MIRs of *Ae. triseriatus* and *Ae. aegypti* and of *Ae. albopictus* and *Ae. aegypti* also differed statistically ($P \leq 0.001$, $\chi^2 = 23.84$ and 29.23, respectively). Midgut infection rates for the three species were ordered as follows *Ae. triseriatus* MIR > *Ae. albopictus* MIR > *Ae. aegypti* MIR.

Disseminated Infection Rates. Female mosquitoes of each species were assayed for disseminated infection by IFA of head tissues after oral infection and subsequent oviposition (Fig. 1B). The DIR for *Ae. triseriatus* was 86% ($n = 130$), for *Ae. albopictus* 41% ($n = 196$), and for *Ae. aegypti* 10% ($n = 544$). The DIRs of *Ae. triseriatus* and *Ae. albopictus*, *Ae. triseriatus* and *Ae. aegypti*, and *Ae. albopictus* and *Ae. aegypti* all

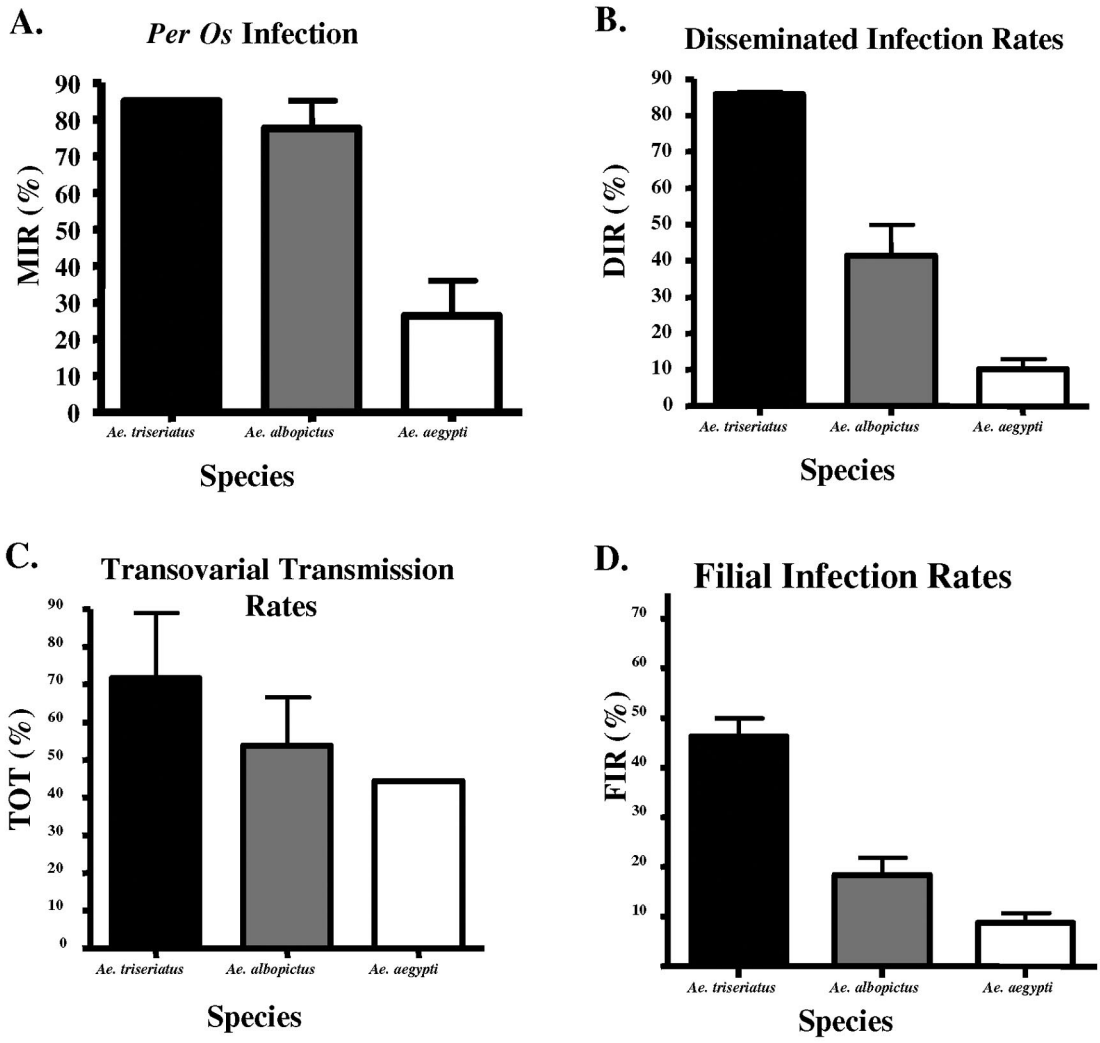

Fig. 1. (A) MIRs of the three mosquito species. In total, 70 *Ae. triseriatus*, 61 *Ae. albopictus*, and 53 *Ae. aegypti* were examined. (B) DIRs of the three mosquito species. In total, 130 *Ae. triseriatus*, 196 *Ae. albopictus*, and 544 *Ae. aegypti* females were examined. (C) TOTRs of the three mosquito species. In total, 62 *Ae. triseriatus*, 52 *Ae. albopictus*, and 50 *Ae. aegypti* LACV antigen-positive females were examined. (D) FIRs of the three mosquito species. In total, 2,083 *Ae. triseriatus*, 594 *Ae. albopictus*, and 1,202 *Ae. aegypti* progeny were examined. All experiments were performed in triplicate. Graphing and error bar calculation of the data were performed using GraphPad Prizm (Graphpad Software Inc., San Diego, CA).

differed statistically ($P \leq 0.001$, $\chi^2 = 65.03, 358.35$, and 107.61, respectively).

Transovarial Transmission and Filial Infection Rates. The TOTR was calculated to be 71% ($n = 62$) for *Ae. triseriatus*, 52% ($n = 52$) for *Ae. albopictus*, and 44% ($n = 50$) for *Ae. aegypti* (Fig. 1C). The differences between the TOTRs of *Ae. triseriatus* and *Ae. albopictus* ($P \leq 0.05$, $\chi^2 = 4.37$) and *Ae. triseriatus* and *Ae. aegypti* ($P \leq 0.01$, $\chi^2 = 8.32$) were significant, whereas that the difference between *Ae. albopictus* and *Ae. aegypti* ($P \leq 1$, $\chi^2 = 0.64$) was not significant. Calculated FIRs for progeny mosquitoes were 46% ($n = 2083$) in *Ae. triseriatus*, 18% ($n = 594$) in *Ae. albopictus*, and 8.8% ($n = 1202$) in *Ae. aegypti* (Fig. 1D). The FIRs differed significantly between *Ae. triseriatus* and *Ae. albopictus*, *Ae. triseriatus* and *Ae. aegypti*, and *Ae. albopictus* and *Ae. aegypti* ($P \leq 0.001$, $\chi^2 = 150.59, 488.01$, and 34.27, respectively).

Transmission Amplification Potential. As an overall measure of vertical transmission potential of the respective mosquito species, the MIR, DIR, TOTR, and FIR scores were used to generate the TAP. Calculated TAP values were 28.5% for *Ae. triseriatus*, 4.1% for *Ae. albopictus*, and 0.4% for Ae. *aegypti* (Fig. 2). Thus, there are major differences in the vertical transmission potential of the three species.

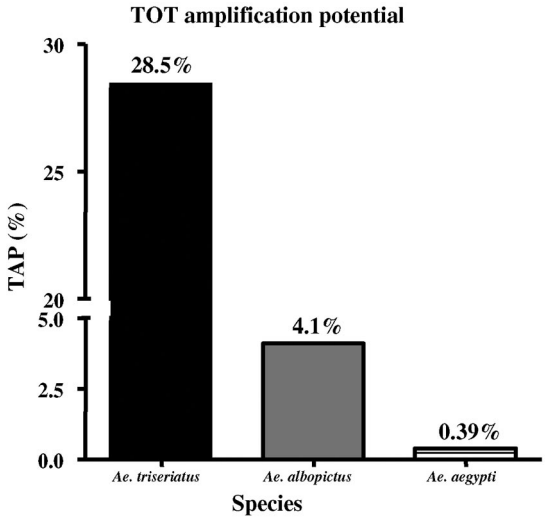

Fig. 2. TAP for the respective mosquito species. TAP scores were calculated from the relative values of DIR, TOTR, and FIR of the individual species as noted in Materials and Methods.

Discussion

All three mosquito species tested in this study were susceptible to LACV infection, but they differed significantly in several important aspects of vector competence. In terms of oral infection of the vectors, *Ae. triseriatus* was most permissive to both midgut infection and dissemination of LACV. *Ae. albopictus* was permissive to midgut infection as well and exhibited a MIR (77%) near to that of *Ae. triseriatus* (94%). However, the DIR for *Ae. albopictus* was less than half that of *Ae. triseriatus*, implying these mosquitoes have a significant midgut escape barrier to LACV infection (Bennett et al. 2005). The molecular basis of this barrier remains to be determined. In contrast, *Ae. aegypti* was significantly less permissive to both midgut and disseminated infection than either *Ae. triseriatus* or *Ae. albopictus* (Fig. 1A and B). The DIR is a major determinant of vector competence for the vectors of Orthobunyaviruses (Paulson et al. 1989). Not surprisingly, DIR is typically greatest in homologous Orthobunyavirus–vector challenge systems. Oral challenge with heterologous bunyaviruses or reassortant viruses with heterologous M segments results in the restriction of virus replication to the midgut (Beaty et al. 1982). Similarly, as shown here, challenge of nonpreferred vectors with LACV resulted in restriction of the virus to the midgut. The DIR in *Ae. aegypti* was approximately half the value of the MIR, and therefore greatly affected the vector competence for LACV.

In terms of vertical transmission, *Ae. triseriatus* mosquitoes had the highest TOTR. Nearly three-quarters of female mosquitoes having disseminated LACV infections transmitted the virus vertically to at least a portion of their progeny. Interestingly, the TOTR of both *Ae. albopictus* and *Ae. aegypti* approached or exceeded 50%. The major difference between the species, however, was in the FIR, which was greatest in *Ae. triseriatus*, intermediate in *Ae. albopictus*, and lowest in *Ae. aegypti*. Significantly, *Ae. aegypti* vertically transmitted the virus to <10% of the progeny.

TAP is representative of the overall permissiveness of the respective vector for midgut infection, dissemination, transovarial transmission, and for filial infection efficiency, all of which are important aspects contributing to the overall vector competence for LACV. *Ae. triseriatus* exhibited the greatest TAP (Fig. 2). *Ae. albopictus* was nearly as permissive to LACV oral infection as *Ae. triseriatus* (Fig. 1A), but the disseminated infection rate was much lower. Midgut escape is therefore a major determinant in the calculated intermediate TAP score for *Ae. albopictus*. *Ae. aegypti* was significantly less susceptible to both midgut and disseminated infection than either *Ae. triseriatus* or *Ae. albopictus*. Both midgut infection and escape were major determinants of the low TAP in *Ae. aegypti*. The overall TAP of *Ae. aegypti* was ≈10-fold less than that of *Ae. albopictus*. Thus, *Ae. albopictus* is a more competent vector of LACV than *Ae. aegypti* in the United States and may provide more of a public health threat for transmission to humans.

Ae. triseriatus can be found as far north as Quebec and Ontario to as far south as Florida and eastern Texas (Walker 1992). Southern strains of *Ae. triseriatus*, however, seem to be less competent vectors for LACV because of a significantly lower FIRs (Woodring et al. 1998). Since its introduction into the United States in 1985, *Ae. albopictus* has spread from Texas to Florida and is found as far north as New Jersey and Illinois (Moore 1999). Because of its catholic feeding behavior, it seems unlikely that *Ae. albopictus* will become a vector in a typical arbovirus cycle with one or a few preferred vertebrate hosts, such as the well characterized *Ae. triseriatus*–chipmunk and tree squirrel cycle (Beaty and Calisher 1991). Nonetheless, if some local strains of *Ae. albopictus* exhibit even greater TAP than that demonstrated here, LACV has the potential to be amplified and maintained in nature in this vector species, posing a potential risk for human infection. Indeed, *Ae. albopictus* are more aggressively anthropophagic than *Ae. triseriatus* and certainly could prove to be a more efficient vector of LACV to humans. It must be noted that these studies were conducted with long-colonized laboratory strains of the vectors. Additional studies using newly colonized strains and more geographically representative *Ae. albopictus* will need to be conducted to confirm these results. More field studies and epidemiological studies of LACV transmission in the southern states are warranted. Indeed, the potential for transmission of LACV by this vector could dramatically change the epidemiology of LAC encephalitis in the American south.

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References Cited

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.

Beaty, B. J., and C. H. Calisher. 1991. Bunyaviridae–natural history. Curr. Top. Microbiol. Immunol. 169: 27–78.

Beaty, B. J., B. R. Miller, R. E. Shope, E. J. Rozhon, and D. H. Bishop. 1982. Molecular basis of bunyavirus per os infection of mosquitoes: role of the middle-sized RNA segment. Proc. Natl. Acad. Sci. U.S.A. 79: 1295–1297.

Bennett, K. E., D. Flick, K. H. Fleming, R. Jochim, B. J. Beaty, and W.C.T. Black. 2005. Quantitative trait loci that control dengue-2 virus dissemination in the mosquito *Aedes aegypti.* Genetics 170: 185–194.

Erwin, P. C., T. F. Jones, R. R. Gerhardt, S. K. Halford, A. B. Smith, L. E. Patterson, K. L. Gottfried, K. L. Burkhalter, R. S. Nasci, and W. Schaffner. 2002. La Crosse encephalitis in eastern Tennessee: clinical, environmental, and entomological characteristics from a blinded cohort study. Am. J. Epidemiol. 155: 1060–1065.

Gerhardt, R. R., K. L. Gottfried, C. S. Apperson, B. S. Davis, P. C. Erwin, A. B. Smith, N. A. Panella, E. E. Powell, and R. S. Nasci. 2001. First isolation of La Crosse virus from naturally infected *Aedes albopictus.* Emerg. Infect. Dis. 7: 807–811.

Jones, T. F., A. S. Craig, R. S. Nasci, L. E. Patterson, P. C. Erwin, R. R. Gerhardt, X. T. Ussery, and W. Schaffner. 1999. Newly recognized focus of La Crosse encephalitis in Tennessee. Clin. Infect. Dis. 28: 93–97.

Kärber, G. 1931. Beitrag zur Kollektiven Behandlung Pharmakologischer Reihenversuche. Archiv fur experimentelle Pathologie und Pharmakologie. 162: 480–483.

McJunkin, J. E., E. C. de los Reyes, J. E. Irazuzta, M. J. Caceres, R. R. Khan, L. L. Minnich, K. D. Fu, G. D. Lovett, T. Tsai, and A. Thompson. 2001. La Crosse encephalitis in children. N. Engl. J. Med. 344: 801–807.

Moore, C. G. 1999. *Aedes albopictus* in the United States: current status and prospects for further spread. J. Am. Mosq. Control Assoc. 15: 221–227.

Paulson, S. L., P. R. Grimstad, and G. B. Craig, Jr. 1989. Midgut and salivary gland barriers to La Crosse virus dissemination in mosquitoes of the *Aedes triseriatus* group. Med. Vet. Entomol. 3: 113–123.

Rust, R. S., W. H. Thompson, C. G. Matthews, B. J. Beaty, and R. W. Chun. 1999. La Crosse and other forms of California encephalitis. J. Child. Neurol. 14: 1–14.

Rutledge, L. C., M. A. Moussa, C. A. Lowe, and R. K. Sofield. 1978. Comparative sensitivity of mosquito species and strains to the repellent diethyl toluamide. J. Med. Entomol. 14: 536–541.

Tesh, R. B., and D. J. Gubler. 1975. Laboratory studies of transovarial transmission of La Crosse and other arboviruses by *Aedes albopictus* and *Culex fatigans.* Am. J. Trop. Med. Hyg. 24: 876–880.

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.

Walker, N. 1992. The eastern treehole mosquito, *Aedes triseriatus.* Wing Beats 3: 17.

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

Woodring, J., L. Chandler, C. Oray, M. McGaw, C. Blair, and B. Beaty. 1998. Short report: diapause, transovarial transmission, and filial infection rates in geographic strains of La Crosse virus-infected *Aedes triseriatus.* Am. J. Trop. Med. Hyg. 58: 587–588.

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