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Vertical Transmission of St. Louis Encephalitis Virus in *Culex quinquefasciatus* (Diptera: Culicidae) in Córdoba, Argentina

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Abstract

Arbovirus vector transmission is interrupted when vector abundance decreases or disappears in temperate regions during the winter season. Although the primary overwintering mechanism for many arboviruses in nature remains unknown, vertical transmission is one potential mechanism. Vertical transmission functions as an overwintering mechanism for St. Louis encephalitis virus (SLEV) in temperate areas of Argentina, where SLEV is endemic. The aim of this project was to detect vertical transmission of SLEV in *Culex quinquefasciatus* mosquitoes. Under laboratory conditions, SLEV vertical transmission (3.4 minimum infection rates) was detected in larvae (1:256) and adults F_1 (1:406). There were no positive larvae for SLEV among over 2011 analyzed individuals collected in nature. This is the first study to confirm experimental vertical transmission of SLEV in *Cx. quinquefasciatus* populations from Argentina, though additional overwintering mechanisms (e.g., nontraditional vectors such as ticks and nondiapausing female mosquitoes) should be considered.

Key Words: *Culex quinquefasciatus*—SLEV—Overwinter—Vertical transmission.

Introduction

Numerous flaviviruses (e.g., yellow fever virus, dengue virus, West Nile virus, St. Louis encephalitis virus [SLEV], Japanese encephalitis, and Murray Valley encephalitis) have continued to emerge on a global scale (Solomon and Mallewa 2001). Argentina is currently facing the reemergence of dengue virus (serotypes I, II, and III), yellow fever virus, and SLEV, and the emergence of West Nile virus (Avilés et al. 2003, Diaz et al. 2006, 2008, Morales et al. 2006, 2008).

SLEV was first discovered in 1933 during an outbreak of human encephalitis in St. Louis, MO, and is widely distributed throughout the Americas. In the United States, this virus is maintained through transmission cycles involving *Culex* mosquitoes (e.g., *Cx. quinquefasciatus*, *Cx. Nigripalpus*, and *Cx. tarsalis*) and birds (e.g., house sparrows [*Passer domesticus*], house finches [*Carpodacus mexicanus*], and mourning doves [*Zenaida macroura*]) (Reisen 2003).

In Argentina, SLEV was first detected in 1957 and is widely distributed from the subtropical northern to cold southern regions (Sabattini et al. 1998). Viral isolations have been made from *Culex* mosquitoes, rodents belonging to the genera *Mus* and *Calomys*, as well as from febrile humans (Sabattini et al. 1998). Serologic evidence of SLEV infection has been reported from horses, cattle, goats, wild birds, and domestic fowl (Sabattini et al. 1998). In Argentina, human seroprevalence has ranged from 3% to 50% (Sabattini et al. 1998). As with other regions of Central and South America, symptomatic cases of SLEV infection have rarely been reported in Argentina. In 2002, SLEV reemerged in the central regions of Argentina, where the virus is endemic (Spinsanti et al. 2003). In 2005, an outbreak of encephalitis in humans caused by SLEV occurred in Córdoba Province, during which two genoptype III strains were isolated from *Cx. quinquefasciatus* (Diaz et al. 2006). Moreover, SLEV genotype V actively circulated in mosquito populations through 2001–2004 in Córdoba City (LAD, unpublished data). Since the climate of Córdoba Province is temperate with a dry-cold winter season, an overwintering mechanism appears to facilitate the SLEV endemic activity.

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In tropical areas, arbovirus activity is homodynamic, since vector populations remain active year-round. On the other hand, vector transmission of arboviruses is interrupted when vectors decrease in abundance or disappear in temperate regions during winter season (Kramer and Ebel 2003). However, there is evidence to support the existence of mechanisms that allow year-round of arbovirus transmission in temperate areas. Although the primary overwintering mechanism of SLEV remains unknown, existing data suggest that different mechanisms regarding viral persistence may occur. For example, vertical transmission or horizontal transmission in nondiapausing vectors or viral persistence in host populations, and re-introduction may occur via migratory birds, bats, or wind-dispersed mosquitoes (Reisen 2003). Therefore, the aim of this project was to assess vertical transmission of SLEV genotype V strain in *Cx. quinquefasciatus* mosquitoes collected in Córdoba City as a possible overwintering mechanism.

Materials and Methods

Viral strain

Genotype V SLEV 78V-6507 strain, isolated from *Cx. quinquefasciatus* collected in Argentina, was used. The viral suspension was prepared from a 10% dilution of infected suckling-mouse brain and was titered by Vero cell plaque assay as described in Early et al. (1967). The viral titer was expressed as plaque-forming units per milliliter (PFU/mL).

Viremia profile assays

Chicks (*Gallus gallus*) (24 h old) seronegative for SLEV (by plaque reduction neutralization test) were subcutaneously inoculated in the peritoneal region with 0.1 mL of virus suspension containing 300 PFU. Chicks were bled daily from the jugular vein using 28-gauge needles over a 6-day period. Whole blood (100 μL) was diluted in 0.9 mL of minimum essential medium with 10% fetal calf serum (FCS), and 1% gentamycin, and centrifuged at 1500 *g* for 15 min; the supernatant was stored at −80°C. Viremia titers were determined by plaque assay on Vero cells and expressed as PFU/mL. The minimum detection threshold was 2 $\log_{10}$ PFU/mL.

Mosquito collection

Cx. quinquefasciatus female mosquitoes were from a laboratory colony originating from immature specimens collected in Córdoba City. The rearing methodology followed the guidelines of Gerberg et al. (1994). Mosquitoes were kept in an insectarium at 25°C under a 12 h light–dark cycle. Larvae were reared in plastic trays (1000 mL) and fed on powdered liver (0.25 mg/larvae/day). Adults were housed in entomological cages (30 cm^3), and provided with 10% sugar-soaked cotton pads as a food source. An immobilized quail (*Coturnix coturnix*) was offered to the female mosquitoes as blood source for egg development.

Experimental vertical transmission

Approximately 100 two- to three-day old colonized female mosquitoes were allowed to feed overnight on chicks that had been inoculated 60–72 h earlier. Mosquitoes were maintained alive for 7 days at 25°C, and then allowed to oviposit over a 3-day period. All eggs from this first oviposition were discarded. After oviposition, some mosquitoes were allowed to feed on a second noninfected chick. These re-fed mosquitoes females were transferred to individual cages and held for 4 days at 25°C for egg development and oviposition. After oviposition, all mosquitoes were frozen to −80°C until testing.

The F_1 progeny were reared individually at 25°C, and minimum infection rates (MIRs) were determined for larval and adult progeny. Progeny from each re-fed female were tested separately as follows: 50% as larvae and 50% reared to adulthood and subsequently tested. Third- and fourth-instar larvae were pooled in groups of ≤50 for testing. Each pool was rinsed thoroughly with distilled water and then triturated in 1.0 mL minimum essential medium supplemented with 10% fetal calf serum and 1% gentamycin, and centrifuged at 1500 *g* for 15 min at 4°C. Supernatants were stored at −80°C until testing. The larvae that were reared to adulthood were pooled by sex in groups of ≤25 for testing, triturated, and stored at −80°C. Infectious virus in adult mosquito and larvae homogenates was detected by Vero cell plaque assay.

The chicks used for the second blood feeding were bled every 24 h after exposure, and the sera were tested by Vero cell plaque assay to assess viremia. Detection of viremia indicated transmission of virus by mosquito.

Natural vertical transmission

Cx. quinquefasciatus larvae were collected during the winter (June–August 2008) in southern areas of Córdoba City (31°28′ 33.6″S and 64°12′ 8.4″W) using a plastic dipper. Larvae were identified under stereomicroscope, and sorted by pools containing 50 individuals each. All pools were analyzed by reverse transcription (RT)-nested polymerase chain reaction assay specific for SLEV (Ré et al. 2008) and by Vero cell plaque assay.

Results

Overall, all of five inoculated baby chicks developed viremia titers that ranged from 3 to 6.61 log PFU/mL during the 2nd and 5th day postinoculation (dpi). Since the highest viremia was observed on the 3rd day postinoculation, *Cx. quinquefasciatus* female mosquitoes were allowed to feed on viremic chicks on at this time point. Donor chicks had circulating virus between 5.60 and 6.61 log PFU/mL at the time females fed on them. Eighty of 88 (90.9%) females that fed on these chicks had evidence of infection after 15 days of incubation at 25°C. All chicks became viremic and seroconverted to SLEV after being bitten by infected females. Viremia titers ranged from 3 to 5 log PFU/mL between the second and fourth postfeeding days. These data suggest that *Cx. quinquefasciatus* were competent vectors for the SLEV strain used.

Among 80 SLEV-infected female mosquitoes, 11 failed to oviposit and 23 laid eggs that resulted in larvae that died during the first or second instar. From the remaining 46 mosquitoes, 3270 F_1 offspring were obtained, of which 2051 (62.72%) were grouped as larvae composing 95 pools, and 1219 (37.28%) were adults (683 males and 536 females) constituting 97 pools separated by sex.

A total of 11 pools were SLEV positive by plaque assay, 8 from larval pools and 3 from F_1 adult pools (Table 1). These pools were obtained from nine parental females: seven had only one positive pool each (two adults and five larvae), and

TABLE 1. VERTICAL TRANSMISSION IN LARVAE AND ADULT F_1 *CULEX QUINQUEFASCIATUS* MOSQUITO FEMALES INFECTED *PER OS* WITH 78V-6507 ST. LOUIS ENCEPHALITIS VIRUS STRAIN

	Analyzed F_1	*Positive pools/ analyzed pools*	*Ratio*[a]	*MIR*
Larvae	2051	8/95	1:256	3.9
Adults	♂ 683	2/50	1:341	2.9
	♀ 536	1/47	1:536	1.9
	1219	3/97	1:406	2.5
Total	3270	11/192	1:297	3.4

[a]Proportion of positive mosquitoes by uninfected mosquitoes.
MIR, minimum infection rate as ratio.

two females had two positive pools each (two larval pools in one case and one larval pool and one adult pool in the other). From three positive adult pools, there were two males and one female showing an MIR of 2.9 (1:341) and 1.9 (1:536), respectively (Table 1).

Discussion

Although vertical transmission as an overwintering mechanism has been documented for SLEV in *Culex* spp. in the United States (Francy et al. 1981, Hardy et al. 1984, Nayar et al. 1986), this is the first experimental study to confirm vertical SLEV transmission by indigenous *Cx. quinquefasciatus* populations in Argentina. Although the MIR detected in the present study seems low, it is higher than those detected by Hardy et al. (1984) and Nayar et al. (1986). Hardy et al. (1984) obtained a ratio of 1:500 in larvae of *Cx. quinquefasciatus* collected in California and then intrathoracically inoculated with SLEV. Nayar et al. (1986) detected a ratio of 1:1120 for *Cx. quinquefasciatus* mosquitoes inoculated *per os* with SLEV Fort Washington strain. Infection of egg with SLEV appears to occur when virus enters the micropyle so that the infected/uninfected egg ratio remains low (Francy et al. 1981, Monath 1990). In addition, in the present study, a diminishing infection rate was observed between the larvae (0.39) and the adult stages (0.25). Although the reasons for this are unknown, some researchers have postulated that viral inactivation or alteration may occur during metamorphosis or when adults emerge (Hardy et al. 1984, Rosen et al. 1989a).

SLEV was not detected in 2011 *Cx. quinquefasciatus* larvae collected from natural breeding sites in Córdoba City (June–August 2008). Hardy et al. (1984) also failed to detect natural SLEV infection in 4798 larvae of *Cx. quinquefasciatus* collected in California. The lack of evidence of SLEV-infected larvae in nature could be due to the small number of individual mosquitoes analyzed (Rosen et al. 1989b). An intensive field effort to collect more larvae might provide a more accurate representation of the occurrence of vertical transmission in mosquito populations of Córdoba City.

The continuous circulation of genotype V SLEV strains detected over multiple years (LAD, unpublished data) and the nondiapausing *Cx. quinquefasciatus* females that continue to actively feed on hosts during winter (Almirón and Brewer 1996) support the hypothesis that some overwintering mechanisms have allowed SLEV to become endemic in Córdoba City. Moreover, a *Cx. quinquefasciatus* male mosquito collected during fieldwork in Tucumán Province (northwestern Argentina) was naturally infected with SLEV, supporting that vertical transmission occurs in nature (LAD, unpublished data). According to these data, indigenous *Cx. quinquefasciatus* should be competent vectors of SLEV and vertical transmission is a plausible overwintering mechanism for this virus. Nondiapausing *Cx. quinquefasciatus* populations could act as an overwintering mechanism maintaining vectorial transmission during winter in Argentina. However, this could occur in conjunction with vertical transmission (i.e., transovarial), enabling SLEV endemicity in Córdoba City.

Although the experimentally determined MIR was low in the present study, vertical transmission could have a significant impact in nature especially if enhanced by alternative infection routes (Shroyer 1986, 1990a), including venereal transmission (Shroyer 1990b). Vertically infected *Aedes albopictus* females were more efficient vertical transmitters than females infected by inoculation (i.e., horizontally) (Shroyer 1986, 1990a). On the other hand, our results demonstrated male mosquitoes that were born infected (Table 1), and similar results have been reported by Francy et al. (1981) and Hardy et al. (1984).

In conclusion, vertical transmission is a possible overwintering mechanism for the maintenance of SLEV in nature, since a *Cx. quinquefasciatus* male was naturally infected with the virus and experimental transmission was demonstrated in autochthonous *Cx. quinquefasciatus* originating from Córdoba City. Further studies should be performed to assess the possibility of additional overwintering mechanisms, such as ticks as alternate vectors.

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Ethical Approval

Not required.

Authorship Statement

F.S.F., G.P.B., and L.A.D. analyzed the samples; F.S.F., L.A.D., and M.S.C. carried out the analysis and interpretation of the data; F.S.F., G.P.B., and W.R.A. collected and identified mosquito samples; F.S.F., L.A.D., G.P.B., W.R.A., and M.S.C. draft the article.

Disclosure Statement

No competing financial interests exist.

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