Title: *Culex saltanensis* and *Culex interfor* (Diptera: Culicidae) are susceptible and competent to transmit St. Louis encephalitis virus (Flavivirus: Flaviviridae) in central Argentina.

Short title: *Culex* competent vectors for the SLEV

Authors: Beranek MD[1, 2*], Quaglia AI[2], Peralta GC[3], Flores FS[2, 4], Stein M[1], Diaz LA[2, 5], Almirón WR[5] and Contigiani MS[2]

Affiliations:

1. Área Entomología, Instituto de Medicina Regional, CONICET, Universidad Nacional del Nordeste, Resistencia, Chaco, Argentina.
2. Laboratorio de Arbovirus y Arenavirus, Instituto de Virología " Dr. *J. M. Vanella*", CONICET, Facultad de Medicina, Universidad Nacional de Córdoba, Córdoba, Argentina.
3. Instituto de Diversidad y Ecología Animal, CONICET, Universidad Nacional de Córdoba, Córdoba, Argentina.
4. Instituto Nacional de Tecnología Agropecuaria, CONICET, Rafaela, Santa Fe, Argentina.
5. Instituto de Investigaciones Biológicas y Tecnológicas, CONICET, Universidad Nacional de Córdoba, Córdoba, Argentina.

*__Corresponding author information__:

E-mail: mauricioberanek@gmail.com (MDB)

Phone number: 54 9 353 4111643

Abstract

Infectious diseases caused by mosquito-borne viruses constitute health and economic problems worldwide. St. Louis encephalitis virus (SLEV) is endemic and autochthonous in the American continent. *Culex pipiens quinquefasciatus* is the primary urban vector of SLEV; however, *Culex interfor* and *Culex saltanensis* have also been found naturally infected with the virus, suggesting their potential role as vectors.

OBJETIVE The aim of this study was to determine the vector competence of *Cx. interfor* and *Cx. saltanensis* for SLEV from central Argentina in comparison to a recognized vector.

METHODS Adult female mosquitoes of the three *Culex* species were orally infected by feeding on viremic chicks that had been inoculated with SLEV CbaAr-4005. Then, abdomens, legs and saliva blood-fed mosquitoes were analyzed by viral plaque assay and the presence of cytopathic effect on the cell culture monolayer.

RESULTS Mosquitoes were permissive to orally acquire infections, to virus dissemination, and to viral replication in the salivary glands. *Culex saltanensis* and *Cx. interfor* are potential vectors of SLEV.

CONCLUSIONS Our results demonstrate that in Argentina both *Culex saltanensis* and *Culex interfor* are susceptible to SLEV and competent for its transmission. Moreover they are abundant during SLEV activity period in urban area, positive for this virus in nature, and found to feed on natural hosts.

Key words

Arbovirus, Argentina, Culicidae, *Flavivirus*, Infectious diseases, Vector competence.

Introduction

St. Louis encephalitis virus (SLEV) is an endemic neurothropic flavivirus in temperate and subtropical areas of the New World that is maintained between multiple avian hosts and vector mosquito species [1]. In 2002, SLEV reemerged in the central area of Argentina and southern Brazil causing neurological diseases in humans. In 2005, the first outbreak occurred in Córdoba City with 47 confirmed cases and nine fatalities. Factors that promoted this emergence in Argentina include the introduction of a more virulent SLEV strain into a highly susceptible avian hosts community along with possible land use changes (urbanization, agriculture) [2]. Moreover, after 11 years of apparent absence, SLEV was detected as the causative agent of human encephalomyelitis in Arizona and California. Phylogenetic analysis suggested that the SLEV strain detected in Arizona and California, CbaAR-4005, was potentially introduced from Argentina belonging to the same genotype as the reemerging Argentinean virus [2].

SLEV, a similar to other arboviruses transmitted by mosquitoes, is dependent upon a complex interaction with the tissues of mosquito vectors. The virus-vector interaction is essential for its effective transmission and is influenced by both viral and vector components. There are two critical factors in mosquitoes that affect the likelihood for viral transmission after ingesting an infectious bloodmeal. The first occurs after a bloodmeal and infection of the midgut epithelial cells followed by dissemination to the hemocele. Following dissemination, infection of the salivary gland epithelial cells must occur, followed by the subsequent transmission through the saliva [3]. The intrinsic limitations of vector competence are the midgut infection barrier (MIB), the midgut escape barrier (MEB), the salivary gland infection barrier (SGIB) and the salivary gland escape barrier

(SGEB) [3, 4]. Mosquito vector competence [susceptibility, extrinsic incubation period (EIP), transmission rate], longevity, and host bloodmeal preferences are included among intrinsic factors. Each of these factors is affected by extrinsic factors such as larval density and nutrition, temperature, rainfall, host availability and host immunity [4].

In Argentina, the Eared Dove (*Zenaida auriculata*) and Picui Ground-Dove (*Columbina picui*) are natural hosts of SLEV, while *Culex pipiens quinquefasciatus* is an efficient vector for the virus [1, 5]. For example, SLEV was detected and isolated in *Cx. p. quinquefasciatus* mosquitoes during an outbreak of encephalitis in humans in Córdoba City [6]. These mosquitoes also have been detected during periods without reports of clinical disease symptoms in Santa Fe province between 1978-1983 [7] and Córdoba between 2001-2004 [8]. In addition viral isolations from field collected mosquitoes, and the population abundance throughout the City [6], laboratory studies have confirmed horizontal transmission [5, 9], and the infrequent vertical transmission of SLEV in *Cx. p. quinquefasciatus* [10]. The presence of naturally SLEV-infected *Culex interfor* and *Culex saltanensis*, dominant species in urban-vegetated sub-assemblage that frequently feed on competent hosts, suggest they could participate as vectors in the transmission network of SLEV [1, 5, 6, 11]. Based on these evidences we argue that *Cx. saltanensis* and *Cx. interfor* could be playing a role as vectors of SLEV in central Argentina; yet vector competence experiments have not been conducted on these species. Therefore, we evaluated the vector competence of *Cx. interfor* and *Cx. saltanensis* against SLEV from central Argentina compared to the natural vector, *Cx. p. quinquefasciatus*.

Materials and Methods

Capture and maintenance of mosquito colonies

Egg rafts of *Cx. p. quinquefasciatus*, *Cx. interfor* and *Cx. saltanensis* were collected during 2015 at the Bajo Grande sewage treatment plant (31°24'13"S 64°06'08"W) located in east of Córdoba City. The site is surrounded by aquatic vegetation, reservoirs, low income human settlements and crop lands (vegetables and fruits). Authorization for mosquito field collections was obtained from the Municipality of Córdoba.

Egg rafts were collected with a dipper (350 ml) and transferred to polypropylene bottles with a fine brush and transported to the Instituto de Virología "Dr. *J. M. Vanella*" Facultad de Medicina, Universidad Nacional de Córdoba (FM-UNC). Mosquitoes were maintained 27°C, 70% humidity, and 12:12 h light: dark (L: D) photoperiodic. Adult F1 females from the field collected rafts were used in the vector competence assay (Table 1). Egg rafts were placed in a plastic container with distilled water and hatched larvae were fed with suspension of liver powder (0.25 mg/0.5 ml distilled water) every 48 h. After identification, larvae were grouped by species, pupae of the same species were placed inside screened cages (26 x 22 cm) until emergence, are adults provided a 10% sugar solution by soaked cotton balls. Identification of adult females and 4th instars were based on morphological keys [12]. Adult males were also collected to confirm the taxonomic identification based on the genitalia morphology [13, 14].

Viral stock

Adult female mosquitoes were orally infected by feeding than on viremic chicks that had been inoculated with SLEV CbaAr-4005, a strain isolated from *Cx. p. quinquefasciatus*

mosquitoes during the 2005 human encephalitis outbreak in Córdoba City [6]. Viral stock was obtained from collections stored at the Instituto de Virología "Dr. *J. M. Vanella*" (FM-UNC). Viral stock was prepared from brain of a infected Swiss albino suckling mice homogenized in 10% P/V solution in Eagle's minimal essential medium (MEM) (Gibco, Ireland), supplemented with 10% fetal bovine serum (FBS) (Natocor, Argentina) and 1% of gentamycin (Klonal, Argentina). Viral titration was carried out by viral plaque assay in VERO cell monolayer (African green monkey kidney, *Cercophitecus aethiops*). We inoculated 0.1 ml of the samples onto VERO cell monolayer on a 12-well plate and incubated the plates for 60 min at 37°C with 5% CO_2 and a humid atmosphere to favor the adsorption of the virus to the cell. After incubation 0.5 ml of MEM 2x was mixed with 1% methylcellulose and added to plate followed by incubation at 37°C for 7 days, until the formation of lytic plaques. The plates were then fixed with 10% formalin solution for 2 h and stained with crystal violet for the observation and plaque forming units (PFU) counts. Viral concentrations were then expressed as $\log_{10}$ PFU per milliliter (PFU/ml).

Vector competence assay

Twenty-four h-old chicks (*Gallus gallus*) were inoculated intraperitoneally with 0.1 ml of a viral suspension containing approximately 400 PFU of SLEV. In accordance with viremia kinetic [5], 48 hr post-inoculation starved female mosquitoes were fed on chicks (Table 1). Prior to and following blood feeding by mosquitoes, 0.1 ml of blood was taken from the chick jugular vein to determine the viremia titer (potential viral load ingested by mosquitoes). The chick blood was diluted in 0.45 ml of MEM supplemented with 10% FBS and 1% gentamycin, followed by centrifugation at 4°C for 20 min at 2,300 g before supernatant stored at -80°C. Titers were determined by plaque assay and expressed as the

mean viremia load before and after feeding (log_{10} PFU/ml). Mosquitoes were anesthetized by cold and fully engorged females were maintained in a screened cage at 27°C, H° 70%, 12:12 h L: D photoperiod and provided a 10% sugar solution. After 14 days EIP, the females were aspirated and anesthetized for 2 min at 4°C, placed on a refrigerated plate where the legs and wings were gently removed. Saliva samples were recovered after live females were placed on a flat surface with adhesive tape and the proboscis inserted for 30 min into a capillary tube with 0.001 ml glycerin (Todo Droga, Argentina) [15]. The abdomen was then removed, and saliva, legs and abdomen samples individually stored at -80°C with 1 ml of MEM supplemented with 10% FBS and 1% gentamycin. Legs and abdomen were individually homogenized by agitation with glass beads for 4 min and centrifuged at 11,200 g for 30 min. Subsequently, 0.1 ml of the sample was inoculated with two cellular and viral controls and infective virus particles detected by plaque assay as described above.

SLEV-negative saliva samples from females with positive abdomen samples were re-analyzed to reduce false negatives due to dilution. Thus, 0.2 ml of each sample was inoculated in two cell monolayer plates (‘A’ and ‘B’ plates) using the same controls as mentioned above. ‘A’ plates followed the same culture protocol described above. For ‘B’ plates, MEM, FBS (2%) and gentamycin (1%) were added, and after 96 h the cytopathic effect (cell detachment, rounding and non confluent monolayer) was determined under an inverted microscope. To amplify viral particles not detected in the first passage, monolayer from the ‘B’ plates were harvested on the fourth day post inoculation, by collecting 0.75 ml from each of the duplicates wells followed by centrifugation at 9,300 g for 30 min (passage 1). Finally, another plate was inoculated (duplicate, same ‘B’ plates protocol) with 0.2 ml

of the samples without cytopathic effect in the ‘B’ plates. Therefore, PFU/ml was recorded in ‘A’ plates and the cytopathic effect presence in ‘B’ plates.

Ethical Guidelines

The protocol used was approved by Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) and Instituto de Virología "Dr. *J. M. Vanella*" (FM-UNC). The chicks were maintained in cages with a substrate of wood shavings and with a 12:12 h L: D photoperiod, 40-80% humidity and 20-26□ temperature. Food and water were available *ad libitum* (balanced concentrate feed, Gepsa Feeds, Argentina). The experimental procedures used were to minimize or eliminate pain and distress. Awareness were taken to avoid chicks suffering, as anesthetic protocols were not used. Euthanasia was carried out with neck dislocation by a trained laboratory technician.

Statistics analysis

Abdomens, legs, and saliva were considered positive when they showed at least one PFU or the presence of cytopathic effect on the cell culture monolayer. Infection rates were defined as the numbers of positive mosquito abdomens of the total number of blood feed mosquitoes. Dissemination rates were calculated as the number of mosquitoes with positive legs out of the number of mosquitoes fed analyzed. Transmission rates were determined by the number of mosquitoes with positive saliva of the total number of mosquitoes that blood fed. Variations in the infection, dissemination and transmission rates between the three *Culex* spp., were analyzed using a generalized linear model (GLM) with binomial distributions of errors and logit link function. *Culex p. quinquefasciatus* was used as the control SLEV vector as infections and transmission has been determined [5]. Differences in

viremia levels (range: 2.4-3.4 $\log_{10}$ PFU/ml) between feeding trials could be a source of variation confounding the outcomes analyzed, therefore viremia level was added as a co-variable. Significance at infections, dissemination, and transmission models were evaluated by Likelihood Ratio test (α=0.05, type II error) [16]. Contrast between species using the Tukey test, where the odds ratio (OR) informed the magnitude for the differences between species for each significant rate. Normal distribution and homogeneity of the variance of the residuals for each model were graphically explored [17]. All analyses and graphical presentations were made in the R [18] with car [19], factorplot [20] and ggplot2 [21] packages.

Results

The numbers of eggs collected *Cx. p. quinquefasciatus*, *Cx. interfor*, *Cx. saltanensis* and females that fed and were positive for SLEV is shown in Table 1. There was a narrow range of viremia during blood feeding (*Cx. p. quinquefasciatus*=2.9 $\log_{10}$ PFU/ml, *Cx. interfor*=3.5 $\log_{10}$ PFU/ml and *Cx. saltanensis*=3.2 $\log_{10}$ PFU/ml) not contribute to the variation in infection (LR X^2=1.01, df=2, n=76, p=0.604), dissemination (LR X^2=4.94, df=2, n=76, p=0.084) and transmission (LR X^2=1.20, df=2, n=76, p=0.548) (Table 2).

Infection rates were not significantly difference among the three species (LR X^2=3.42, df=2, n=76, p=0.181), although infections rates of *Cx. saltanensis* being slightly higher (8/12, 67%), followed by *Cx. interfor* (14/25, 56%), and lastly *Cx. p. quinquefasciatus* (13/39, 33%) (Figure). Viral loads (range=1.3-5.3 $\log_{10}$ PFU/ml) were evaluated in 89% (31/35) of the three species mosquitoes.

Dissemination rates were significantly higher in *Cx. saltanensis* (8/12, 67%; p=0.01; OR=8.9, 95% CI [2.12-44.10]) than *Cx. p. quinquefasciatus* (7/39, 18%) and *Cx. interfor* (10/25, 40%; p=0.09). Both, *Cx. p. quinquefasciatus* and *Cx. interfor* showed similar dissemination rates (p=0.813). Among infected mosquitoes, dissemination was achieved in 100% of those individuals tested (8/8) for *Cx. saltanensis*, while are 71% (10/14) of *Cx. interfor* and 54% (7/13) of *Cx. p. quinquefasciatus* demonstrated disseminated infections. These results represent a potential midgut barrier in *Cx. p. quinquefasciatus* (Figure). Viral load ranged from 1.0-5.4 $\log_{10}$ PFU/ml for 76% (19/25) of the three species mosquitoes with viral dissemination.

Transmission rates between *Cx. interfor* (20%, 5/25), *Cx. p. quinquefasciatus* (18%, 7/39) and *Cx. saltanensis* (17%, 2/12); (LR X^2=2.82, df=2, n=76, p=0.093) were not significantly different (Figure). All *Cx. p. quinquefasciatus* females with disseminated infections demonstrated SLEV in their saliva (7/7); this rate was only 50% (5/10) in *Cx. interfor* and 25% (2/8) in *Cx. saltanensis*, indicating a potential salivary gland barrier in both *Cx. interfor* and *Cx. saltanensis* (Figure). The saliva viral load range was 1.1-2.3 $\log_{10}$ PFU/ml in *Cx. saltanensis* and *Cx. interfor* (5/7, 71%). Because lysis plaques in *Cx. p. quinquefasciatus* were not confluent the SLEV load in saliva was not able to measure.

Discussion

Culex p. quinquefasciatus, *Cx. interfor* and *Cx. saltanensis* were competent vectors for SLEV based on 1) acquired infections, 2) disseminated virus, and 3) transmission of SLEV in the saliva. The three *Culex* species displayed similar infection susceptibility and comparable salivary gland infections, whereas dissemination patterns appeared to be

species specific. While, *Cx. saltanensis* had a greater chance of becoming infected and disseminating (67%) SLEV than *Cx. interfor* (infection=56%; dissemination=40%), and both with respect to *Cx. p. quinquefasciatus* (infection=33%; dissemination=18%) they were not observed differences.

To be considered a vector, a mosquito species must fulfill several biological and ecological characteristics [4]. The coevolution between viruses and arthropods determine the vector competence, and thus the ability to acquire, maintain and eventually transmit an arbovirus [22]. To systemically infect the vector, the virus must cope with innate immune responses and overcome tissue barriers associated with the midgut and the salivary glands [3]. The tested species showed similar infection and transmission rates, but *Cx. p. quinquefasciatus* and *Cx. saltanensis* demonstrated differences in dissemination rates that could be related to a species-specific tissue barriers affecting the infection-transmission kinetics [23-25]. In *Cx. saltanensis* the SGIB/SGEB likely partially limited the transmission of SLEV since (100%, 8/8) of the infected mosquitoes demonstrated disseminated virus, transmission, while only 25% (2/8) transmitted it. Turell et al. found that *Psorophora cingulata* and *Coquillettidia venezuelensis* were susceptible to infection by Venezuelan equine encephalitis virus; however they had low transmission rates, even after intrathoracic inoculation, which indicated the presence of SGIB/SGEB in these species [26]. A salivary gland barrier of *Cx. p. quinquefasciatus* was not observed since 100% those disseminated with to SLEV transmitted the virus in saliva, while *Cx. interfor* was intermediate vector. Reisen et al. quantified the viral particles of SLEV expectorated in the saliva of *Culex tarsalis* (1.1-2.2 $\log_{10}$ PFU) [27] and we obtained similar data for *Cx. interfor* (range=1.1-2.3 $\log_{10}$ PFU) and *Cx. saltanensis* (1.8 $\log_{10}$ PFU). However, the number of tested mosquitoes was low, thus our results are not conclusive. Further studies are needed to have

deeper understanding regarding the SLEV infection and dissemination barriers in these three *Culex* species.

Our results corroborate the findings reported by Diaz et al. on the susceptibility of *Cx. p. quinquefasciatus* for SLEV infection [5]. These authors observed an infection rate of 70% using the same viral strain CbaAr-4005 and feeding viremia level of 5.2 $\log_{10}$ PFU/ml. In our study only one third of the *Cx. p. quinquefasciatus* females became infected. This difference could be related to the lower viremia level that the mosquitoes were exposed to in this assay (2.9 $\log_{10}$ PFU/ml). Mitchell et al. obtained a transmission rate of (90.5%, 19/21) with strain 78V-6507 for *Cx. p. quinquefasciatus* from Santa Fe Province [9]. In our study, the transmission rate was (18%, 7/39). Dissemination and transmission rate formulation in Mitchell and herein are different. Even through dissemination rates are higher with a higher infectious dose (Mitchell, 4.1-4.8 $\log_{10}$ PFU/ml vs our study, 2.9 $\log_{10}$ PFU/ml), adjusting the formulation for rates the dissemination between Mitchell and the current study would be similar.

This is the first completed vector competence evaluation of *Cx. interfor* and *Cx. saltanensis* against SLEV. Future studies to determine the Minimum Infection Threshold (MIT) and the EIP are needed. Furthermore, we were able to obtain an approximation of the MIT and EIP for *Cx. p. quinquefasciatus*, *Cx. interfor* and *Cx. saltanensis* of 2.9, 3.5 and 3.2 $\log_{10}$ PFU/ml, respectively, because the infected mosquitoes transmitted SLEV 14 days after infection. Mitchell et al. estimated an MIT in *Cx. p. quinquefasciatus* for SLEV and determined a value of 2.9 log PFU/ml and 3.5 log PFU/ml for 79V-2533 and 78V-6507 strains, respectively [28]. The low MIT likely was caused by the low viremia titers of the hosts [29]. For the species used in our study regardless of the host's viremia, we did not find differences between infection, dissemination and transmission rates. The effect of

viremia could be affected by the small number of individuals used in this study, caused by low feeding success and high mortality, which limited the sample size. Future studies to determine the MIT for different time periods are needed, even though our findings showed at least 14 days of incubation were sufficient for transmission.

The maintenance of SLEV in nature is complex and requires the coexistence in time and space of mosquito vectors and avian hosts. The detection of naturally infected mosquitoes does not represent, by itself, a reliable proof of their role as a competent vector. In the case of *Cx. p. quinquefasciatus*, *Cx. interfor* and *Cx. saltanensis*, there is evidence supporting their intervention as vectors in SLEV transmission. *Culex p. quinquefasciatus* is considered the primary vector, because it was abundant and *pools* assayed were positive for SLEV [6-8, 11]. In 2004, prior to the outbreak of encephalitis in Córdoba City, SLEV-infected *Cx. interfor* were detected [8]. In 2010, there were small outbreaks of SLEV in provinces, e.g., Buenos Aires, Córdoba and San Juan [30]. Not long thereafter, SLEV-infected *Cx. saltanensis* were detected for first time in Córdoba City [11]. The local populations of *Culex* spp. increase in abundance with peaks in summer, with are temporal distribution of *Culex* spp. coinciding with the activity peaks of SLEV in human infection [1, 31]. Adult mosquitoes belonging to the species *Cx. saltanensis* and *Cx. interfor* have been found in increasing numbers in Córdoba City, with a higher abundance in urban and periurban areas where vegetation is more robust. This differs from *Cx. p. quinquefasciatus*, which is predominant throughout a vast range of city-type environments [31]. As far as vector competence is concerned, all three species were essentially identical in transmitting SLEV (ranged 17-20%). Our results support the hypothesis that SLEV is transmitted by multiple sympatric *Culex* spp., and that both *Cx. saltanensis* and *Cx. interfor* can be considered potential vectors of SLEV. In the United States, this has been observed as well;

however, different mosquito species serve as the primary vector transmitting SLEV in different geographical areas. *Cx. quinquefasciatus* and *Cx. nigripalpus* are vectors for the virus in Florida, *Cx. tarsalis* in the western and *Cx. pipiens* in the northern United States [32].

SLEV is a multi-host and multi-vector flavivirus in the process of an ongoing reemergence in Argentina. Further studies are required to understand the spatial compartmentalization of these mosquito species in the transmission network of SLEV by performing vector capacity studies. By having insights into its ecoepidemiology, we will have a better understanding of which factors are causing this reemergence and how biological and environmental factors interact with and affect its activity. In addition, knowledge of the potential mosquito species vectors of SLEV will provide information to be used by different public agencies related to human health for the control of vector mosquito populations and improve efficiency in SLEV prevention programs.

Acknowledgements

We thank Kristin Sloyer for sharing suggestions and improving the English of the manuscript.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

Funding Statement

Specific grants from funding agencies, commercial, or not-for-profit sectors were not received. Mr. Beranek, a doctoral Biological Sciences, National University of Córdoba, was a recipient of the CONICET doctoral scholarship of Argentina.

References

1. Diaz LA, Flores FS, Quaglia A *et al*. Intertwined arbovirus transmission activity: Reassessing the transmission cycle paradigm. *Front Physiol* 2013: 3: 493.
2. Diaz A, Coffey LL, Burket-Cadena N *et al*. Reemergence of St. Louis encephalitis virus in the Americas. *Emerg Infect Dis* 2018: 24: 2150–2157.
3. Franz AWE, Kantor AM, Passarelli AL *et al*. Tissue barriers to arbovirus infection in mosquitoes. *Viruses* 2015: 7: 3741–3767.
4. Kramer LD & Ebel GD. Dynamics of *Flavivirus* infection in mosquitoes. *Adv Virus Res* 2003: 60: 187–232.
5. Diaz LA, Flores FS, Beranek M, *et al*. Transmission of endemic St Louis encephalitis virus strains by local *Culex quinquefasciatus* populations in Córdoba, Argentina. *Trans R Soc Trop Med Hyg* 2013: 107: 332–334.
6. Diaz LA, Ré V, Almirón W *et al*. Genotype III Saint Louis encephalitis virus outbreak, Argentina, 2005. *Emerg Infect Dis* 2006: 128: 1752–1754.
7. Sabattini MS. Historical, epidemiological and ecological aspects of arboviruses in Argentina: Flaviviridae, Bunyaviridae and Rhabdoviridae. An overview of Arbovirology in Brazil and neighbouring countries (ed. by Travassos da Rosa APA,

Vasconcelos PFC & Travassos da Rosa JFS). Brasil: Instituto Evandro Chagas; 1998. 113–134 p.

8. Diaz LA, Albrieu Llinás G, Vázquez A *et al*. Silent circulation of St Louis encephalitis virus prior to an encephalitis outbreak in Córdoba, Argentina (2005). *PLoS Negl Trop Dis* 2012: 6: 1489.
9. Mitchell CJ, Monath TP & Sabattini MS. Transmission of St. Louis encephalitis virus from Argentina by mosquitoes of the *Culex pipiens* (Diptera: Culicidae) Complex. *J Med Entomol* 1980: 17: 282–285.
10. Flores FS, Diaz LA, Batallán GP *et* al. Vertical transmission of St. Louis encephalitis virus in *Culex quinquefasciatus* (Diptera: Culicidae) in Córdoba, Argentina. *Vector Borne Zoonotic Dis* 2010: 10: 999–1002.
11. Batallán GP. Binomía de *Culex interfor* Dyar en Córdoba, Argentina. [theses]. Córdoba: Universidad Nacional de Córdoba; 2013. 197 p.
12. Darsie RF. Mosquitoes of Argentina. Part I. Keys for identification of adult females and fourth stages larvae in English and Spanish (Diptera: Culicidae). *Mosquito Systematics* 1985: 17: 153–253.
13. Harbach RE, Jakov WL & Peyton EL Recognition of *Culex bidens* Dyar and *Culex interfor* Dyar (Diptera: Culicidae) as separate species. *Mosquito Systematics* 1986: 18: 139–144.
14. Laurito M, Visintin AM & Almirón WR. *Culex saltanensis* morphological redescription of the inmature and adult stage. *J Am Mosq Control Assoc* 2008: 24: 203–210.
15. Anderson SL, Richards SL & Smartt CT A Simple method for determining arbovirus transmission in mosquitoes. *J Am Mosq Control Assoc* 2010: 26: 108–111.

16. Zuur AF. GLM and GAM for Absence–Presence and Proportional Data. New York: Springer; 2009. Chapter 19, Mixed Effects Models and Extensions in Ecology with R. Statistics for Biology and Health; p. 245–259.

17. Zuur AF, Ieno EN & Elphick CS. A protocol for data exploration to avoid common statistical problems. *Methods in Ecology and Evolution* 2010: 1: 3–14.

18. R Core Team. R: A language and environment for statistical computing. [cited 2015]. https://www.R-project.org.

19. Fox J and Weisberg S. An R Companion to Applied Regression. 2nd Ed. Thousand Oaks CA: Sage Publications, 2011. http://socserv.socsci.mcmaster.ca/jfox/Books/Companion

20. Armstrong D. factorplot: factorplot. R package version 1.1-1. 2014. https://CRAN.R-project.org/package=factorplot.

21. Wickham H. ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag, 2009.

22. Kuno G & Chang GJ. Biological transmission of arboviruses: reexamination of and new insights into components, mechanisms, and unique traits as well as their evolutionary trends. *Clin Microbiol Rev* 2005: 18: 608–637.

23. Weaver SC, Scott TW & Lorenz LH. Patterns of eastern equine encephalomyelitis virus infection in *Culiseta melanura* (Diptera: Culicidae). *J Med Entomol* 1990: 27: 878–891.

24. Reisen WK, Barker CM, Fang Y *et al*. Does variation in *Culex* (Diptera: Culicidae) vector competence enable outbreaks of West Nile virus in California? *J Med Entomol* 2008: 45: 1126–1138.

25. Richards SL, Anderson SL, Lord CC *et al*. Relationships between infection, dissemination, and transmission of West Nile virus RNA in *Culex pipiens quinquefasciatus* (Diptera: Culicidae). *J Med Entomol* 2012: 49: 132–142.

26. Turell MJ, Jones JW, Sardelis MR *et al*. Vector competence of Peruvian mosquitoes (Diptera: Culicidae) for epizootic and enzootic strains of Venezuelan equine encephalomyelitis virus. *J Med Entomol* 2000: 37: 835–839.

27. Reisen WK, Chiles RE, Kramer LD *et al*. Method of infection does not alter response of chicks and house finches to western equine encephalomyelitis and St. Louis encephalitis viruses. *J Med Entomol* 2000: 37: 250–258.

28. Mitchell CJ, Gubler DJ, Monath TP. Variation in infectivity of Saint Louis encephalitis viral strains for *Culex pipiens quinquefasciatus* (Diptera: Culicidae). *J Med Entomol* 1983: 20: 526–533.

29. Diaz LA, Flores FS, Quaglia IA *et al*. Evaluation of Argentinean birds species as amplifying hosts for St. Louis encephalitis virus (Flavivirus, Flaviviridae). *Am J Trop Med Hyg* 2018: 99: 216–221.

30. Vergara Cid C, Spinsanti L, Rivarola M *et al*. Detección de infecciones humanas por *Flavivirus* en la ciudad de Córdoba durante el año 2010. *Rev Argent Microbiol* 2011: 43: 37.

31. Batallán GP, Estallo EL, Flores FS *et al*. St. Louis encephalitis virus mosquito vectors dynamics in three different environments in relation to remotely sensed environmental conditions. *Acta Trop* 2015: 146: 53–59.

32. Reisen WK. Epidemiology of St. Louis encephalitis virus. *Adv Virus Res* 2003: 61: 139–184.

Table 1: The egg collections and number females of *Cx. p. quinquefasciatus*, *Cx. interfor* and *Cx. saltanensis* in Córdoba City.

Specie	N. egg rafts*	Female total†	Female fed‡	Female infected§
Culex p. quinquefasciatus	12	64	39	13
Culex interfor	15	32	25	14
Culex saltanensis	9	18	12	8

***Number of egg rafts collected for each mosquito species, assuming that each raft was from a different female. Number of eggs not determined. Male/female ratio of hatched individuals was ~50%.**

† Number of females provided blood meals on viremic chicks.

‡Number of engorged females after feeding on viremic chicks.

§Number of females positives for SLEV.

Table 2: Vector competence for St. Louis encephalitis virus of *Culex p. quinquefasciatus*, *Cx. interfor* and *Cx. saltanensis*

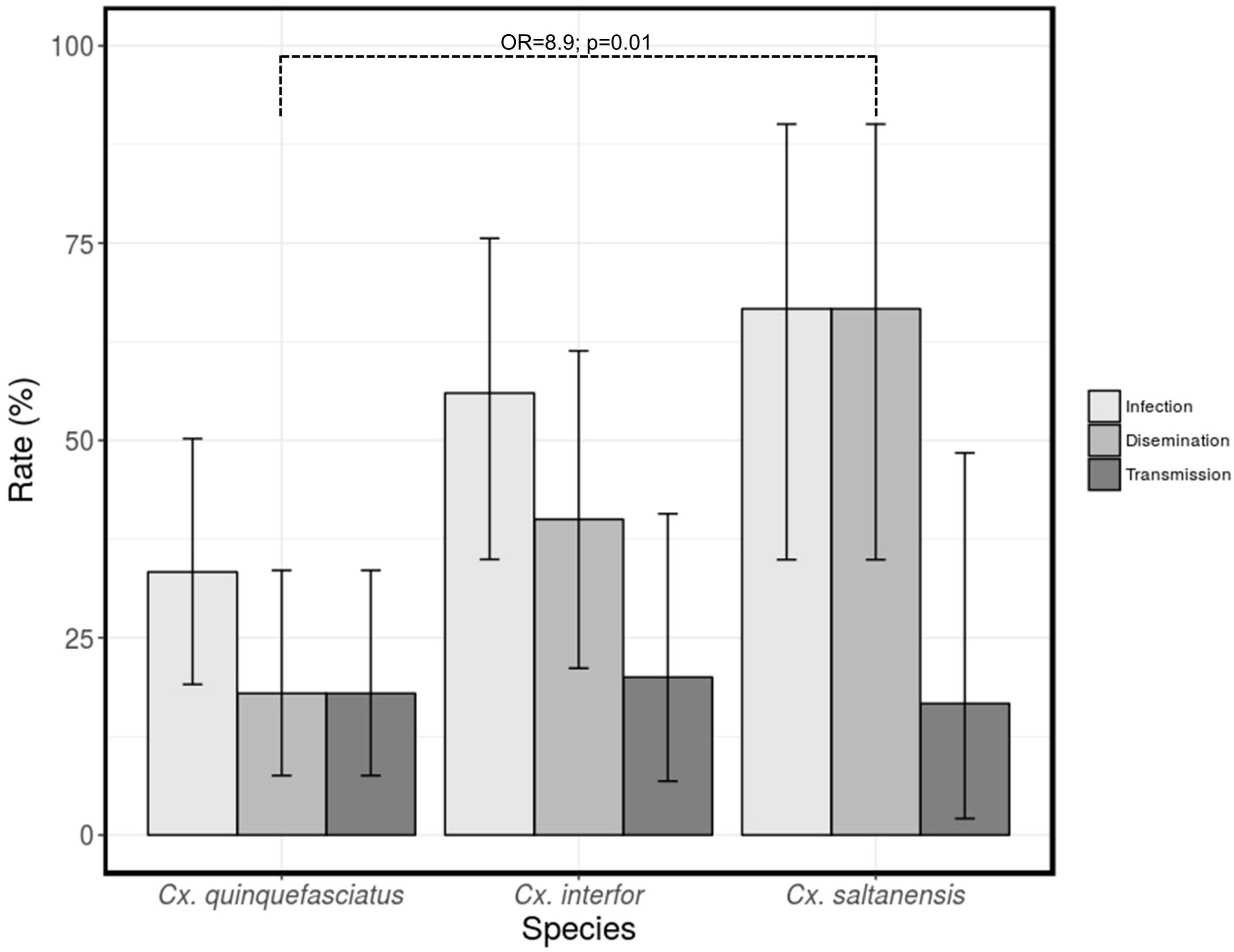
Specie	Rate	N*	Rate† (0.95%CIs)	Viral load‡ (0.95%CIs)
Culex p. quinquefasciatus	Infection	13/39	33 (19.1-50.2)	2.4 (1.3-2.9)
Culex p. quinquefasciatus	Dissemination	7/39	18 (7.5-33.5)	2.9
Culex p. quinquefasciatus	Transmission	7/39	18 (7.5-33.5)	No data
Culex interfor	Infection	14/25	56 (34.9-75.6)	4.8 (2.4-5.3)
Culex interfor	Dissemination	10/25	40 (21.1-61.3)	4.8 (1-5.4)
Culex interfor	Transmission	5/25	20 (6.8-40.7)	1.8 (1.1-2.3)
Culex saltanensis	Infection	8/12	67 (34.9-90.1)	4.8 (3.8-5.1)
Culex saltanensis	Dissemination	8/12	67 (34.9-90.1)	4.6 (1.6-5.1)
Culex saltanensis	Transmission	2/12	17 (2.1-48.4)	1.8

***Number of mosquitoes positive for SLEV/total number of mosquitoes assayed**

†Infection rate (number of positive mosquito abdomens/total number of mosquitoes fed); dissemination rate (number of mosquitoes with positive legs/total number of mosquitoes fed) and transmission rate (number of mosquitoes with positive saliva/total number of mosquitoes fed) with their 0.95% CIs.

‡ Average SLEV titers (log_{10} PFU/ml) in abdomen, legs and saliva of each mosquito species (0.95% CIs).

Figure: Vector competence for SLEV of *Cx. p. quinquefasciatus*, *Cx. interfor* and *Cx. saltanensis*. Infection rate (number of positive mosquito abdomens/total number of mosquitoes fed) in light gray, dissemination rate (number of mosquitoes with positive legs/total number of mosquitoes fed) in gray and transmission rate (number of mosquitoes with positive saliva/total number of mosquitoes fed) in dark gray; with their 0.95% CIs. Dashed line indicates significance difference based on pairwise comparison between species (Tukey Test). OR: Odds ratio.


OR=8.9; p=0.01
100
75
50
25
0
Rate (%)
Infection
Disemination
Transmission
Cx. quinquefasciatus
Cx. interfor
Cx. saltanensis
Species