Author's response to reviews

Title: Parvovirus B19 infection and severe anaemia in Kenyan children: a retrospective case control study

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Author's response to reviews:

Point by point responses to reviewers:

Reviewer Giogio Gallinella

Minor essential revisions

1. Background section text amended as suggested

2. It is not possible to include information on what B19 genotypes were detected as sequencing was not performed on the PCR products

3. Information on the serological status of PCR+ patients has been added in table 4.

4. Suggested tables have been included - (tables 2 and 3). A graph illustrating the IgG data in table 1 has been included as figure 2.

Reviewer Thomas Tolfvenstam

Minor essential revisions

1. The error bars in figure 1 indicate the 95% confidence interval. This has been stated in the figure legend.

2. All the samples were not tested by PCR because of equipment failure during the study. This is stated in the methods section.

Reviewer Giogio Bedogni

Minor essential revisions
1. Spelling corrected in text. McNemar test was used as the study tested matched pairs.

2. It is correct that the main conclusion of the report is that the highest OD values in B19 IgM testing were found among the cases. Specifically there were 7 of these among the cases and 0 among the controls. I have been advised that because the number of controls in this instance is 0, the odds ratio and 95% CI are not able to be estimated, and so only the p value is included.

3. The samples tested in this study were archival, having been collected previously from children being admitted to Kilifi district hospital. Most of these children had microscopy of a blood sample done for malaria parasites (all species) on admission. These results were then used for our analysis.

4. Results from our previous study is now included at the beginning of the discussion.

5. The interval around the point estimate in figure 1 is the 95% confidence interval. This is now stated in the caption.

Reviewer Claudio Lunardi

Discretionary revisions

1. The initial intention was for all samples to be matched and tested for both IgM and IgG. There are 2 major reasons why this did not happen. Firstly one of the 96 well kits for testing IgG gave invalid results, and there were no spare kits available. Thus some of the matched samples did not get a valid IgG result. Secondly, the identity of the samples to be tested was generated by KEMRI IT staff from a database of admissions, with the team doing the lab testing blinded to the case or control status of the sample numbers generated. Unfortunately, on numerous occasions the selected sample would be missing from the freezer (probably taken elsewhere for another study or already used) These missing samples meant that some samples were tested that did not have a corresponding match.

2. A large proportion of the children 0-6 months with B19 IgG are likely to have maternal IgG, which would disappear by 6 months of age. Looking at the children over 6 months, the prevalence of B19 IgG does generally increase with age.

3. An extra row has been added to table 1 to show how many children were analysed in total. The same sera were tested for B19 IgM and IgG at the same time, and this has been stated in the text. The IgG prevalence in the matched pairs is 14.2% and in all tested samples is 14.6% (see table 1) which is similar.

Minor point - KDH has been changed to Kilifi district hospital in the text.