Author's response to reviews

Title: Immune response to live-attenuated Japanese encephalitis vaccine (JE CV) neutralizes recent Japanese encephalitis virus isolates from South-East Asia and India

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

Thank you for your review of our manuscript. The authors appreciate the time and effort by the reviewers on our manuscript. We found the reviewers' comments very helpful and think we have been able to address all concerns, resulting in a stronger manuscript. Our response to each comment is detailed below.

Thanks again for considering this manuscript for publication in the BMC Infectious Diseases. Please let us know if you need any further information.

Sincerely yours,

Mark Boaz

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Reviewer #1
In the present MS, Matthew Bonaparte et al. assessed the neutralization capacity of a chimeric JEV vaccine (JE-CV)-induced antibodies against the recent isolated wild type JEVs, and suggested that the results were consistent with previously generated data on the neutralization of wt JEV isolates, and no differences were observed between genotype 1 and 3 JEV. However, it seems that several important points need to be revised first.

Major Compulsory Revisions
1) In Methods, although five different titer categories were created, including the increasing titers from low (titers 40-80, two pools) through medium (titers 160-320, two pools), high (titers 640-2560, two pools) and very high (titers >5120, one pool), I think at least three independent pools of each category should be included for evaluating their neutralization sensitivities to distinct JEV strains.

The serum used was from children and therefore the volume was limited. This precluded creating additional sera pools however as 3 independent results were obtained and grouped for analysis for each pool, we feel that this was a robust approach and is reflected in the manuscript. The results section (geometric mean titers) was updated for clarity concerning these 3 independent results.

2) For the Statistical methods, the authors ranked the neutralization sensitivity for seven JEV strains within each sample pool using GMT, which is reasonable. However, the subsequent rank across all sample pools was performed using the average GMT. Whether it is a scientific statistical method? And, how to explain the differences between the average rank (overall GMT) and those ranks analyzed based on any of a single sample with low, medium, high and/or very high GMTs?

Given the lack of statistical difference between neutralization sensitivity of these strains and potential over-interpretation of the overall sensitivity ranking we agree and have removed Table 4 that highlighted the overall ranking of neutralization sensitivity. We comment on comparisons by using the 95% confidence intervals within the manuscript to also address this.

3) For JEV viruses, the authors stated that they used the recent isolated viruses. However I noted that the latest isolates, JEV-SM1 and JEV-057434, were isolated in 2003 or 2005, respectively. These isolates were obtained at least 8 years ago. Importantly, the genotype 1 strain of JEV-SM1 is derived from pig. May be it is better to use a human-derived G1-JEV in the present study, why not?

We acknowledge the reviewer’s comment though in the context of both available JEV and those strains used routinely within the field - including the other published work that we reference which used strains isolated up to 1984, 2002 and 2009 (references 23, 20 and 21 respectively) – we consider these to be relatively recent. We did not have any human-derived G1-JEV available.

4) Finally, I suggest the author to revise the present MS to be a brief report or a short communication, which may be more suitable for publishing on BMC infectious disease.

We feel this work is interesting and a relevant addition to the field and prefer not to reduce this to a shorter communication. Though in view of the reviewer's comment we have made this manuscript more...
accessible by performing further copyedit and removing some content (Table 4 and summary text on overall ranking).

**Minor Essential Revisions**
1) On page 6, the last sentence, revise 2007 to 2005? It is not consistent with Table 1.

Thank you we have corrected this for isolate JEV-057434 to indicate 2005.

**Reviewer # 2**
Bonaparte and coauthors tested the cross-neutralization against recent genotypes 1 and 3 JEV strains in children immunized with JE-CV, a novel licensed JEV vaccine, and based on the PRNT results, the authors concluded that there was no difference in neutralization capability against JEV isolates in JE-CV-immunized children. JEV genotype shift is continuing in Asia, while all vaccine products are based on genotype III. The manuscript represents an interesting and important concept of the area, and the reviewer has the following comments.

**Major Compulsory Revisions**
1. The authors stated that no difference in neutralization titers, however, as shown in Figure 1 and Table 1, the GMT variations are too big. Actually, the reviewer noticed there is BIG different for selected samples. For example, sample 7 in Figure 1, PRNT50 for JE-902/97, together with SA14-14-2 and Nakayama, was clearly lower than that for JE-CV.

We have updated the results section (geometric mean titers) to address this. We agree that on an individual basis there are some neutralization differences among the viruses for the sample 7, though these differences are not consistent when viewed across the range of samples or when considering normal assay variability.

2. Statistically analysis is critically needed. Only eight samples were subjected to PRNT assay and comparison, the sample size may be too small for statistic analysis.

The authors acknowledge the statistical limitations and highlight comparisons by using 95% confidence intervals within the manuscript to address this. The serum used was from children and therefore the volume was limited which precluded creating additional sera pools. However, as 3 independent results were obtained and grouped for analysis for each pool, we feel that this was a robust approach and is reflected in the manuscript. The results section (geometric mean titers) was updated for clarity concerning these 3 independent results.

**Minor Essential Revisions**
3. The reviewer noticed that neutralization titers for JE-CV and SA14-14-2 varied. Since JE-CV derived from SA14-14-2 and contained the same prM-E domains, what factors caused the difference. The authors can discuss it.

We have updated the discussion section to add a comment and thoughts on this.

4. The reviewer suggests the authors be caution about their results description and conclusion. JE-VC does induce protective immune response against all strains tested, however, neutralization titer variations among different does exist. Especially, the abstract should be improved to avoid misleading.

We agree and have updated the abstract accordingly.
5. Genome sequence of JE-TVP236 should be sequenced and submitted to Genbank, supporting the genotype clustering. We are trying to determine whether this isolate has actually been sequenced to address this point. A typographical error has been corrected to indicate this strain is TVP-8236.

6. The reviewer suggests the authors be caution about their results description and conclusion. JE-VC does induce protective immune response against all strains tested, however, neutralization titer variations among different does exist. Especially, the abstract should be improved to avoid misleading. We agree and have updated the abstract accordingly.

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**Editor's comment:**

"We received review from two well-regarded experts in area. I agree with them - manuscript requests major rewriting and implementing all the major compulsory revisions. If they will be answered by authors and implemented them into manuscript (and again re-review from both reviewers), than manuscript could be publish in BMC Infectious diseases.

So there are two options for authors and I personally feel that re-submission of the manuscript is still possible. They have very nice and educational review how to achieve the higher level of manuscript. They could get from both reviewers lot of inspiration how to do it (abstract, discussion and especially conclusion). Nevertheless they have still possibility to avoid extensive revisions and they could publish it in research notes."

**Editorial requirements:**
-- Please clarify ethics statement to include specific institute names. This has been added.

-- Requesting copyedit: We recommend that you copyedit the paper to improve the style of written English. If this is not possible, you may need to use a professional language editing service. We have performed a copyedit of the paper to improve the style of written English."