PEER REVIEW HISTORY

BMJ Open publishes all reviews undertaken for accepted manuscripts. Reviewers are asked to complete a checklist review form (**http://bmjopen.bmj.com/site/about/resources/checklist.pdf)** and are provided with free text boxes to elaborate on their assessment. These free text comments are reproduced below.

ARTICLE DETAILS

TITLE (PROVISIONAL)	Efficacy, immunogenicity, and safety of a recombinant tetravalent dengue vaccine (CYD-TDV) in children aged 2 to 17 years: systematic review and meta-analysis
AUTHORS	Rosa, Bruno Alves da Cunha, Antonio; Medronho, Roberto

VERSION 1 – REVIEW

REVIEWER	Ryan Maves Naval Medical Center San Diego, San Diego, California, USA
REVIEW RETURNED	09-May-2018

GENERAL COMMENTS	Thank you for the opportunity to review this manuscript, which performs a valuable analysis of the immunogenicity and efficacy of CYD-TDV. The issue with this paper is that it has been overtaken by events since its likely initial presentation. In order for this paper to have any real validity at this point, it will need to address subsequent findings from follow-up studies demonstrating increased risks for hospitalization. These have led to new recommendations by the Global Advisory Committee on Vaccine Safety (GACVS) and are summarized at http://www.who.int/vaccine_safety/committee/GACVS-StatementonDengvaxia-CYD-TDV/en/ . These data were likely not available at the time of this initial systematic review but are clearly relevant to its findings. Separate from this observation, I believe that the analysis is well-conducted and informative. Our challenge going forward is to reconcile this RCT data with the follow-on findings. There are a number of minor grammatical and typographical errors that I defer to the editors. Minor specific comments: 1. Page 9 line 51. - The authors report that investigators on three trials refused to disclose patient outcome data. The use of the word “refused” may be a little inflammatory (although no doubt accurate). I would consider rephrasing this specific word. Page 11 line 55 - “justifying to evaluate” - this is ungrammatical. Please rephrase.

REVIEWER	Moffat M Malisheni National University of Singapore, Singapore
REVIEW RETURNED	13-May-2018

GENERAL COMMENTS	TITLE

	What is the difference between preliminary efficacy and efficacy? Line-3 ABSTRACT Add background information even if it is a sentence before objective. Line-4 STUDY ELIGIBILITY Do studies that assessed immunogenicity also qualify? You have only stated efficacy and safety. Line-13 – 16 OUTCOME MEASURES Revisit the sentence, e or and? Line-19 RESULTS Revisit neutralizing antibodies. Line- 35 – 36 CONCLUSION Revisit the conclusion. Immunize or protect children? Only against DENV3 and 4? Only for a year? Line- 45 – 50. Page 3 STRENGTHS AND LIMITATIONS OF THIS STUDY Are you sure about this statement? Line-8-9 INTRODUCTION Revisit line- 39 – 45. Have a look at antibody dependent enhancement (ADE). What do you mean by serologically related viruses? Line-52 Page 4 Agents of DNA replication? Line-19 What is significant immune response? Line- 24 – 25 Revisit protection rate. Line-32 Page-6 Data or dada? Line-11 Page-7 Efficacy expressed as 1-RR but presented as a percentage (%)? Line-30 Page-9 Table-1. It would be better if the selected articles were arranged by year. Page-10 Overall rate (why is rate being used?)? Serious AE was 5.2% or 5.1%? line-26 Page-11 Edema reported but not presented in the table. Line-37 DISCUSSION Line- 55, 3 – 34 could be moved to introduction and limitations section. Page-12 Line 35 – 38 reference required. Line- 40 – 45 sentence not clear. Page-13 Revisit line- 5 – 8 Revisit line- 27 – 32 Line- 12 – 16 and line- 38 – 42 look the same but have different references. In addition, the implications of the findings of the study have not been discussed. Justification of the study should be presented in the introduction. What could be the possible explanation behind high antibody levels and low efficacy for DENV2. What could be the possible explanation behind the high heterogeneity between the selected studies? Is there a reason why studies that were very diverse were combined? Why were the overall and serotype specific WMDs not reported?

	Any explanation why the overall Risk Ratio and CI from figure 2 and the one reported different in the text different (0.42 vs 0.46)? How do you suggest the efficacy of the vaccine should be improved? Why do you suggest that the vaccine is only protective for one year? Have vector control measures failed or just insufficient on their own? Any reason why fixed and random effects models were used for RR and WMD respectively? Has safety of the vaccine considering only studies with long follow up been assesses? If yes, what are the findings? I suggest you have a look this, "World Health Organization. Safety of CYD-TDV Dengue Vaccine: Weekly Epidemiological Record. Geneva: WHO (2016). p. 421–8. Available from: http://www.who.int/vaccine_safety/committee/reports/wer9034.pdf"

REVIEWER	Mohammadreza Mohebbi Deakin University, Australia
REVIEW RETURNED	28-Jun-2018

GENERAL COMMENTS	In Figure 1, add an explanation for how 22 records were screened and the rest were disregarded Is it possible to extract 95% cI for preliminary efficacy ratio? How RRs and their 95% CIs were estimated, please specify in the stats method? How WMDs and their 95% CIs were estimated, please specify in the stats method? The rate of serious AE in control group (6.4%) is for placebo controls or all control groups? How about other AE reports for controls? I^2 statistic for Serotype-specific efficacy analysis range from 36% to 96% , comment about consequences of these high level of heterogeneity in the analyses. Seme comment is also applicable to Serotype-specific efficacy analysis. Are fixed effects model still appropriate for analyses with high I^2. Some indexes and information presented in Figure 2, and 3 without explaining them in the stats methods or results. If this information added more to what has been presented in the manuscript text please explain them in more details otherwise omit theme. Currently the presented information in Figure 2 and 3 is not self-explanatory.

VERSION 1 – AUTHOR RESPONSE

Answers to Reviewer 1 comments

Bruno R. Rosa

The Answers to Reviewer 1 comments are into document in red.

Reviewer 1 comments to author

1) Please leave your comments for the authors below

Thank you for the opportunity to review this manuscript, which performs a valuable analysis of the immunogenicity and efficacy of CYD-TDV. The issue with this paper is that it has been overtaken by events since its likely initial presentation. In order for this paper to have any real validity at this point, it will need to address subsequent findings from follow-up studies demonstrating increased risks for hospitalization. These have led to new recommendations by the Global Advisory Committee on Vaccine Safety (GACVS) and are summarized at http://www.who.int/vaccine_safety/committee/GACVSStatementonDengvaxia-CYD-TDV/en/ . These data were likely not available at the time of this initial systematic review but are clearly relevant to its findings.

R: We have included a statement in the discussion (page 13) about new data presented to GACVS (below).

The WHO Global Advisory Committee on Vaccine Safety (GACVS) have presented new evidences that indicates that the increased risk of hospitalization (and severe disease) by dengue affects vaccinated subjects who are naive to wild dengue infection prior to vaccination. This corroborates prior hypotheses suggesting that immune priming from natural or other stimulation such as immunization with the dengue vaccine can lead to a higher risk of severe dengue disease on secondary exposure to wild dengue viruses.

World Health Organization. Global Vaccine Safety. GACVS Statement on Dengvaxia ® (CYD-TDV). World Health Organization, December 7, 2017. Available at: http://www.who.int/vaccine_safety/committee/GACVSStatementonDengvaxia-CYD-TDV/en/ (accessed July 15, 2018).

2) Separate from this observation, I believe that the analysis is well-conducted and informative. Our challenge going forward is to reconcile this RCT data with the follow-on findings. There are a number of minor grammatical and typographical errors that I defer to the editors.

R: Thanks. Due to little bit time we have, we will not be able to review the grammatical and typographical issues (we have contracted a specialized company for this manuscript translation). However, we are committed to do a large review to entire manuscript after the re-evaluation of our responses by the editor and reviewers.

Minor specific comments:

3) Page 9 line 51. - The authors report that investigators on three trials refused to disclose patient outcome data. The use of the word “refused” may be a little inflammatory (although no doubt accurate). I would consider rephrasing this specific word.

R: Page 11 - We change the statement “refused to disclose patient outcome data” by “but they did not respond”.

4) Page 11 line 55 - “justifying to evaluate” - this is ungrammatical. Please rephrase.

R: Page 13 – The statement: “Two reasons justifying to evaluate the effects of CYD-TDV exclusively in individuals under 18 years of age” was changed by the statement: “There are two reason to evaluate the effects of CYD-TDV exclusively in individuals under 18 years of age”.

Answers to Reviewer 2 comments

Bruno R. Rosa

The Answers to Reviewer 2 comments are into document in red.

Reviewer 2 comments to author

1) TITLE

What is the difference between preliminary efficacy and efficacy? Line-3 R: Preliminary efficacy refers to previous evaluate of an intervention. We have rephrased that (we draw the word “preliminary”) because this term is not applied to our research question.

2) ABSTRACT

Add background information even if it is a sentence before objective. Line-4 R: Background information was added (page 2).

3) STUDY ELIGIBILITY

Do studies that assessed immunogenicity also qualify? You have only stated efficacy and safety. Line-13 – 16

R: The term “Immunogenicity” was included in the issue “studies eligibility criteria” (page 2).

4) OUTCOME MEASURES

Revisit the sentence, e or and? Line-19

R: We have rephrased this sentence (page 2).

5) RESULTS

Revisit neutralizing antibodies. Line- 35 – 36

R: We have rephrased this statement (page 2).

6) CONCLUSION

Revisit the conclusion. Immunize or protect children? Only against DENV3 and 4? Only for a year? Line- 45 – 50.

R: We have rephrased this statement (pages 2-3, below):

CYD-TDV is considered safe and able to partially protects children and adolescents against four serotypes of dengue virus for one year. Despite this, research should give priority to improvements in vaccine efficacy to provide higher long-term protection against all virus serotypes.

Page 3

7) STRENGTHS AND LIMITATIONS OF THIS STUDY

Are you sure about this statement? Line-8-9

R: No. We have rephrased this statement. Checked.

8) INTRODUCTION

Revisit line- 39 – 45. Have a look at antibody dependent enhancement (ADE).

R: We have rephrased this statement (page 4).

9) What do you mean by serologically related viruses? Line-52

We just wanted to say that the vaccine should protect against all four DENV serotypes simultaneously.

Page 4

10) Agents of DNA replication? Line-19

R: PAGE 4 - We have corrected this statement (page 4). We would like to say “Agents of RNA replication”.

11) What is significant immune response? Line- 24 – 25

R: PAGE 4 - We have rephrased this statement. We change the word

“significant” for “important”.

12) Revisit protection rate. Line-32

R: PAGE 4 - We decided to rephrase this statement (below):

“Preliminary results of a phase 2 clinical trial involving 4000 Thai schoolchildren show that CYD-TDV is safe and provided 60% protection against DENV-1, and 80–90% protection against DENV-3 and DENV-4 [15]”.

Page-6

13) Data or dada? Line-11

R: PAGE 6 - This is “data”. We have rephrased this word.

Page-7

14) Efficacy expressed as 1-RR but presented as a percentage (%)? Line-30 R: PAGE – 8. We have rephrased this statement.

Page-9

15) Table-1. It would be better if the selected articles were arranged by year.

R: PAGE 10 – Table 1 was arranged by year.

Page-10

16) Overall rate (why is rate being used?)?

R: PAGE 11 – We have rephrased this word.

17) Serious AE was 5.2% or 5.1%? line-26

R: PAGE 12 – We have corrected this data. The correct proportion is 5.2%.

Page-11

18) Edema reported but not presented in the table. Line-37

R: PAGE 12 – Edema was presented in the table as swelling (but we have changed this word).

DISCUSSION

19) Line- 55, 3 – 34 could be moved to introduction and limitations section. R: DISCUSSION – We have moved first paragraph to the end of introduction; on the other hand, we have moved the second paragraph to the limitation subheading.

Page-12

20) Line 35 – 38 reference required.

R: PAGES 12 - 13 – These findings were extracted from our own results. We have not references in the literature for this statement.

21) Line- 40 – 45 sentence not clear.

R: We decided to remove this statement of the text.

Page-13

22) Revisit line- 5 – 8

R: We decided to remove this statement of the text.

23) Revisit line- 27 – 32

R: We have rephrased this statement (we have removed the part “in the body”). Checked.

24) Line- 12 – 16 and line- 38 – 42 look the same but have different references. In addition, the implications of the findings of the study have not been discussed. Justification of the study should be presented in the introduction.

R: PAGE 14 – We have rephrased and clarified this statement (since they are two identical statements, we also have removed one of that). In addition, we have corrected the reference.

25) What could be the possible explanation behind high antibody levels and low efficacy for DENV2?

R: We have included an explanation for that in the PAGE 14 (lines 33 - 34) and PAGE 15 (lines 1 - 3). The phenomenon of viral diversity may explain this issue.

26) What could be the possible explanation behind the high heterogeneity between the selected studies?

R: We have included an explanation for that in the PAGE 14 (lines 11 - 16). A hypothesis to explain this finding may be the different populations of studies. Differences between studies in terms of methodological factors (e.g. use of blinding and concealment of allocation), clinical issues (e.g. different populations) or if there are differences between studies in the way the outcomes are defined and measured, may be expected to lead to differences in the observed intervention effects.

27) Is there a reason why studies that were very diverse were combined? R: Yes. Meta-analysis should only be considered when a group of studies is sufficiently homogeneous in terms of participants, interventions and outcomes to provide a meaningful summary (Higgins 2011).

Higgins JPT, Green S (editors). Cochrane Handbook for Systematic Reviews of Interventions Version 5.1.0 [updated March 2011]. The Cochrane Collaboration, 2011. Available from www.cochrane-handbook.org.

28) Why were the overall and serotype specific WMDs not reported?

R: The serotype specific WMDs (with 95% CI) are reported in the text (PAGE 11) and figure 3. Overall WMD (with 95% CI) are also reported in figure 3. We have also included the overall WMD (with 95% CI) in both abstract and text.

29) Any explanation why the overall Risk Ratio and CI from figure 2 and the one reported different in the text different (0.42 vs 0.46)? R: We have corrected this statement (both figure 2 and text – PAGES 2 and 11). First overall analysis (RR 0.42) was carried out based on four DENV serotype-specific analyses (whose 3 studies used to person-years at risk as incidence density measure). On the other hand, second overall analysis (RR 0.46) was calculated with a different measure (we have considered the final number of enrolled participants in the analyses). This difference led to divergent conclusions. In addition, we have changed the models (from fixedeffects model to random-effects model). For this reason, we have a final RR = 0.40.

30) How do you suggest the efficacy of the vaccine should be improved? R: Future research should focus to increase efficacy of vaccine between both baseline seronegative individuals and against DENV-2 (on the last one, a proposal could include an increase in the number of viral particles of DENV-2 in the vaccine).

31) Why do you suggest that the vaccine is only protective for one year? R: Because the follow-up of all studies included in the efficacy analysis lasted for about one year, we can only limit our analyzes and conclusions regarding this period.

32) Have vector control measures failed or just insufficient on their own?

R: We have considered both assertions are true. We need to improve the management of vector control programs and create innovative methods for these programs.

33) Any reason why fixed and random effects models were used for RR and WMD respectively?

R: In the draft stage (protocol) we had hoped to find low heterogeneity among the studies (three RCT were alike on several issues - e.g. intervention, outcomes). According to Higgins (2011), random-effects method and the fixed-effect method will give identical results when there is no heterogeneity among the studies. However, where there is heterogeneity, confidence intervals for the average intervention effect will be wider if the random-effects method is used rather than a fixed-effect method, and corresponding claims of statistical significance will be more conservative. Because we have found heterogeneity among studies in the overall CYD-TDV vaccine efficacy we decided to change the model (from fixed-effect model to random-effect model).

Higgins JPT, Green S (editors). Cochrane Handbook for Systematic Reviews of Interventions Version 5.1.0 [updated March 2011]. The Cochrane Collaboration, 2011. Available from www.cochrane-handbook.org.

34) Has safety of the vaccine considering only studies with long follow up been assesses? If yes, what are the findings?

R: Any other long-term studies that have evaluated safety of CYD-TDV have not been included on review because they are not randomized controlled trials.

Hadinegoro SR, Arredondo-García JL, Capeding MR, et al. Efficacy and long-term safety of a dengue vaccine in regions of endemic disease. *N Engl J Med* 2015; 373(13):1–12.

35) I suggest you have a look this, “World Health Organization. Safety of CYD-TDV Dengue Vaccine: Weekly Epidemiological Record. Geneva: WHO

(2016). p. 421–8. Available

from: http://www.who.int/vaccine_safety/committee/reports/wer9034.pdf” R: Thanks. We have included some parts of this reference in the manuscript.

Bruno R. Rosa

The Answers to Reviewer 3 comments are into document in red.

Reviewer 3 comments to author

1) In Figure 1, add an explanation for how 22 records were screened and the rest were disregarded

R: In the figure 1 we have explained the reasons for 22 records were screened and the rest were disregarded.

2) Is it possible to extract 95% CI for preliminary efficacy ratio?

R: We have rephrased the statement “preliminary efficacy”. In fact, we have evaluated efficacy of CYD-TDV (based on pooled assessment of three RCT).

We have calculated RR with your 95% CI.

3) How RRs and their 95% CIs were estimated, please specify in the stats method?

R: PAGE 8 - We have included a statement in the issue “Summary measures” (below):

Dichotomous outcomes (efficacy and safety) were assessed using the relative risk (RR). A 2x2 table was used to calculate RR. The Mantel-Haenszel random effects model have estimated the pooled RR and associated 95% confidence interval (M-H, 95% CI). The M-H method provides a pooled relative risk across the strata of fourfold data.

4) How WMDs and their 95% CIs were estimated, please specify in the stats method?

R: PAGE 8 - We have included a statement in the issue “Summary measures” (below):

Continuous outcomes (immunogenicity) were assessed using mean difference (MD) analyses (a difference between two means). A fixed effects model with the inverse variance method was used to estimate the pooled MD and associated 95% confidence interval (I-V, 95% CI).

5) The rate of serious AE in control group (6.4%) is for placebo controls or all control groups? How about other AE reports for controls? R: PAGES 7 AND 12 - The rate of serious AE was estimated for all interventions used to control group (including placebo).

6) I^2 statistic for Serotype-specific efficacy analysis range from 36% to 96%, comment about consequences of these high level of heterogeneity in the analyses.

R: FIGURES 2 AND 3 - I^2 statistic for serotype-specific efficacy analysis was no range. It was 0% in each of the four analyses. However, this ranging refers to I^2 statistic for serotype-specific immunogenicity. We have included a statement about this issue in both results and discussion headings (PAGES 11 and 14).

7) Some comment is also applicable to Serotype-specific efficacy analysis. Are fixed effects model still appropriate for analyses with high I². R: In the discussion (PAGE 14) we have rephrased the statement on efficacy of vaccine. We have included comments addressed to serotype-specific efficacy of CYD-TDV outcomes (below):

Serotype-specific efficacy of vaccine ranged from 34% (DENV-2) to 77% (DENV-4). Satisfactory efficacy against DENV-1 and DENV-2 serotypes of dengue virus was not observed. In the two largest studies (Asian and Central/South American trials) all four dengue serotypes contributed to the overall efficacy during the active phase and although immunogenicity had have higher for DENV-2 this serotype was the one against which CYD-TDV had the lowest protective effect (34% - 95% CI 0.66 [0.50-0.86]). The weak efficacy of the DENV-2 component of CYD-TDV may be explained by the phenomenon of viral diversity [40]. Viral diversity represents the geographic variants of same viruses, being characterized by the presence of structural or genetic variation. This heterogeneity may compromise the DENV optimal recognition by the antibody [40].

8) Some indexes and information presented in Figure 2, and 3 without explaining them in the stats methods or results. If this information added more to what has been presented in the manuscript text please explain them in more details otherwise omit theme. Currently the presented information in Figure 2 and 3 is not self-explanatory.

R: We have clarified the statement on analysis (PAGE 8). We have better explained information about chi-square test (Chi^2) and *z* test (below):

A meta-analysis was performed when the same outcome was assessed in at least two RCTs. Chi^2 assessed whether observed differences in results are compatible with chance alone. A low *p* value provides evidence of heterogeneity of intervention effects (variation in effect estimates beyond chance between both groups). The *z* test refers to the interventions summary effect in a meta-analysis and value equal to zero indicates there is no effect (or no effect on average in a random-effects meta-analysis).

VERSION 2 – REVIEW

REVIEWER	Ryan Maves Naval Medical Center, San Diego, California, USA; Uniformed Services University, Bethesda, Maryland, USA
REVIEW RETURNED	23-Aug-2018

GENERAL COMMENTS	I again thank the editors and authors for the opportunity to review this revised manuscript. My specific concerns regarding the published safety of CYD-TDV in dengue-naïve children and the GAVCS findings have been addressed. I appreciate that the authors will use a professional translation service to resolve any remaining grammar and stylistic issues.

REVIEWER	Moffat Malisheni Ministry of Health, Zambia
REVIEW RETURNED	12-Aug-2018

GENERAL COMMENTS	Consistency is required throughout the manuscript.

	- The reviewer also provided a marked copy with additional comments. Please contact the publisher for full details.

REVIEWER	Mohammadreza Mohebbi Deakin University, Australia
REVIEW RETURNED	21-Aug-2018

GENERAL COMMENTS	My raised issues have been addressed properly, I had a look and other reviewers' and editorial comments and they also seemed to addressed accordingly. Written English still can be improved.

VERSION 2 – AUTHOR RESPONSE

Reviewer: 1 - I again thank the editors and authors for the opportunity to review this revised manuscript. My specific concerns regarding the published safety of CYD-TDV in dengue-naïve children and the GAVCS findings have been addressed. I appreciate that the authors will use a professional translation service to resolve any remaining grammar and stylistic issues.

Answer: We revised the entire manuscript for british english language and stylistic issues.

Reviewer: 2

Reviewer Name: Moffat Malisheni - Consistency is required throughout the manuscript.

Answer: We revised the entire manuscript for consistency throughout the document.

Reviewer: 3 - My raised issues have been addressed properly, I had a look and other reviewers' and editorial comments and they also seemed to addressed accordingly. Written English still can be improved.

Answer: We revised the entire manuscript for british english language. We have corrected wrong issues about that.