Author's response to reviews

Title: Aedes aegypti from temperate regions of South America are highly competent to transmit dengue virus.

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Version: 3 Date: 17 December 2013

Author's response to reviews:

Dear Editor

The authors thank a lot the reviewers for the suggestions to improve the manuscript. We agreed with suggestions and made all minor corrections pointed by the reviewers.

All changes are lightened in the new revised version. Please find below specific replies to all reviewers’ comments.

Sincerely

Ricardo Lourenço de Oliveira

REVIEWER 2

Reviewer's report

Title: Aedes aegypti from temperate regions of South America are highly competent to transmit dengue virus.

Reviewer: Lauren Carrington

Reviewer's report:

The authors have addressed the comments of each of the reviewers with great care and attention. They have done a good job to satisfy majority of my concerns. While a few still remain, these are minor.

Questions:

Line 148: What is the reference (Ref 20) actually referring to? Is it the method for sorting? Please give just a bit more information here.
Reply: Reference #20 is a book that includes keys for mosquito species identification. We changed the text to make it clear: “Obtained adults were identified [20], exclusively consisting of Ae. aegypti. (line 148)

Lines 171-180: So this technique is an fluorescent focus assay? If so, please say this.

Reply: Done as suggested

Line 180-181: Given that you just describe what I think is an FFA, you then say that you will report the DIR, a proportion. It seems strange that you go to the trouble of doing serial dilutions for the FFA when you could just do the IFA (a simple qualitative determination for each mosquito) to obtain the same information.

Reply: We made profit of using FFA for both determining DIR (qualitative) and viral titer in mosquito head and saliva (quantitative).

DIR, TR and TE: Your description in your response to the reviewers was very detailed and much appreciated. It is a shame that such detail cannot be included in the manuscript for the benefit of other readers though. Can I just confirm that: DIR = # females with dissemination / # females tested; TR = # females with positive saliva / # females with dissemination; TE = # females with positive saliva / # females tested. Is this correct? If so, perhaps a very simply table or summary as above would help the reader.

Reply: Yes, the reviewer is correct. Detailed descriptions of these rates are included in the ms, as follows:

Line 180: The disseminated infection rate (DIR) corresponded to the proportion of mosquitoes with virus detected in heads among the tested ones.”

Lines 188-194: “TR corresponds to the proportion of mosquitoes with infectious saliva among mosquitoes able to ensure viral dissemination beyond the midgut barrier (...). TE represents the proportion of mosquitoes with infectious saliva among those exposed to the infectious blood meal (...).”

Line 220: You are referring to the DIR here?

Reply: No, we are describing viral titer, as follows "The median virus titer in mosquito heads tended to be higher at day 21 pi than at day 14 pi (Fig. 2)." As said above, we used FFA for both determining DIR (qualitative) and viral titer (quantitative) in mosquito heads.

Lines 265-266: This “highest virus titers in the head” and the “highest dissemination of infection” to me appear the same thing. Or, is the second referring to the proportion of mosquitoes with the dissemination? Please clarify this.
Reply: Yes, the first (“highest virus titers in the head”) refers to the quantification of virus concentration while the second (“highest dissemination of infection”) refers to proportion of mosquitoes with dissemination. We think it is clear in the text, as follows:

“(…) female mosquitoes sampled in the subtropics (ACO) are more efficient than those of temperate regions (SAL and BUE), displaying the highest virus titers in the head and presenting the highest dissemination of infection and transmission efficiency rates”.

Line 278: Please add after how many days infectious viral particles were detected in the saliva.

Reply: Done as suggested.

Line 284: At 26°C, the EIP for Aedes aegypti under large fluctuations tended to increase, no decrease. Please address this.

Reply: Corrected as asked. Our intention was not compare 20°C to 26°C. It was changed to “(...) it has been recently demonstrated that a great diurnal temperature fluctuation regime at an average of 20°C increases DIR and reduces the extrinsic incubation period of DENV in Ae. aegypti compared to a constant exposition at 20°C.”

Line 323: Is the TE that is higher in mosquitoes at 20°C relative to mosquitoes at 15°C? If so, please state this.

Reply: Done as suggested.

Line 377: Is there a definitive value for the household index? I didn’t see this in the reference indicated.

Reply: Data on house index (HI) are available from Table 1 (page 1085) of the cited reference. HI were as high as 6.2 and 11.6 in February and March (summer) in Salto, respectively.

Lines 115-120: Because Aedes albopictus is not mentioned again after this single paragraph (in reference to being a vector), even in the discussion, I think this paragraph could be removed entirely.

Reply: We prefer to keep this small paragraph because both the geographical distribution and the status as dengue vector of Ae. albopictus in the Southern Cone of South America is very poorly know. For instance, this is the first formal publication on this subject considering Uruguay. Moreover, we are updating data published in 2008 about this mosquito in Argentina.

Line 225-226: I feel this sentence would be more suitable at the start of the paragraph.

Reply: Done as suggested.
Line 300: Please include a reference for the environmental factors.
Reply: Done as suggested.

Line 338: Perhaps this could be changed to: “are expected to be capable to transmit DENV”?
Reply: Done as suggested.

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Minor grammatical changes:
Reply: All changes pointed below were done as suggested (highlighted in the text).
Line 91: Change “DENV strains were…” to “These strains were…”
Line 138: Please adjust first sentence to: “The mosquitoes used in this study were…”
Line 141: Please adjust the sentence to: “…were collected from March to May”
Line 157: In reference to FFU, the abbreviation should come after the term has been defined, not before. Please put the words first, then followed by the FFU abbreviation in brackets.
Line 164: Mosquitoes being “constantly incubated” sounds strange. Perhaps rephrase to “Fully engorged females were incubated at 28°C constant temperature…”
Lines 194, 276, 327, 331, and anywhere else I’ve missed: As mentioned in the previous review, the word order of “viral infectious particles” is inaccurate. It should be “infectious viral particles. Please correct this.
Line 199-200: Please adjust the sentence to: “Statistical significance was established when p-values were lower than 0.05.”
Line 208-209: Please adjust the sentence to: …the ACO population from northern subtropical Argentina performed the best in measures of DIR, TR and TE compared to the temperate…”
Line 211: No need to redefine DIR, especially when you have just used the abbreviation only 2 lines above.
Lines 290, 295 and anywhere else I’ve missed: The correct terminology is “vectorial capacity”, not “vector capacity”. Please fix this.
Lines 216, 225, 232: Unless the journal has specific guidelines stating otherwise, numbers below 11 should be written in full, rather than the number itself. I refer not to time points for example “Day 14 pi”, but to text such as “3 populations”.
Line 221: Please adjust the sentence to: “… when considering each mosquito population…”

Line 234: No need to say “significantly lowest”. The p-value indicates this already.

Line 242: “title” should be “titer”?

Line 308: Salto, not Santo.

Line 317: “In doing so…”, not “For doing so…”

Line 393: Is this author initial supposed to be GW?

Line 230: No need to say “In addition”.

Reply: We prefer to keep as it is, just to emphasize that other index were distinct and to avoid star the sentence with a abbreviation.

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Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.

REVIEWER 3

Reviewer’s report

Title: Aedes aegypti from temperate regions of South America are highly competent to transmit dengue virus.

Version: 2 Date: 9 December 2013

Reviewer: Cheong Huat Tan

Reviewer’s report:

Discretionary Revisions

The authors might want to mention their future plans to conduct further studies to include DENV strains that are currently circulating in South America.

Reply: We prefer to avoid the inclusion of statements of potential future studies or potential future results in a publishing manuscript.

Level of interest: An article of importance in its field
Quality of written English: Acceptable

Statistical review: Yes, but I do not feel adequately qualified to assess the statisti