Journal of the American Mosquito Control Association, 19(2):159–162, 2003

EXPERIMENTAL TRANSMISSION OF ST. LOUIS ENCEPHALITIS VIRUS BY *OCHLEROTATUS J. JAPONICUS*[1,2,3]

MICHAEL R. SARDELIS,[4,5] MICHAEL J. TURELL[6] AND RICHARD G. ANDRE[4]

ABSTRACT. *Ochlerotatus japonicus japonicus,* a newly discovered nonindigenous mosquito species in North America, and a colonized strain of *Culex pipiens* were compared for their vector competence for St. Louis encephalitis virus (SLE). Infection rates in *Oc. j. japonicus* were 0–33% after feeding on chickens with viremias between $10^{4.1}$ and $10^{4.7}$ plaque-forming units (PFU)/ml of blood. In comparison, infection rates were 12–94% for *Cx. pipiens* that fed on the same chickens. When fed on chickens with viremias between $10^{5.3}$ and $10^{5.6}$ PFU/ml of blood, infection rates for *Oc. j. japonicus* and *Cx. pipiens* were similar, 96% and 100%, respectively. After 12–14 days of extrinsic incubation at 26°C, all 34 infected *Oc. j. japonicus* had a disseminated infection. In contrast, only 23 (43%) of 54 infected *Cx. pipiens* had a disseminated infection after feeding on the same chickens. If they developed a disseminated infection, both species efficiently transmitted (≥87%) SLE. Estimated transmission rates at viral doses sufficient to infect both of the tested species were 29–84% for *Oc. j. japonicus* and 30–50% for *Cx. pipiens.* Because of its continued geographic expansion, field and laboratory evidence incriminating it as a vector of the closely related West Nile virus, and its ability to transmit SLE in the laboratory, *Oc. j. japonicus* should be considered as a potential enzootic or epizootic vector of SLE.

KEY WORDS *Ochlerotatus j. japonicus,* St. Louis encephalitis, vector competence

INTRODUCTION

The recent introduction of *Ochlerotatus japonicus japonicus* (Theobald) into the northeastern and mid-Atlantic regions of the USA (Peyton et al. 1999, Sardelis and Turell 2001, Scott et al. 2001) has heightened concerns of increased future transmission of arboviruses of human health importance by this species in the eastern USA. Repeated evidence has been found of West Nile virus (WN) infection, including both viral isolates and detection of WN genome, in *Oc. j. japonicus* collected in New York and New Jersey in 2000 (CDC 2000). Additionally, laboratory studies have shown *Oc. j. japonicus* to be a highly efficient laboratory vector of WN (Turell et al. 2001, Sardelis and Turell 2001).

St. Louis encephalitis is enzootic in most of the mainland USA. St. Louis encephalitis virus (SLE), the cause of the disease, is a member of the genus *Flavivirus,* family *Flaviviridae,* and is closely related antigenically and epidemiologically to WN. In the eastern USA, SLE primarily cycles between birds and mosquitoes of the *Culex pipiens* complex and has been responsible for both urban and widespread epidemics (Powell and Blakey 1977, Levy et al. 1978, Brinker et al. 1979, Zweighaft et al. 1979, Bleed et al. 1992).

The objective of this study was to assess the vector competence of *Oc. j. japonicus* for two strains of SLE. For comparison, *Culex pipiens* (L.) was tested in conjunction with *Oc. j. japonicus.*

MATERIALS AND METHODS

Mosquitoes: Ochlerotatus j. japonicus used in this study were from eggs collected in oviposition traps (Zeichner and Perich 1999) set throughout Frederick County, Maryland, during May and June of 2001. The ovistrips containing eggs were placed in 31 × 19 × 6-cm plastic pans and flooded with dechlorinated tap water. The resulting larvae were provided ground catfish chow (AquaMax Pond Plus 3000, Purina Mills, Inc., St. Louis, MO) for nutrition, and reared at 26°C, 80–85% relative humidity, and a 16:8 h light:dark photoperiod. Adults were provided water-soaked gauze pads and apple slices and maintained in 3.8-liter cardboard cartons with netting covering one end in the same environmental chamber with the immature stages.

Culex pipiens were reared from a colony established in 1999 from specimens collected in Westchester County, New York (Turell et al. 2000). They were maintained under similar conditions as *Oc. j. japonicus.*

To minimize age-related effects, only 4- to 10-day-old adult mosquitoes were used in these ex-

[1] The views of the authors do not necessarily reflect the position of the Department of Defense or the Department of the Army.

[2] In conducting research using animals, the investigators adhered to the *Guide for the care and use of laboratory animals,* as prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (National Institutes of Health publication 86-23, revised 1996). The facilities are fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care, International.

[3] This study was done as part of the requirement for M.R.S. to attain a doctoral degree at the Uniformed Services University of the Health Sciences, Bethesda, MD.

[4] Division of Tropical Public Health, Department of Preventive Medicine and Biometrics, Uniformed Services University of the Health Sciences, 4301 Jones Bridge Road, Bethesda, MD 20814.

[5] Present address: U.S. Army Medical Research Unit–Kenya, Unit 64109, APO AE 09831-4109.

[6] Department of Vector Assessment, Virology Division, U.S. Army Medical Research Institute of Infectious Diseases, 1425 Porter Street, Frederick, MD 21702-5011.

periments. One day before feeding on chickens, the apple slices were removed and the mosquitoes were transferred to 0.5-liter cartons with netting over one end.

Virus and virus assay: The Fort Washington (FTWASH) strain of SLE was isolated from overwintering *Cx. pipiens* collected in Fort Washington, MD, in 1977 (Bailey et al. 1978) and had been passaged 3 or fewer times in Vero cell culture. The TBH-28 strain was isolated from a fatal case of SLE in Tampa Bay, FL, in 1962 (Coleman et al. 1968) and had been passaged 12 times in mouse brains and once in Vero cell culture. Viral stocks, triturated mosquito suspensions, and chicken blood samples were tested for infectious virus by plaque assay on Vero cells as described by Gargan et al. (1983), except that the 2nd overlay, containing neutral red stain, was added 5 days after the 1st overlay.

Viremia profile studies: Preliminary studies were done to determine SLE viremia profiles in white leghorn chickens (*Gallus gallus*). Five chickens that were less than 1 day old were inoculated subcutaneously with 0.1 ml of a suspension containing 10^4 plaque-forming units (PFU) of each of the SLE strains. These chickens were bled from the jugular vein (0.1 ml of blood) at 24, 48, 72, and 96 h after inoculation. The blood was diluted in 0.9 ml of diluent (10% heat-inactivated fetal bovine serum in Medium 199 with Earle's salts, $NaHCO_3$, and antibiotics) plus 10 units of heparin per milliliter and stored at −70°C until tested for virus.

Vector competence studies: Mosquitoes were allowed to feed on 1- to 2-day-old restrained chickens that had been inoculated with 10^4 PFU of 1 of the SLE strains 1–2 days earlier. Immediately after mosquito feeding, blood was obtained from the jugular vein of each chicken as described above to determine the viremias at the time of mosquito feeding. After feeding on a viremic chicken, engorged mosquitoes were transferred to 3.8-liter screen-topped cardboard cages and held at 26°C with a 16:8 h light:dark photoperiod. An oviposition substrate was added to each cage about 5 days later to stimulate oviposition, and collected eggs were discarded. After an incubation period of 12–14 days, the mosquitoes were allowed to refeed on chickens that were less than 2 days old, either individually or in groups of 5 to determine if they could transmit virus by bite. Immediately after the transmission attempt, the mosquitoes were killed by freezing at −20°C and the feeding status was determined visually under a dissecting microscope or by the presence of blood when the mosquito was triturated. The mosquitoes' legs and bodies were triturated separately in 1 ml of diluent. Infection was determined by recovery of virus from the mosquito tissue suspension. If virus was recovered from its body, but not its legs, the mosquito was considered to have a nondisseminated infection limited to its midgut. In contrast, if virus was recovered from both the body and leg suspensions, the mosquito was considered to have a disseminated infection (Turell et al. 1984). Infection and dissemination rates were defined as the percentages of mosquitoes tested that contained virus in their body or legs, respectively. Chickens used in the transmission attempts were bled from the jugular vein 1 or 2 days after mosquito feeding and the blood was handled as described above. Recovery of virus from this blood indicated transmission.

To examine viral transmission more efficiently, some of the original, unfed mosquitoes were inoculated intrathoracically (Rosen and Gubler 1974) with 0.3 µl of a viral suspension containing SLE at $10^{4.3}$ to $10^{4.6}$ PFU/ml ($10^{0.8}$ to $10^{1.1}$ PFU/mosquito), held 7–9 days, and allowed to feed on chickens that were less than 2 days old. Mosquitoes and blood specimens from these chickens were processed as described for the orally exposed mosquitoes.

In order to estimate transmission rates for the 2 species, the percentage of mosquitoes with a disseminated infection (after either oral exposure or by intrathoracic inoculation) that transmitted virus by bite was determined. This value was then multiplied by the percentage of mosquitoes that developed a disseminated infection after feeding on a host with a particular viremia to yield an estimated transmission rate for those mosquitoes.

Data analysis: Infection and dissemination rates were compared by chi-square or Fisher exact tests as appropriate and significant differences were determined at the 95% confidence level (SAS Institute Inc. 1999).

RESULTS

Both strains of SLE replicated in young chickens; however, the FTWASH strain produced significantly ($T \geq 10.7$, df = 8, $P < 0.001$) higher viremias 2–4 days after infection (Fig. 1). Both *Oc. j. japonicus* and *Cx. pipiens* were susceptible to infection with SLE and infection and dissemination rates increased as viremia titers increased. When fed on chickens with a viremia of $10^{4.1}$ PFU/ml of blood, the infection rate was higher, although not significantly (Fisher's exact test, $P \geq 0.06$), in *Cx. pipiens* than in *Oc. j. japonicus* (Table 1). At viral titers between $10^{4.4}$ and $10^{4.7}$ PFU/ml of blood, significantly fewer ($\chi^2 = 26.91$, df = 1, $P < 0.001$) *Oc. j. japonicus* (33%) became infected than *Cx. pipiens* (94%). In contrast, when viremias were $10^{5.3}$ to $10^{5.6}$ PFU/ml of blood, no significant difference (Fisher's exact test, $P = 0.56$) was found between the infection rates for *Oc. j. japonicus* (96%) and *Cx. pipiens* (100%). All of the SLE-infected *Oc. j. japonicus* developed a disseminated infection, regardless of the original infectious dose. In contrast, only about one half of infected *Cx. pipiens* developed a disseminated infection.

Nearly all *Oc. j. japonicus* and *Cx. pipiens* with a disseminated infection transmitted SLE by bite.

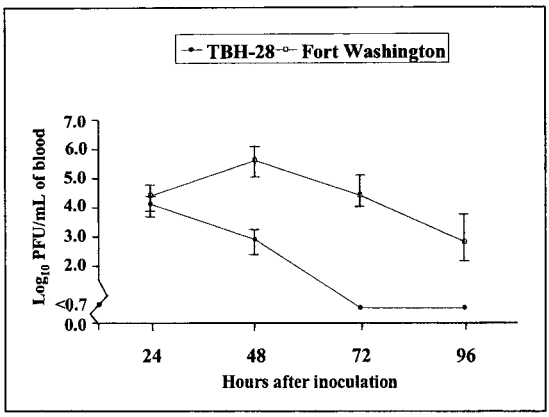

Fig. 1. Viremias in chickens (*Gallus gallus*) 24, 48, 72, and 96 h after subcutaneous inoculation with 0.1 ml of a suspension containing approximately 10^4 plaque-forming units (PFU) of either the TBH-28 or Fort Washington strain of St. Louis encephalitis virus (SLE). Five chickens were inoculated with each of the 2 strains of SLE when they were less than 1 day old, and all chickens were bled daily. Bars indicate range of viremias.

Because neither route of infection nor viral strain significantly affected transmission of virus by mosquitoes with disseminated infections (Fisher's exact test, $P > 0.29$), these data were combined. Overall, 88% of *Oc. j. japonicus* ($n = 34$) and 87% of *Cx. pipiens* ($n = 15$) with a disseminated infection transmitted SLE by bite (Table 2). However, because dissemination rates were generally higher in *Oc. j. japonicus* than in *Cx. pipiens,* estimated transmission rates for *Oc. j. japonicus* for SLE were similar to those for *Cx. pipiens* when mosquitoes fed on a host circulating about $10^{4.5}$ PFU/ml of blood and nearly 2 times greater than those for *Cx. pipiens,* when mosquitoes fed on a host with a higher viremia titer (Table 1).

Table 2. Transmission of virus by bite for mosquitoes with a disseminated infection after either oral exposure to or intrathoracic inoculation with either the TBH-28 or the Fort Washington strain of St. Louis encephalitis virus.[1]

Species	Route of exposure: Oral	Intrathoracic inoculation	Combined
Ochlerotatus j. japonicus	88 (8)	88 (26)	88 (34)
Culex pipiens	50 (2)	92 (13)	87 (15)

[1] Values given are percent transmitting (number feeding).

DISCUSSION

This study showed that *Oc. j. japonicus* was moderately susceptible to infection with SLE and, depending on the viremia level, between 0 and 84% of orally exposed *Oc. j. japonicus* would be expected to transmit virus by bite if allowed to refeed 12–14 days later. Compared to *Cx. pipiens, Oc. j. japonicus* needed to feed on hosts with a slightly higher viremia to become infected. However, virus was more likely to escape the midgut and become disseminated in *Oc. j. japonicus.* The combined effects of lower infection rates, but higher dissemination rates, resulted in similar estimated transmission rates for *Oc. j. japonicus* and *Cx. pipiens* under the conditions in this study. The viremias at the time of mosquito feeding in this study, between $10^{3.5}$ and $10^{5.6}$ PFU/ml of blood, are similar to those produced in a number of epidemiologically important avian hosts (i.e., robins, cardinals, and house sparrows) experimentally infected with SLE (McLean et al. 1985, Savage et al. 1994). Thus, the rates observed in this study should reflect those that would occur in nature.

This is the 1st evidence of laboratory transmission of SLE by *Oc. j. japonicus.* Because *Oc. j.*

Table 1. Infection, dissemination, and estimated transmission rates for *Ochlerotatus j. japonicus* and *Culex pipiens* orally exposed to St. Louis encephalitis virus (SLE).

Species	Viremia titer at time of feeding[1]	Viral strain	No. tested	Infection rate[2]	Dissemination rate[3]	Estimated transmission rate[4]
Oc. j. japonicus	3.5	TBH-28	14	0	0	0
	4.1	TBH-28	40	0	0	0
	4.4–4.7	FTWASH	33	33	33	29
	5.3–5.6	FTWASH	24	96	96	84
Cx. pipiens	3.5	TBH-28	13	0	0	0
	4.1	TBH-28	26	12	0	0
	4.4–4.7	FTWASH	34	94	35	30
	5.3–5.6	FTWASH	19	100	58	50

[1] Logarithm_{10} plaque-forming units per milliliter of blood in chickens (*Gallus gallus*) inoculated 24 h previously with SLE.
[2] Percentage of mosquitoes containing virus in their bodies.
[3] Percentage of mosquitoes containing virus in their legs.
[4] The estimate transmission rate is the percentage of mosquitoes that developed a disseminated infection 12–14 days after ingesting SLE multiplied by the percentage of individuals with a disseminated infection that transmitted virus by bite. The percentage of individuals with a disseminated infection that transmitted virus by bite was previously determined to be 88% for *Oc. j. japonicus* and 87% for *Cx. pipiens.*

japonicus is expanding into areas of SLE activity, no data are available on its potential to serve as a natural vector of SLE. However, *Oc. j. japonicus* is known to be an efficient experimental vector of 2 other flaviviruses: Japanese encephalitis virus (Takashima and Rosen 1989) and WN (Sardelis and Turell 2001, Turell et al. 2001).

Presently, predicting the extent to which *Oc. j. japonicus* will become involved in the transmission cycle of SLE is difficult. Little is known about the behavioral and other biological characteristics of *Oc. j. japonicus* in the USA. In 2000, evidence of WN infection was reported in field-collected *Oc. j. japonicus* (CDC 2000). The enzootic cycles of WN and SLE in urban settings in the eastern USA are similar. Thus, as the distribution of *Oc. j. japonicus* extends into areas where SLE is enzootic, the likelihood of contact between the 2 will increase. Based on expansion of its range into areas where extensive SLE viral activity exists, recent studies indicating that it is involved in the WN transmission cycle in the eastern USA, and our study indicating that it is a competent laboratory vector of SLE, *Oc. j. japonicus* should be considered a potential secondary vector in the enzootic cycle of SLE and a possible bridge vector of this virus.

ACKNOWLEDGMENTS

We thank J. Blow, D. Dohm, M. O'Guinn, and K. Kenyon for their critical reading of the manuscript, and M. Delgado and D. Schachner for their assistance with the mosquito rearing. This research formed part of a Ph.D. dissertation by the senior author.

REFERENCES CITED

Bailey CL, Eldridge BF, Hayes DE, Watts DM, Tammariello RF, Dalrymple JM. 1978. Isolation of St. Louis encephalitis virus from overwintering *Culex pipiens* mosquitoes. *Science* 199:1346–1349.

Bleed DM, Marfin AA, Karabatsos N, Moore P, Tsai T, Olin AC, Lofgren JP, Higdem B, Townsend TE. 1992. St. Louis encephalitis in Arkansas. *J Ark Med Soc* 89: 127–130.

Brinker KR, Paulson G, Monath TP, Wise G, Fass RJ. 1979. St Louis encephalitis in Ohio, September 1975: clinical and EEG studies in 16 cases. *Arch Intern Med* 39:561–566.

CDC [Centers for Disease Control and Prevention]. 2000. Update: West Nile activity—eastern United States, 2000. *Mor Mortal Wkly Rep* 49:1044–1047.

Coleman PH, Lewis AL, Schneider NJ, Work TH. 1968. Isolations of St. Louis encephalitis virus from post-mortem tissues of human cases in the 1962 Florida epidemic. *Am J Epidemiol* 87:530–538.

Gargan TP II, Bailey CL, Higbee GA, Gad A, El Said S. 1983. The effect of laboratory colonization on the vector pathogen interaction of Egyptian *Culex pipiens* and Rift Valley fever virus. *Am J Trop Med Hyg* 32:1154–1163.

Levy JS, Carver HD, Moseley IK, Calisher CH, Francy DB, Monath TP. 1978. St. Louis encephalitis in Memphis–Shelby County, Tennessee, 1975: epidemiologic aspects of human cases. *South Med J* 71:633–637.

McLean RG, Francy DB, Campos EG. 1985. Experimental studies of St. Louis encephalitis virus in vertebrates. *J Wildl Dis* 21:85–93.

Peyton EL, Campbell SR, Candeletti TM, Romanowski M, Crans WJ. 1999. *Aedes (Finlaya) j. japonicus* (Theobald), a new introduction into the United States. *J Am Mosq Control Assoc* 15:238–241.

Powell KE, Blakey DL. 1977. St Louis encephalitis. The 1975 epidemic in Mississippi. *J Am Med Assoc* 23: 2294–2298.

Rosen L, Gubler D. 1974. The use of mosquitoes to detect and propagate dengue viruses. *Am J Trop Med Hyg* 23: 1153–1160.

Sardelis MR, Turell MJ. 2001. *Ochlerotatus j. japonicus* in Frederick County, Maryland: discovery, distribution, and vector competence for West Nile virus. *J Am Mosq Control Assoc* 17:137–141.

SAS Institute Inc. 1999. *SAS/STAT MULTITEST software release 8.00* Cary, NC: SAS Institute.

Savage HM, Smith GC, Mitchell CJ, McLean RG, Meisch MV. 1994. Vector competence of *Aedes albopictus* from Pine Bluff, Arkansas, for a St. Louis encephalitis virus strain isolated during the 1991 epidemic. *J Am Mosq Control Assoc* 10:501–506.

Scott JJ, Carle FL, Crans WJ. 2001. *Ochlerotatus japonicus* collected from natural rockpools in New Jersey. *J Am Mosq Control Assoc* 17:91–92.

Takashima I, Rosen L. 1989. Horizontal and vertical transmission of Japanese encephalitis virus by *Aedes j. japonicus* (Diptera: Culicidae). *J Med Entomol* 26:454–458.

Turell MJ, Gargan TP II, Bailey CL. 1984. Replication and dissemination of Rift Valley fever virus in *Culex pipiens*. *Am J Trop Med Hyg* 33:176–181.

Turell MJ, O'Guinn M, Dohm DJ, Jones JW. 2001. Vector competence of North American mosquitoes (Diptera: Culicidae) for West Nile virus. *J Med Entomol* 38:130–134.

Turell MJ, O'Guinn M, Oliver J. 2000. Potential for New York mosquitoes to transmit West Nile virus. *Am J Trop Med Hyg* 62:413–414.

Zeichner BC, Perich MJ. 1999. Laboratory testing of a lethal ovitrap for *Aedes aegypti*. *Med Vet Entomol* 13: 234–238.

Zweighaft RM, Rasmussen C, Brolnitsky O, Lashof JC. 1979. St. Louis encephalitis: the Chicago experience. *Am J Trop Med Hyg* 28:114–118.