Author's response to reviews

Title: Emergence of chikungunya seropositivity in healthy Malaysian adults residing in outbreak-free locations

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Author's response to reviews: see over
Dear Editor,

Thank you very much for reviewing our manuscript. Below are our replies to the reviewer:

Reviewer 1.
Thank you very much for reviewing our work. Below are our replies to your questions:

Major Compulsory Revision:

**Question 1:** Maps/spatial analysis. This is the biggest issue with this manuscript. This manuscript should not be published unless there issues are resolved or the discussion about spatial analysis and the maps are removed.

a) Spatial analysis. Did you do a quantitative analysis? If so, which methodology did you use and what were your results?

**Answer 1(a):** Yes, we performed a quantitative analysis. In this study, we used the point pattern analysis tool “Average Nearest Neighbourhood” (ANN) in the ArcGIS software (Environmental System Research Institute, Inc., Redland, CA) to determine whether the distribution of CHIK IgG seropositive subjects is clustered or dispersed. With this method, the presence of significant cluster(s) in the study area could also be determined. Several sparsely distributed clusters were found in Negeri Sembilan, while more centralized clusters were found in Kuala Lumpur (pages 8-9 of manuscript).

b) What made of ‘cluster’ in your analysis? Was this quantitative or qualitative?

**Answer 1(b):** Clusters are quantitative and defined by the R value in the ANN analysis. R values which are less than 1 indicate clustered distributions; while R values which are more than 1 indicate either dispersed or uniform distributions. A random distribution is indicated by R =1 (page 7 of manuscript).

c) The maps, as currently prepared are unclear. A small locator map, included as part of the larger maps would be very helpful. The colour scheme of the maps is also unclear. What do the boundary lines represent? Are all of the small geographic units shown on the maps necessary? It is unclear to me, with the current maps, where the boundaries between the different regions are. Indicating the location of previous outbreaks would be useful.

**Answer 1(c):** Thank you for your suggestion. We have corrected the maps (Figure 1).
Minor Essential Revisions

**Question 1:** The writing a bit wordy throughout and could be use careful revision for grammar

**Answer 1:** Thank you for your suggestion. We have revised our manuscript according to your suggestion and corrected the grammar mistakes.

**Question 2:** Malaysian Cohort. The objectives and methods behind the cohort are not clearly explained.

**Answer 2:** We have revised our manuscript according to your suggestion by providing more information about the Malaysian Cohort Program in the text (Page 5).

**Question 3:** Logistic regression. As written, it is not entirely clear to me how you did your logistic regression. Also, how did you build your multivariate models. Were all factors included in these models?

**Answer 3:** The multivariate model was built using all significant single predictors or risk factors determined in the univariate logistic regression (page 6 of manuscript).

**Question 4:** Please include some discussion of the regional and global implications of the finding from this study. As it stands the discussion is fairly Malaysia-centric. This study would be strengthened by further discussion of how its finding help us better understand this disease and its spread.

**Answer 4:** The manuscript has been revised to include discussions on the regional and global implication of our findings (page 10 of manuscript).

**Question 5:** Please include a section in this paper discussing its limitations

**Answer 5:** We have include a section discussing study limitations in this manuscript (Page12 of manuscript).
Discretionary Revisions

**Question 1(a):** Comparison of chikungunya seroprevalence rates. The first paragraph of discussion section compares the seroprevalence rates from your study with other studies. I think that this comparison is misleading, as your study was looking at seroprevalence in non-epidemic areas, but the other studies cited looked at seroprevalence rates post-epidemic.

**Answer 1(a):** Thank you for your suggestion. We have revised our manuscript accordingly (page 9 of manuscript).

**Question 1(b):** Similarly, I think that comparing the urban/rural rates in Malaysia with those of Lamu Island may not be appropriate, since the differences between urban and rural Malaysia is very different from the differences between urban and rural in Lamu.

**Answer 1(b):** Thank you for your suggestion. We have revised our manuscript accordingly (page 11 of manuscript).

**Question 2:** How mobile is the population? Does the fact that a study participants doesn’t currently live in an epidemic area tell us that they likely were not in the epidemic area in previous years?

**Answer 2:** Given the ease of transportation, populations from both previously outbreak free and outbreak areas are becoming more mobile. Therefore, we could not identify subjects in our population who have never left their state of residence. The mobility of the study participants might have contributed to the spread of CHIK into the outbreak-free area, and this has been highlighted in the manuscript (page 12 of manuscript).
Reviewer 2.
Thank you very much for reviewing our work. Below are our replies to your questions:

**Question 1:** There is confusing wording in the methods section on power calculation: reconsider changing to: The samples size for this seroprevalence study was calculated under the assumption OF 50% prevalence for CHIK infection with a ± 5% error using StatCalc Epi Info Version 6 software (CDC, Atlanta, USA).

**Answer 1:** Thank you for your suggestion, we have made the corrections as suggested.

**Question 2:** Methods such discuss the definition of a cluster in the mapping section since in the results section, this statement should be better supported:

**Answer 2:** Thank you for your suggestion. We have revised our methods section where the definition of a cluster had been added (page7 of manuscript).

**Question 3:** Now that clusters have been detected, the authors might consider discussion wether these represent foci of local transmission or whether there are other risk factor links among these, all same ethnicity, all migrant workers, all rural?

**Answer 3:** Our present data could not provide definite answers about local CHIK transmission; nevertheless, we have revised our manuscript and added a paragraph to discuss the possible factors involved in the transmission (pages 11-12 of manuscript).

**Question 4:** The appearance of mapping results, specifically the very small dots representing the 850 CHIK negative patients homes in inadequate-larger dots or another mapping presentation method is needed.

**Answer 4:** Thank you for your suggestion. A new map has been provided in the revised manuscript (figure 2).

Thank you very much and we hope the revised manuscript will receive your kind consideration for publication.

Best Wishes,
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