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SHORT COMMUNICATION

Transmission of endemic St Louis encephalitis virus strains by local *Culex quinquefasciatus* populations in Córdoba, Argentina

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Background: St Louis encephalitis virus (SLEV) is a re-emerging human pathogen widely distributed in the American continent. Although it is not fully understood, the SLEV transmission network may involve *Culex quinquefasciatus* mosquitoes as vectors and Columbidae species as hosts.

Methods: To calculate infection rates, we inoculated *Cx. quinquefasciatus* mosquitoes from Córdoba, Argentina by feeding them on viremic chicks.

Results: We observed differences in infection rate among the viral strains, the highest rate (78/87 mosquitoes, 90.8%) being seen in strain 78V-6507. After re-feeding on susceptible chicks, mosquitoes were able to transmit the virus.

Conclusion: Our findings suggest that *Cx. quinquefasciatus* populations are susceptible to and able to transmit different SLEV strains.

Keywords: St Louis encephalitis virus, *Culex quinquefasciatus*, Mosquitoes, Viral strains, Vector competence, Córdoba, Argentina

Introduction

The St Louis encephalitis virus (SLEV) is a member of the Japanese encephalitis serogroup (genus *Flavivirus*, family Flaviviridae). It is widely distributed throughout the American continent from Canada to Argentina.[1] SLEV shows molecular and biological variation across its geographical distribution.[2,3] In Argentina, genotypes II, III, V and VII are found in mosquitoes and rodents.[4–6] Since 2002 Argentina has been experiencing a re-emergence of SLEV, with the virus causing febrile illness and encephalitis outbreaks in humans. By 2005, the first SLEV human encephalitis outbreak was reported in the central region of the country.[7] At this time two SLEV strains of the genotype III were isolated from *Culex quinquefasciatus* mosquitoes.[5] However, the role of this mosquito species in the transmission cycle of SLEV is not completely understood and requires further study.

It has been hypothesized that the transmission network of SLEV in Argentina includes *Cx. quinquefasciatus* as vector and Columbidae species as hosts.[4] One study has shown that *Cx. quinquefasciatus* mosquitoes from Santa Fe province are able to transmit the virus.[8] However, the importance of this mosquito as a SLEV vector is unknown for other locations in Argentina. The aim of the present research was to evaluate the susceptibility of local populations of *Cx. quinquefasciatus* to infection with and transmission of different native SLEV viral strains in Córdoba, Argentina.

Materials and methods

In order to analyze the viremia profile of different SLEV, three groups of five 24 h-old baby chicks were subcutaneously inoculated. The 0.1 ml virus inoculum contained 300 plaque-forming units (PFU) for every assayed strain (CbaAr-4005 [Genotype III],[5] 78V-6507 [Genotype V][4] and CorAn-9275 [Genotype VII][4]). Chicks were daily bled from the jugular vein over a 7-day period. Whole blood (0.1 ml) was diluted in 0.9 ml of minimum essential medium (MEM) 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 $\log_{10}$ PFU/ml. The minimum detection threshold was 2 $\log_{10}$ PFU/ml. The viremia profile obtained was used to determine when viraemia peaked, in order to select the best day to infect mosquitoes.

Cx. quinquefasciatus mosquitoes were exposed to SLEV by allowing them to feed on previously inoculated chicks. For each viral strain, approximately 100 2–3-day-old colonized female mosquitoes from Córdoba city were allowed to feed for 2 h on one viremic chick. The feeding procedure was carried out on the 3rd day post-inoculation. In order to detect the viral load that mosquitoes were exposed to, we bled each chick immediately before feeding.

After feeding, mosquitoes were anesthetized and only fully engorged mosquitoes were assayed. Mosquitoes for assay were killed, placed individually in 1.5 ml plastic tubes and homogenized in 1.0 ml of MEM supplemented with 10% FCS and 1% gentamycin. Mosquito homogenates were centrifuged at 11 000 g for 15 min at 4°C. Supernatants were stored at −80°C until tested. Infectious viral particles in adult mosquito homogenates were detected by Vero cell plaque assay. We used a χ^2 test to evaluate the hypothesis that different SLEV strains produce different infection rates.

After the first blood feed, selected *Cx. quinquefasciatus* mosquitoes were re-located in cardboard containers for 15 days at 25°C before a second feed on susceptible chicks, to detect vectorial transmission. The chicks were bled 3 days after exposure to infectious mosquitoes and the sera tested to assess viremia.

Results and Discussion

The three viral strains tested (78V-6507, CbaAr-4005, CorAn-9275) differed with respect to the viremia profile produced in chicks. All chicks inoculated with the strains 78V-6507 and CbaAr-4005 developed detectable viremia between the 2nd and the 5th day post-inoculation, with the highest viremia titer observed on the 3rd day post-inoculation for both strains. Titers were 3–6.61 $\log_{10}$ PFU/ml and 3.30–7.50 $\log_{10}$ PFU/ml, in 78V-6507 and CbaAr-4005 respectively. The differential capacity to produce viremia among viral strains is not exclusive to SLEV; it was also demonstrated for West Nile virus in captured house sparrows.[9] Chicks inoculated with CorAn-9275 did not develop detectable viremia, so they were not included in the mosquito infection assay. This undetectable replication of the CorAn-9275 strain in chicks coincides with the low viremia titers found in house sparrows.[2] The study on house sparrows and our own results on chicks support the hypothesis that SLEV strain CorAn-9275 does not replicate in birds, leaving mammals as the potential main reservoir in an alternative transmission cycle.[4]

Cx. quinquefasciatus mosquitoes from Córdoba city fed on viremic chicks became infected and able to transmit SLEV strains under laboratory conditions. However, the infection rate varied between the viral strains ($\chi^2 = 8.45$; degrees of freedom = 1; $p = 0.0037$). The 78V-6507 SLEV strain showed a higher infection rate than the CbaAr-4005 strain (Table 1). These differences may be caused by intrinsic factors of the viral strain, such as infection efficiency and replicative capacity. Activity dominance of this genotype (V) over other co-circulating genotypes (II, III, VII) was previously detected in Córdoba city,[6] and hypothesized to be the result of this strain being more efficient in infecting vectors and replicating in the avian host. Our results support this hypothesis.

After re-feeding on susceptible chicks, mosquitoes were able to transmit the virus. Susceptible chicks developed viremia (3–5 $\log_{10}$ PFU/ml) higher than the oral infectious $dose_{50}$ for *Cx. quinquefasciatus* mosquitoes.[10] There is evidence of variation in vector competence among mosquito populations for a given vector species.[11,12] For example, Mitchell and colleagues[8] showed that Argentinean populations of *Cx. quinquefasciatus* have significantly higher vectorial transmission efficiency for Argentinean SLEV strains (90.5%) than US populations of the same mosquito species (40%). Comparing our results with those obtained by Mitchell and colleagues,[8,10] similar infection rates were observed between *Cx. quinquefasciatus* populations from Santa Fe and Córdoba provinces. Taken together, these results suggest that SLEV strains are better adapted to the *Cx. quinquefasciatus* mosquito populations they coexist with.

We showed that, although different SLEV strains differed in the degree to which they are infectious to mosquitoes, *Cx. quinquefasciatus* is indeed competent to transmit Argentinean SLEV strains that amplify in birds. This supports previous studies suggesting that this mosquito species is susceptible to infection and is able to transmit the virus.[8,10,13] In addition to our findings, SLEV-infected *Cx. quinquefasciatus* mosquitoes were detected in several natural populations across Argentina, including locations associated with human encephalitis cases.[5,6] Furthermore, *Cx. quinquefasciatus* populations are abundant during February and March, when the number of individuals infected with SLEV often increases.[7]

Therefore we conclude that *Cx. quinquefasciatus* meets several of the criteria needed to be considered a vector, and that this mosquito species probably plays a part in the natural transmission of SLEV in Argentina.

Table 1. Infection percentages in *Culex quinquefasciatus* mosquitoes inoculated per-orally with two strains of St Louis encephalitis virus

Viral strain	Titer[a] ($\log_{10}$ PFU/ml)	Infected mosquitoes (n)	Total mosquitoes assessed (n)	Infection rate[b]	p
78V-6507	5.30	79	87	90.80	<0.05
CbaAr-4005	5.18	59	80	73.75	<0.05

PFU: plaque-forming unit.
[a]Viral titer of chick blood fed on by mosquitoes.
[b]No. of infected mosquitoes/Total no. of mosquitoes assessed.

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