Peer Community In Zoology

Multiple vector species may be responsible for transmission of Saint Louis Encephalatis Virus in Argentina

Anna Cohuet *based on peer reviews by 2 anonymous reviewers*

Beranek MD, Quaglia AI, Peralta GC, Flores FS, Stein M, Diaz LA, Almirón WR and Contigiani MS (2019) Culex saltanensis and Culex interfor (Diptera: Culicidae) are susceptible and competent to transmit St. Louis encephalitis virus (Flavivirus: Flaviviridae) in central Argentina. bioRxiv, ver. 6, peer-reviewed and recommended by Peer Community in Zoology. https://doi.org/10.1101/722579

Submitted: 03 August 2019, Recommended: 10 January 2020

Cite this recommendation as:
Cohuet, A. (2020) Multiple vector species may be responsible for transmission of Saint Louis Encephalatis Virus in Argentina. *Peer Community in Zoology*, 100002. 10.24072/pci.zool.100002

Published: 10 January 2020

Medical and veterinary entomology is a discipline that deals with the role of insects on human and animal health. A primary objective is the identification of vectors that transmit pathogens. This is the aim of Beranek and co-authors in their study [1]. They focus on mosquito vector species responsible for transmission of St. Louis encephalitis virus (SLEV), an arbovirus that circulates in avian species but can incidentally occur in dead end mammal hosts such as humans, inducing symptoms and sometimes fatalities. *Culex pipiens quinquefasciatus* is known as the most common vector, but other species are suspected to also participate in transmission. Among them *Culex saltanensis* and *Culex interfor* have been found to be infected by the virus in the context of outbreaks. The fact that field collected mosquitoes carry virus particles is not evidence for their vector competence: indeed to be a competent vector, the mosquito must not only carry the virus, but also the virus must be able to replicate within the vector, overcome multiple barriers (until the salivary glands) and be present at sufficient titre within the saliva. This paper describes the experiments implemented to evaluate the vector competence of *Cx. saltanensis* and *Cx. interfor* from ingestion of SLEV to release within the saliva. Females emerged from field-collected eggs of *Cx. pipiens quinquefasciatus*, *Cx. saltanensis* and *Cx. interfor* were allowed to feed on SLEV infected chicks and viral development was measured by using (i) the infection rate (presence/absence of virus in the mosquito abdomen), (ii) the dissemination rate (presence/absence of virus in mosquito legs), and (iii) the transmission rate (presence/absence of virus in mosquito saliva). The sample size for each species is limited because of difficulties for collecting, feeding and maintaining large numbers of individuals from field populations, however the results are sufficient to

show that this strain of SLEV is able to disseminate and be expelled in the saliva of mosquitoes of the three species at similar viral loads. This work therefore provides evidence that *Cx saltanensis* and *Cx interfor* are competent species for SLEV to complete its life-cycle. Vector competence does not directly correlate with the ability to transmit in real life as the actual vectorial capacity also depends on the contact between the infectious vertebrate hosts, the mosquito life expectancy and the extrinsic incubation period of the viruses. The present study does not deal with these characteristics, which remain to be investigated to complete the picture of the role of *Cx saltanensis* and *Cx interfor* in SLEV transmission. However, this study provides proof of principle that that SLEV can complete it's life-cycle in *Cx saltanensis* and *Cx interfor*. Combined with previous knowledge on their feeding preference, this highlights their potential role as bridge vectors between birds and mammals. These results have important implications for epidemiological forecasting and disease management. Public health strategies should consider the diversity of vectors in surveillance and control of SLEV.

References:

[1] Beranek, M. D., Quaglia, A. I., Peralta, G. C., Flores, F. S., Stein, M., Diaz, L. A., Almirón, W. R. and Montigiani, M. S. (2020). Culex saltanensis and Culex interfor (Diptera: Culicidae) are susceptible and competent to transmit St. Louis encephalitis virus (Flavivirus: Flaviviridae) in central Argentina. bioRxiv 722579, ver. 6 peer-reviewed and recommended by PCI Entomology. doi: [10.1101/722579](https://dx.doi.org/10.1101/722579)

Reviews

Evaluation round #3

Version of the preprint: Third

Authors' reply, 18 December 2019

Download author's reply
Download tracked changes file

Decision by Anna Cohuet, posted 18 December 2019

PCI entomology : revision requested

Dear authors

I ask for only minor corrections before I'll write the recommendation

Line 31 : change « objetive » to « objective »

Line 32 : remove "a" before Cx

Line 38 : change "acquire" to "acquired"

Line 39 : change "Culex saltanensis » to « Cx. Saltanensis » because already cited above

Lines 41-42 : change "Culex saltanensis and Culex interfor" to "Cx. saltanensis and Cx. interfor"

Line 51 : rephrase "Due to the hematophagous habit of females, many mosquito species are vectors of infectious…" (add "many" because not all species are vectors, remove "competent" because trophic behaviour is a parameter of vectorial capacity not of vector competence"

Line 54 : replace "one" by "few" because the cited virus can be transmitted not only by one species

Line 56 : add "are" in "arboviruses are generalist"

Line 86 : replace "competence" by "capacity"

Line 90 : add " infected " Culex p. quinquefasciatus if this actually what is meant
Line 210 : Table 2 instead of Table 3
Line 470 : rephrase the legend and explain better what is presented : p values , test and what is compared. Reverse the order of the columns so that they are ranked in the same order than the lines.

Evaluation round #2

Version of the preprint: Second

Authors' reply, 11 December 2019

Download author's reply

Decision by Anna Cohuet, posted 14 November 2019

PCI entomology : revision requested

Dear Dr Beranek and co-authors I acknowledge the improvement of the revised manuscript according to the suggestions of the reviewers. However, I notice that the main comment from the first reviewer was not fully considered. Indeed, the first reviewer highlighted weakness in the statistical analyses. Each of the mosquito species was fed on a different chick individual for exposure to virus. This means that mosquito species and viremia are confounded and interpreting the levels of infection/dissemination/transmission between species as differences of vector competences does not make sense. Only the ratio between the 3 steps (infection/dissemination/transmission) could be compared. However, the sample size being very limited I do not recommend it. The manuscript reports for the first time experimental observations of the susceptibility of Culex interfor and Culex saltanensis for SLEV, from ingestion to release of viral particles in the saliva. This deserves attention by itself. Comparing the vectorial competences between species is not feasible with the current data, which is fine. I therefore strongly recommend to present the data without extrapolation on competence levels. The GLM analysis should be removed and the text and figure changed accordingly. Also, I strongly recommend the revised version to be corrected by an native English speaker. The new version of the discussion includes very long sentences; some of them are difficult to understand. Please make sure that the row data are available to readers through an open data repository

Evaluation round #1

DOI or URL of the preprint: https://doi.org/10.1101/722579

Authors' reply, 02 November 2019

Download author's reply

Decision by Anna Cohuet, posted 17 September 2019

PCI entomology : revision requested

The reviewers highlighted the interest of the manuscript but also pointed out some limits that need to be considered/corrected before recommendation can be considered. I invite the authors to revise the manucript accordingly and to re-submit.

Reviewed by anonymous reviewer 1, 16 September 2019

The results reported in this study are interesting and are a worthwhile contribution to the field of medical entomology. Three Culex mosquitoes (Cx. saltanensis, Cx. interfor, Cx quinquefasciatus) were experimentally infected with a strain of St. Louis encephalitis virus; and the vectorial competence of these three species was characterized using 3 traits: (i) infection rate (presence/absence of virus in mosquito abdomen), (ii) dissemination rate (presence/absence of virus in mosquito legs), and (iii) transmission rate (presence/absence of virus in mosquito salivary glands). The results indicate that these three species are competent for the development of this strain of SLEV. In particular, the authors found that 8/12 abdomens of Cx. saltanensis were infected vs 14/25 in Cx. interfor and 13/39 in Cx quinquefasciatus. The virus successfully disseminated to the mosquito legs with the following prevalence: 8/12 for Cx. Saltanensis, 10/25 for Cx. interfor and 7/39 for Cx quinquefasciatus. Finally, the virus invaded mosquito salivary glands at the following rates: 2/12, 5/25, and 7/39 in Cx saltanensis, Cx interfor and Cx quinquefasciatus, respectively.

While previous studies already characterized the competence of Cx quinquefasciatus, this is the first time that the intrinsic competence of Cx saltanensis and Cx interfor is examined. This work therefore provides proof of principle that Cx saltanensis and Cx interfor are permissive species for the development of SLEV.

The authors also analyzed statistical difference in infection, dissemination and transmission rates among the three mosquito species. Although it would have been interesting, such comparisons cannot be derived from the current dataset. Analyses examining such differences are misleading for two reasons.

First, while the sample sizes used in this experiment are sufficient to provide proof of principles that Cx saltanensis and Cx interfor are permissive species, they are too low to draw robust interspecific differences. Second, and most importantly, each mosquito species received different infectious blood-meals in this experiment. Unless I misunderstood, lines 200-203 and the raw data (excel file) indicates that Cx saltanensis mosquitoes were fed on an infected chick with a viremia of 3.2 log10 PFU/ml, Cx interfor mosquitoes were fed on another infected chick with a viremia of 3.5 log10 PFU/ml, while Cx. p. quinquefasciatus mosquitoes were fed on an infected chick with a viremia = 2.9 log10 PFU/ml). Because the three mosquito species were not fed on the same infected chicks during the experiments, no competence comparison among species can be made. I apologize if this is a misunderstanding and if mosquitoes from different species indeed fed on the same individual chicks.

On a similar note, because mosquito species seems confounded with chick viremia (e.g. all mosquitoes from the species saltanensis fed on a chick with a viremia of 3.2 log10 PFU/ml) one cannot account for viremia in the binomial model. Using the raw data provided by the authors and the model described in the statistic section (lines 184-196), the following model: glm(Results~Specie+viremia,family=binomial) do not allow to derive any statistics for the effect of viremia (same is true when viremia is considered a categorical variable with three levels instead of a numeric variable).

The whole discussion is developed around the idea that, because Cx saltanensis and Cx interfor are abundant and permissive to the dissemination of SLEV in their salivary glands, they are possible vectors and may transmit the disease. This could be true but one critical factor must be fulfilled: real contact rate between competent vertebrate hosts and these vectors. What is the trophic preference and blood-feeding pattern of these two species? In fact, Cx saltanensis and Cx interfor will have the potential to ensure transmission, provided that they can feed on competent vertebrate hosts in natural conditions. A paragraph about the blood-feeding behavior of these two species would be great.

If contacts do occur, then what would be the most likely scenario and their role in disease transmission? Would Cx saltanensis and Cx interfor mostly maintain transmission among non-human reservoirs? could they act as a bridge vector between birds and human ? or could they even ensure robust human transmission ? Finally, what was the mortality rate of mosquitoes from 1 to 14 dpi? The Mat&Meth section mentions this was recorded. It would be important to report any lifespan difference among mosquito species as this is a major trait of vectorial capacity.

Minor comments:

- 20 infected chicks were obtained (lines 133) but only 3 were used (Lines 200-202 “There was a narrow 201 range of viremia during blood feeding (Cx. p. quinquefasciatus=2.9 log10 PFU/ml, Cx. 202 interfor=3.5 log10 PFU/ml and Cx. saltanensis=3.2 log10 PFU/ml”)? This is unclear. -Line 42 : consider replacing « activity » by « epidemic »

- Line 61: “as similar to”

- Line 91: “we evaluated the vector competence of Cx. interfor and Cx. saltanensis against SLEV from central Argentina compared to the natural vector, Cx. p. quinquefasciatus”. This sentence suggests that Cx interior and saltanensis are “artificial” vectors. However, as stated in the introduction they can be naturally infected. Consider replacing “the natural vector” by “the primary urban vector” -Line 82: I do not really understand what “horizontal transmission” refers to here (sexual transmission between mosquitoes?) -Table 1: replace Specie by Species -Line 109: replace “are” by “and” in: “…emergence, are adults provided” -Line 115: delete “than” -Line 119: “of a infected Swiss albino” replace “a” by “an” -line 200: SLEV is shown replace « is » by « are » -line 200 to 204. This is unclear. Does this means that (i) the different chicks used to perform the oral mosquito infection carried about the same virus titers and (ii) that this possible source of variability (different infection doses) did not affect the infection, dissemination and transmission rate?

- line 205: significantly difference by « different » -line 208-209 Viral loads (range=1.3-5.3 log10 PFU/ml) were 209 evaluated in 89% (31/35) of the three species mosquitoes. How did it vary among the three mosquito species? Was it different between species? -line 216: “These results represent a potential midgut barrier in Cx. p. quinquefasciatus” consider changing by “These results suggest the possible existence of a midgut barrier to SLEV in Cx. p. quinquefasciatus -Line 217-218: “Viral 217 load ranged from 1.0-5.4 log10 PFU/ml for 76% (19/25) of the three species mosquitoes with viral dissemination”. How did it vary among the three species? Was it different between species? Lines 224-225: The saliva viral load range was 1.1-2.3 log10 PFU/ml in Cx. saltanensis and Cx. interfor (5/7, 71%) How did it vary among the three species? Was it different between species?

- Line 235-236 : « …they were not observed differences”. Do you mean this was not statistically different? If yes please reword. In addition, increasing the sample size would perhaps make these stat different. -Line 246-247: …of SLEV since (100%, 8/8) of the infected mosquitoes demonstrated disseminated virus, transmission, while only 25% (2/8) transmitted it”. Consider deleting the word “transmission”

- lines 274-276: “ 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 log10 PFU/ml, respectively, because the infected mosquitoes transmitted SLEV 14days after infection” MIT and EIP (even rough values) cannot be derived from this experiment. This would require (i) infecting the mosquitoes with a wide range of viremia (and look at the threshold above which mosquitoes become infected) and (ii) dissect mosquitoes at several time points. Perhaps the EIP of this viral strain is 2 days, we simply cannot tell until mosquitoes are dissected and checked for the presence of virus at this time point.

Reviewed by anonymous reviewer 2, 12 September 2019

Review of ‘Culex saltanensis and Culex interfor (Diptera: Culicidae) are susceptible and competent to transmit St. Louis encephalitis virus (Flavivirus: Flaviviridae) in central Argentina’ by Beranek et al.

This study compares the efficacity of three different species of mosquitoes from the Culex genus as vectors of St Louis encephalitis virus (SLEV). Culex saltanensis and Culex interfor are thought to be new vectors, whereas Culex pipiens quinquefasciatus is considered the established and most common vector. Mosquitoes of each species were inoculated with the virus, and viral presence confirmed in the haemocoel, legs and salivary glands. Presence of the virus in the salivary glands was taken to mean that transmission could take place. They found

that all 3 species became infected with SLEV, with no differences in levels detected in the haemocoel and salivary glands. Thus, they conclude that all species may be competent vectors.

I enjoyed reading the manuscript and think the subject is suitable for PCI Entomology. However, revision is required before it can be recommended. Please see below recommendations that I hope will improve the clarity of the manuscript.

I think the Introduction needs more information describing the ecology of the virus, notably that it has many vertebrate hosts and Culex vectors. As presented, it seems that Cx. saltanensis and Cx interfor are new potential vectors, but then later in the Discussion it is revealed that there are actually many known Culex vectors. Another concern is that the study doesn't measure actual transmission. This is fine, but please provide information about whether presence in the salivary glands means that transmission can occur. If viral particles from the salivary glands infect cell culture, does this mean that they can also be transmitted in to the blood. Describe somewhere about viral replication in the mosquito.

There are a number of grammatical mistakes throughout the ms that need to be corrected.

Abstract

Line 32: Put the species name instead of 'a recognized vector'.

Line 34: Does the strain name need to be here? If so maybe add the importance of the strain, namely that it is the same strain detected in the US and Argentina, and responsible for human disease.

Introduction I would start the introduction with a more general paragraph setting the scene in the context of parasite-vector interactions being more or less specialist/generalist, and consequences for parasite transmission/epidemiology, before discussing the specific system.

Lines 49 – 60: Add in this paragraph whether detection in humans is recent. Is transmission possible from humans, or are they just a spillover host? The second half of the paragraph could be more concise, stating that re-emergences have occurred in the US (add the year) and Argentina (2002), with a particularly large outbreak in Cordoba City, and that the same strain is probably responsible.

Lines 61 – 65: These sentences should be referenced.

Lines 68 – 71: This repeats the previous 3 sentences. Just state these terms whilst describing these stages the first time round (lines 65 – 68).

Line 74: Do you mean the avian host here?

Line 72: These measures are proportions, not rates. Please change throughout the manuscript.

Lines 79 – 81: Were these SLEV infected mosquitoes detected during this period?

Lines 81 – 84: This sentence is unclear. Population abundance of what? Horizontal transmission between avian or mosquito hosts?

Materials and Methods Line 101: Did you track how many adult females came from each raft?

Line 115: This sentence should only describe the viral strain used. It is confusing to talk about adult female mosquitoes here.

Line 122: Shouldn't the description of viral titration be a new paragraph. Isn't this how you quantified virus in the mosquitoes?

Line 133: How many chicks?

Line 152: What were the cellular and viral controls?

Lines 181 – 182: State why dissemination rates are important. Does this give a measure of within-vector replication?

Lines 182 – 184: If viral particles are found in the salivary glands, are you sure that the virus can be transmitted? In some species of plant virus, transmission to a non-competent arthropod vector can result in viral particles being found throughout their body, but no transmission to a new host.

Lines 186 – 187: This sentence is not clear.

Line 188: If there were multiple feeding trials this should be included in the statistical model as a random factor. This will account for variance in viremia levels between feeding trials. Why not do a separate model looking to see how viremia levels change in the haemocoel, legs and salivary glands changes for the different

species? Also, if you can track which mosquito was fed on which chick, chick should also be included in the statistical models as a random factor.

Results

Lines 201 -202: Include a measure of variance when you present means such as confidence intervals or standard errors. Please correct this throughout the manuscript.

Line 208: Add that this refers to Figure 1. This needs to be corrected elsewhere in the manuscript.

Lines 208 – 209, lines 216 – 218 and lines 224 – 225: Why were viral loads only measured for a subset of infected mosquitoes? Also, just stating that they were measured tells us nothing. It would be interesting to do a statistical model to see how the viral loads differ between the different species.

Line 210: Please show the results for the main effect of species in the Dissemination model, not just the pairwise comparisons.

Lines 210 – 226: It would also be more informative to measure dissemination and transmission as a proportion of those that became infected, not the total number fed on infected chicks.

Line 216: Why does this suggest a midgut barrier for Cx. p. quinquefasciatus and not Cx. saltanensis? Please elaborate, but this information should be in the Discussion not the Results.

Line 223 – 224: This information about a potential salivary gland barrier should be in the Discussion.

Discussion

Lines 233 – 236: This sentence doesn't make sense.

Lines 240 – 242: It would be interesting to elaborate here and discuss in more detail the midgut and salivary gland barriers, and how they can prevent the transmission of other parasites by mosquitoes.

Line 247 and line 252: It is misleading to say that the virus was transmitted. This study only measures the potential for transmission by showing that the virus could migrate to the salivary glands in the vector.

Lines 247 – 250: Did this study actually measure transmission, or just the presence of the virus in the salivary glands?

Lines 259 – 264: Why do your results corroborate the results of Diaz et al when you find much lower infection rates.

Lines 266 – 267: How are your measures of dissemination and transmission different, and which is better?

Lines 272 – 276: What are the viremia levels presented for the MIT here? Levels measured on day 14? All you can really say about EIP is that it is less than 14 days.

Line 282: This information about mortality and low feeding success should be described in more detail in the methods.

Lines 288 – 291: As far as I can see there is no evidence showing that Cx. interfor and Cx. saltanensis were not previously vectors? Or have they only recently been identified as being infected? Did anyone look before?

Lines 297 – 308: This information should be presented in the Introduction.

Lines 309 – 310: This information should also be presented in the Introduction.

Table 1: You can put most of the information about the different columns, except about the egg rafts, as the column headings. For example, 'no. of egg rafts per species', 'total no. of females fed on chicks', 'no. of engorged females', and 'no. of SLEV positive females'. Also, why were so few, and different numbers of females for each species retained for infection?

Table 2 is difficult to read. Change the stating exactly what is shown. I think this would be something like 'Vector competence of Cp, Ci and Cs for SLEV measured as infection, dissemination and transmission. Table shows the proportion of mosquitoes positive for the virus and viremia levels in the haemocoel, legs and salivary glands of each species. The table would be much simpler, and it would be easier to compare among species, if the species were the columns and infection, transmission and dissemination the rows. Under each species you could have a sub-column for N, Rate (Proportion) and Viral load.

Figure 1 is also unclear. It would be simpler if you had 3 panels, 1 for each species. Or if you want to have only 1 panel, change it so that each of the coloured bars is a different species and infection, dissemination and transmission are on the x-axis. It is also not clear which pairwise comparison the dashed line showing the

odds ratio refers to.