Author’s response to reviews

Title: Dengue type 4 in Rio de Janeiro, Brazil: cases characterization following its introduction in an endemic region

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REBUTTAL LETTER

INFD-D-17-00043
Dengue type 4 in Rio de Janeiro, Brazil: cases characterization following its introduction in an endemic region
Manoela Heringer; Thiara Manuele Souza; Monique Lima; Priscila Nunes; Nieli Faria; Fernanda de Bruycker-Nogueira; Thaís Chouin-Carneiro; Rita Maria Nogueira; Flavia Barreto dos Santos
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Reviewer 1:
Abstract - 1st para, what do the authors mean by 'laboratorial aspects'?
Author's response: The authors aimed to present the data on de laboratorial diagnosis used to analyze the dengue suspected cases.
Results - it's unclear what the authors mean by 'distinct groups' - please elaborate
Author's response: The correction was performed as suggested by the reviewer.
Title should read 'case characterization' not 'cases'
Author's response: Corrected as suggested by the reviewer.

Background –
page 4, line 78: give the species name of the vector
Author's response: Corrected as suggested by the reviewer
Page 5, line 95 - replace with 'despite being known as a mild serotype'
Author's response: Corrected as suggested by the reviewer
Page 5, line 98 - replace with 'occurring'
Author's response: Corrected as suggested by the reviewer
Page 5, line 99 - replace with 'laboratory' instead of 'laboratorial'
Author's response: Corrected as suggested by the reviewer
Page 9, lines 202 - can you explain in the Methods section what adding an antigen-antibody complex dissociation step involved?
Author's response: Information was added as suggested by the reviewer.
Page 9, line 204 - observed IN females (not ON)
Author's response: Corrected as suggested by the reviewer
Table 2 - is the p-value column needed there?
Author's response: Yes, the authors aimed to show the significant differences between the groups with statistical support.
Table 3 - in 2011, the number of samples is very small and so the % may simply be an artefact of that. That needs to be mentioned in the Results text.
Author's response: Despite the lower number of cases in 2011, the authors aimed to characterize the immune response on those cases available on that year. The information on the lower cases and the lack of significant differences between primary and secondary was added in the text as suggested.
Table 4 - can it be explained in the legend why there is only one p-value (test) in the upper part of the Table and which factors are being tested? It's unclear currently.
Author's response: The table was corrected and the information that the statistical analysis was performed for the comparison of the disease severity, to the distinct age groups and type of infection, added as suggested.

Figure 4 - what are A and B? It's not mentioned in the legend.
Author: (A) NS1 antigenemia on DENV-4 cases positive for NS1 ELISA; (B) NS1 antigenemia on DENV-4 cases previously negative by NS1 ELISA and positive after heat-mediated immune-complex dissociation. This information is in the legend.

Figures are generally of very low resolution.
Author's response: Figures were generated again and the quality was improved as suggested.

Reviewer 2:
The manuscript by Manoela Heringer et al. describes an outbreak due to dengue-4 in Rio de Janeiro in 2011-2013 with precise characterization of 3727 human blood samples. Clinical, epidemiological, virological, molecular parameters were described for each confirmed case. 705 dengue 4 cases were detected. The main findings were:

* Females were more infected than males
* Primary cases were more frequent than secondary cases
* Most fatal cases were infected secondarily by dengue-4
* Dengue-4 genotype II was the most prevalent genotype with a possible introduction from Venezuela and Colombia

This study is very well designed and based on 3727 suspected dengue cases with 1593 confirmed cases of dengue and 705 dengue 4.

I suggest to:
- Provide an explanation on the higher number of dengue cases in females

Author's response: The authors agree that there are no explanations at this point. Due to the disease transmission dynamics, this is quite hard to address. For instance, the information on the women's status regarding to work or if they are housewives, if they seek for assistance more often, when they are sick in comparison to men. Despite higher, the differences were not significant.
- Add some information on mosquito populations: main vectors, fluctuations in densities, vector competence of mosquitoes to different serotypes

Author's response: Vectors are not the topic of the the manuscript, however some information was added on the discussion section, as it plays an important role for the virus spread, specially when a new serotype is introduced in a susceptible population.

Reviewer 3:
This is a relatively straightforward paper on the characterization of endemic DENV-4 cases in Rio de Janeiro, Brazil. However, other than identifying that the circulating DENV-4 virus belonged to Genotype II, there was no new information nor insight contributed by this paper to the literature. The authors may wish to highlight potential takeaway points from this study for the readers in the Discussion section.

It is highly recommended that the authors engage the help of a native English speaker to proofread the manuscript. There are many sentences with awkward structures in the prose, which may result in confusion in readers.

Major Revisions
1. Line 165: Real time-PCR generally picks up 10- to 100-fold more viruses, compared to plaque assay. This may possibly be due to the detection of the viral genome of non-viable viruses during PCR and/or the clumping of viral particles during plaque assay. The authors may wish to justify here their preference of real-time PCR over plaque assay.

Author's response: The authors agree with the reviewer. However, the real-time PCR has been used in several studies due to its sensitivity and easy performance. Here, the method was chosen as reliable for the routine laboratorial diagnosis, especially during an outbreak. Moreover, the assay easily allows the viremia determination in the cases being investigated. Despite its usefulness, the plaque assay is laborious and time consuming.

2. Lines 167-173: The authors may wish to elaborate on the antigen-antibody complex dissociation process, which was not mentioned in the Methods section.

Author's response: Information was added as suggested by the reviewer.

3. The results have been rather confusing. With 705 DENV-4 cases in the beginning, the total number of samples tested has not been consistent for real time-PCR, detection of anti-DENV
IgM in both acute and convalescent samples, and detection of NS1 with or without antigen-antibody complex dissociation. Again, the total number of males and females (Line 204), immune response characterization in primary or secondary infections (Line 207), and disease severity characterization (Line 210) did not add up to 705. The authors may wish to elaborate on the differences. One suggestion may be for the authors to include a flow chart to explain how and why certain samples were "dropped out" at each stage of their characterization.

Author's response: Not all analysis, such as those by gender, age, immune response and disease severity were performed in all 705 DENV-4 due to the lack of information in some cases. This information was added in the result section for better clarification. Also, the distinct techniques are performed according to the number of the days of symptoms, therefore, not always all cases were equally tested. However, in all analysis, the number of samples tested by each one, was included in parenthesis.

4. Lines 216-219: The authors made an observation on abdominal pain in DWAS and severe Dengue cases. What is the significance statistically and clinically?

Author's response: According to the classification by WHO in 2009, dengue can be classified as dengue without warming signs, dengue with warming signs and severe dengue, which helps better in the management clinical disease. Abdominal pain is one of the warming signs described by the WHO criteria and in our study we found that it was the most frequent sign in the group dengue with warming signs. Due to the very low number of cases representing those groups, statistics was not performed.

5. Line 224: It was mentioned in the Methods section that a total of 20 samples were quantitated for viremia (Line 164), instead of the 16 samples mentioned here. Please clarify. Also, the authors may wish to elaborate on the statistical reasoning behind choosing 16 or 20 samples to represent a 705-subject cohort.

Author's response: The numbering was corrected as suggested by the reviewer. The number of severe cases was the limitation for this analysis, as the authors wanted to evaluate the differences between those and mild dengue, represented by the majority of the cohort.

6. Lines 229-231: This section is hard to comprehend. Please rephrase and elaborate.

7. Line 238: Again, the authors may wish to elaborate on the statistical reasoning behind sequencing 12 samples to represent a 705-subject cohort.
Author's response: Here, the authors aimed just to characterize the circulating DENV-4 genotype during the period. For that, the choice of representative strains is enough, as the virus circulating during the period does not evolve enough to justify a more robust sequencing for that matter. The results by sequencing a larger number of samples would provide the same information, regarding the genotypes, which was the goal in this study. For molecular and evolutionary studies, on the other hand, the authors agree a larger sampling would be needed.
8. Lines 259-261: This sentence is hard to comprehend. Please rephrase the sentence and briefly elaborate on it where possible.
Author's response: Corrected as suggested by the reviewer.
9. Lines 273-276: Are these 2 percentages tabulated in any of the tables? If so, please include the table number.
Author's response: Corrected as suggested by the reviewer.
10. Lines 278-279: The 2 percentages here differ from what was mentioned in the Results section (Line 212). Please clarify.
Author's response: Corrected as suggested by the reviewer.

Minor Revisions
1. Line 148: Replace "preM" with "prM".
Author's response: Corrected as suggested by the reviewer.
2. Line 195: Insert "(Table 2)" after "... only one sample".
Author's response: Corrected as suggested by the reviewer.
3. The authors may wish to ensure that all their table numbers in the prose are correct.
Author's response: Corrected as suggested by the reviewer.
4. The authors may wish to replace "dengue cases" throughout the prose with "DwoWS" as they have already introduced the abbreviation in Line 113.
Author: Does not fit
5. Line 267: Please elaborate briefly on what was previously described in Ref 9.
Author's response: Corrected as suggested by the reviewer.

6. Table 1: This table does not seem to be referred to in the prose. If this is indeed the case, the authors may wish to consider shifting it under Supplementary Materials.
Author's response: Table 1 is cited on the material and methods section. Line 154 (corrected version).