Author's response to reviews

Title: Identification of Congenital Rubella Syndrome in Sudan

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Author's response to reviews: see over
Dr. Philippa Harris
Executive Editor
BMC Infectious Diseases

MS: 1173958124112553

Dear Dr. Harris,

Please find enclosed our revised manuscript entitled

**Identification of Congenital Rubella Syndrome in Sudan**

for publication as a research article in BMC Infectious Diseases.

We have carefully addressed each one of the reviewers’ comments and have made the revisions accordingly.

Our detailed replies to the reviewers’ comments are shown below. We hope that you will find the revised manuscript suitable for publication.

Yours sincerely,

Prof. Dr. Claude P. Muller
Authors’ Reply to Reviewer Pawel Stefanoff

Comment #1 The article "Identification of Congenital Rubella Syndrome in Sudan" is a concise, well-written description of a survey of children with birth effects admitted to several hospitals in Sudan. The manuscript is suitable and interesting for the international audience because efforts to eliminate rubella and rubella syndrome need to be concerted across all continents. The main weakness of the manuscript is the methods section that do not contain several important details of the study.

Reply from authors: We have revised the Methods Section according to the detailed comments of the reviewer (please see below).

Comment #2 Also, the discussion section should be revised, as often it deviates from strict interpretation of obtained results, and sometimes embarks on unjustified generalizations.

Reply from authors: We have revised the Discussion according to the comments of the reviewer (please see below).

Comment #3 Below are some suggestions on possible improvements to the manuscript:

Major Compulsory Revisions:

1) The aim of the study is stated at the end of the Background. It is clear and straightforward. However, it is important to add the purpose of it, why the authors conducted the research; Was it done just to know how many cases occurred? Or was it done to inform policies, target interventions to prevent further CRS cases?

Reply from authors: We agree with the reviewer and have now added that the data may serve public health authorities as basis for the design of appropriate CRS prevention strategies.

We have revised the text as follows (lines 68-71):

“The present study aimed to identify CRS cases among Sudanese infants presented at different hospitals in Khartoum to obtain more information about the CRS situation in Sudan. These data may help public health authorities to design appropriate CRS prevention strategies.”

Comment #4 2) Methods: it is not clear how the authors selected the hospital units and how were suspected cases recruited; They should thoroughly describe the sampling procedure (the selection of hospitals, selection of children in the hospitals, was the sample size estimated for this study?)

Reply from authors: Based on the reviewer’s suggestions, we have added two additional paragraphs at the beginning of the Methods Section to describe the study setting and participant recruitment. No sample size estimation was done, but at least in the eye hospitals and the echocardiography unit, where 98% of the study participants were recruited, all infants matching
the inclusion criteria and presenting during the 7-months study period were included. For the paediatric hospitals (where only 2% of the participants were recruited) the research team was called by hospital staff as soon as patients matching the inclusion criteria were presented.

We have added the following information (lines 74-93):

“Study settings
This cross-sectional study was conducted between February and September 2010 to identify CRS cases among infants presented to five hospitals in Khartoum, Sudan. Khartoum with an estimated 5 million residents in 2008 [10] is considered to be a major centre for medical facilities, also attracting patients from across the country. The hospitals selected for the present study (two ophthalmology hospitals, two paediatrics hospitals and a paediatrics echocardiography unit) are major specialised hospitals that provide paediatric services for a large number of Sudanese children.

Study participants
During the study period a total of 98 infants aged up to 12 months presenting either with congenital eye defects, heart defects or both as identified by qualified physicians were included in the study. At the ophthalmology hospitals and the echocardiography unit all infants matching the inclusion criteria and presenting during the 7-months study period were included by members of the study team. For the paediatrics hospitals the research team was called upon by hospital staff when patients matching the inclusion criteria were presented. Clinical symptoms compatible with CRS detected during medical examination, extracted from the infants’ medical records or described by their parents were recorded. Based on clinical findings and laboratory results the infants were classified according to WHO case definitions [11] in suspected, clinically-confirmed and laboratory-confirmed CRS cases and congenital rubella infections (Table 1).”

Comment #5 3) Methods: the authors should justify the representativeness of the selected sample (what proportion of Sudanese hospitals was included in the sample frame and how well they represent the country? how the authors did assure that all children with birth defects were screened - did they systematically screen the hospital records? did they maintain logs of all children with birth defects that were subsequently ascertained for inclusion criteria?)

Reply from authors: We involved five major specialized paediatric hospitals in Khartoum in the patient recruitment. While we do not know what proportion of specialized paediatric hospitals they represent in Sudan, we believe that our results are a good approximation of the overall situation in the country since Khartoum is the capital and with about 5 million inhabitants by far the largest agglomeration in this country of 35 million inhabitants. These hospitals attract many patients also from other Sudanese States due to its specialized medical services. In the eye hospitals and the echocardiography unit, where 98% of the study participants were recruited, all infants matching the inclusion criteria and presenting during the 7-months study period were included. For the paediatric hospitals (where only 2% of the participants were recruited) the research team was called upon by hospital staff as soon as patients matching the inclusion criteria were presented. We did not systematically screen the hospital records, but maintained logs of all children with birth defects compatible with CRS. The text has been revised as shown above for comment 4.
Comment #6 4) Methods: Who checked the inclusion criteria? Were there trained collaborators in each participating hospital who saw each suspect child and verified which infant meets the criteria for WHO suspect or clinically confirmed case?

Reply from authors: The inclusion criteria were checked by a qualified physician who did the clinical examination according to the specialty of the respective hospital and at least one member of the research team. It was also the physician who had examined the infant who provided the research team with the clinical findings and/or the medical history related to CRS.

The information has now been included in the text as follows (lines 83-88):

"During the study period a total of 98 infants aged up to 12 months presenting either with congenital eye defects, heart defects or both as identified by qualified physicians were included in the study. At the ophthalmology hospitals and the echocardiography unit all infants matching the inclusion criteria and presenting during the 7-months study period were included by members of the study team. For the paediatrics hospitals the research team was called upon by hospital staff when patients matching the inclusion criteria were presented."

Comment #7 5) Methods: The data collection procedures need to be described in more detail: Did the authors use a questionnaire or did they extract data from medical histories (using predefined data extraction form)?. Did they collect any data from parents (for example on rash or vaccination status)? If yes, did they pilot test the questionnaire? Did they collect the data personally or using trained interviewers (or maybe mail or telephone interviews)? How was data confidentiality assured (were the questionnaires anymized)?

Reply from authors: We have added some information about the data collection process as requested by the reviewer. The questionnaire was not pilot tested, but the clinical data was recorded by a qualified physician and the other information by members of the research team. Access to the completed questionnaires was restricted to research team members.

We have added the following information (lines 103-108):

"A questionnaire including age, gender and place of residence of the infant, clinical signs, age of the mother and maternal history of rash and rubella vaccination was completed for each participating infant. While the clinical data were recorded by the physicians, other data were gathered by the research team from the mother. Access to the completed questionnaires was restricted to members of the research team only."

Comment #8 6) Results: The authors should use more explicitly the WHO classification: which were laboratory confirmed cases, which clinically confirmed and which clinically suspected. It can be deduced from the description, but nevertheless the terms should not be used interchangeably with information such as "[...] were positive for IgM" (line 107). It would be useful to update the Table 1 with the WHO classification of each case. Ideally, particular columns of this table could refer to WHO criteria.
Reply from authors: As suggested by the reviewer we checked the Results Section and included the appropriate WHO classification to describe the cases. The Table includes now the final case classification. The columns describing the clinical symptoms and the laboratory results as used for the case classification are also shown.

Comment #9 7) Discussion: The paragraph included in lines 116-126 is slightly confusing. It would be much clearer if the authors do not start from a general "encyclopaedic" statement about the need for careful evaluation of serologic results. This important interpretation should start from the results followed by sequential argumentation, for example: "XX children were diagnosed by detecting persisting IgG antibodies in absence of IgM antibodies. These antibodies could be persisting maternal antibodies. However, presence of symptoms compatible with CRS and the unvaccinated status of mothers lead to ascertaining these cases as probable CRS cases" or something like that.

Reply from authors: We have revised the paragraph as suggested by the reviewer (lines 158-165):

"Another five infants ≥ 6 months were positive for rubella IgG antibodies and considered potential CRS cases because routine rubella vaccination is not yet practiced in Sudan and postnatal rubella infections seem to be uncommon among infants less than one year of age [14]. In addition, none of the mothers was vaccinated and all infants had clinical signs compatible with CRS. However, it is possible that maternal antibodies acquired after rubella infection still persist in 6–12 months old infants [19]. This may be especially the case in the four 6 months old children in our study (case 3 till case 6), while it is less likely for the 12 months old child (case 7)."

Comment #10 8) Discussion line 134: The authors did not confirm the presence of CRS in Sudan, as it was already confirmed in the previous study. Furthermore, if there is massive circulation of rubella, it is not possible that there is no CRS. I would therefore suggest a subtle modification of the study interpretations (not to confirm the presence of disease, but rather document the burden of disease; This is one of the reasons why documentation of the study representativeness is so important!

Reply from authors: We have changed the wording by stating that we have again documented the presence of CRS in Sudan (line 171):

"The current report again documented the presence of CRS in Sudan."

Comment #11 9) The discussion section should less concentrate on the justification of obtained numbers (why the frequency of CRS was low), and more on the public health aspect of this problem. CRS constitutes a huge burden to societies and is vaccine preventable. The socio-economic consequences of CRS should be emphasized!
Reply from authors: In both the Discussion and the Conclusions we emphasize the need to establish active CRS surveillance as a basis for decisions about rubella vaccine introduction. In addition, we have added some sentences at the beginning of the Discussion to emphasize the current lack of information about CRS and the need for more comprehensive data for public health decision makers. We also added some statements about costs of CRS treatment and rubella vaccination.

The following sentences have been added (lines 153-155):
“In many developing countries, the burden of CRS is under-estimated [2]. Also Sudan lacks robust information about the burden of CRS, although this information is important for the decision to introduce rubella-containing vaccine in the national immunization program.”

The text has been modified as follows (lines 191-202):
“With the current study, we further emphasized CRS as a public health burden in Sudan. However, this study was subject to some limitations including the short participant recruitment period, the limited number of participating hospitals and the lack of clinical examination of hearing deficits, another common symptom of CRS. Despite these limitations, our results together with the findings of other recent studies from Sudan highlight the need to establish active CRS surveillance to obtain a better understanding of the burden of disease and to decide about the cost-benefit of introducing rubella vaccination. Rubella vaccination has been shown to be cost-effective [5], while the treatment of CRS even in poor countries is very costly. The WHO recommends that all countries that have not yet introduced rubella vaccine should consider its inclusion in their national immunization programme [3]. As Sudan has achieved a measles vaccine coverage of >80% during the past few years, the country meets the WHO criteria for introducing rubella vaccine [5].”

Comment #12 10) Discussion lines 136-137: This does not have anything to do with this study results. It can be mentioned as the recommended next step, but cannot be used in the interpretation of the obtained results.

Reply from authors: We have removed the sentence.

Comment #13 11) The discussion should end with a comprehensive paragraph listing the study limitations. The authors already described the limitations of the specimen collection and laboratory investigations. However, the limitations of the epidemiological study should be also listed and interpreted. Each epidemiological study has limitation, typically related to sampling, sensitivity and specificity of case definitions, data quality, etc.

Reply from authors: We have added an additional sentence mentioning limitations of the study related to the epidemiological part.

The following sentence has been added (lines 192-194):
“However, this study was subject to some limitations including the short participant recruitment period, the limited number of participating hospitals and the lack of clinical examination of hearing deficits, another common symptom of CRS.”
Comment #14 12) The references list formatting is incorrect. For example, the journal names should comply with the authors guidelines (e.g. ref. 7 includes the name "Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases"; According to the journal style, the correct abbreviation is "Clin Microbiol Infect". All references should be double-checked.

Reply from authors: We have checked all references and corrected them as necessary.

Comment #15 Minor Essential Revisions:
13) Methods: It would be useful to summarize the case classification used in the study for example in a text box, referring to the WHO materials; Not all readers may be familiar with this classification.

Reply from authors: As suggested we have included the case definitions in the format of a text box (Table 1).

Comment #16 14) Discussion lines 139-141: This statement is unjustified. The authors mention that the study was performed in many different provinces (possibly all country). Thus, the result of 2% laboratory confirmed clinical suspicions do not pertain to Khartoum region, but to the whole country.

Reply from authors: We have removed that statement.

Comment #17 15) Discussion: It would be useful if authors would discuss the potential impact of their results to the current situation in Sudan. For example if they knew that their sample represented 10% of hospitalized children during the study period, than the frequency of CRS could be such and such, leading to such and such socio-economic consequences...

Reply from authors: As mentioned above we have selected 5 major specialized hospitals receiving large numbers of Sudanese infants and at least for 3 of these hospitals (98% of the participants) we have recruited all patients matching the inclusion criteria. Nevertheless, we do not know what percentage of infants with clinical symptoms compatible with CRS was recruited by us and how many children were missed or presented to different hospitals. That is why we discuss our results in combination with other recent studies from Sudan.

The text has been changed as follows (lines 194-202): “Despite these limitations, our results together with the findings of other recent studies from Sudan highlight the need to establish active CRS surveillance to obtain a better understanding of the burden of disease and to decide about the cost-benefit of introducing rubella vaccination. Rubella vaccination has been shown to be cost-effective [5], while the treatment of CRS even in poor countries is very costly. The WHO recommends that all countries that have not yet introduced rubella vaccine should consider its inclusion in their national immunization programme [3]. As Sudan has achieved a measles
vaccine coverage of >80% during the past few years, the country meets the WHO criteria for introducing rubella vaccine [5]."

Comment #18 Discretionary Revisions:
16) Style: the authors should avoid excessive use of the words that contain no information, like "moreover", "nevertheless" or "importantly". They are not needed if the scientific argumentation is provided in a clear and sequential manner.

Reply from authors: We have checked the complete text and removed these words whenever possible.

Authors’ Reply to Reviewer Pat Tookey

Comment #1 Thank you for the opportunity to read this paper. Congenital rubella remains an important cause of death and disability in many parts of the world, including many countries in Africa. Ascertaining the burden of infection in Sudan in order to inform vaccine strategy and policy is extremely valuable, particularly in the context of the high seronegativity rate reported in ref 9. However, I think the study methods need some clarification, both with respect to the study population and the laboratory algorithm and methods.

Reply from authors: We have modified the text according to the detailed comments below.

Comment #2 Major Compulsory Revisions
1. It is unclear how the 98 infants studied were recruited and how representative they were of infants with clinically confirmed or suspected congenital infection in the hospitals selected. Was case classification according to the WHO guidelines carried out according to a specific protocols in each hospital, and how many hospitals were involved? Did many parents decline?

Reply from authors: We have added several paragraphs and sentences to the Methods section to better explain how the infants were recruited, how the case classification was done and how many hospitals were involved. None of the parents declined to participate in the study.

The following text parts address the reviewer’s questions (lines 74- 93):

“Study settings
This cross-sectional study was conducted between February and September 2010 to identify CRS cases among infants presented to five hospitals in Khartoum, Sudan. Khartoum with an estimated 5 million residents in 2008 [10] is considered to be a major centre for medical facilities, also attracting patients from across the country. The hospitals selected for the present study (two ophthalmology hospitals, two paediatrics hospitals and a paediatrics echocardiography unit) are major specialised hospitals that provide paediatric services for a large number of Sudanese children."
**Study participants**

During the study period a total of 98 infants aged up to 12 months presenting either with congenital eye defects, heart defects or both as identified by qualified physicians were included in the study. At the ophthalmology hospitals and the echocardiography unit all infants matching the inclusion criteria and presenting during the 7-months study period were included by members of the study team. For the paediatrics hospitals the research team was called upon by hospital staff when patients matching the inclusion criteria were presented. Clinical symptoms compatible with CRS detected during medical examination, extracted from the infants’ medical records or described by their parents were recorded. Based on clinical findings and laboratory results the infants were classified according to WHO case definitions [11] in suspected, clinically-confirmed and laboratory-confirmed CRS cases and congenital rubella infections (Table 1).”

Comment #3 2. Could you clarify the algorithm for the laboratory tests carried out on the OF and DBS samples? How many samples were available for each test? Are you sure that the sensitivity and specificity of the tests was the same in your lab as in ref 21. Can you provide more information about the sensitivity and specificity of the tests?

Reply from authors: We have now explained how many and which kind of samples were tested for what. Concerning the sensitivity and specificity of the tests, we have added the respective values provided in the manufacturer protocols. We do not have any indication that the kits performed less well in our hands than in previous studies or other labs.

The text was modified as follows (lines 110-120):

“The laboratory confirmation of CRS cases was based on the detection of rubella IgM antibodies. Both OF and DBS samples of all 98 participating infants were investigated for specific IgM antibodies using the Microimmune Rubella IgM capture EIA kit (Microimmune Limited, UK) and the Anti-rubella Virus IgM ELISA kit (Siemens, Germany), respectively. According to the manufacturers these tests have a specificity and sensitivity of at least 96.9%. The DBS samples of the 49 infants aged ≥ 6 months were also screened for rubella IgG antibodies using the Anti-rubella Virus IgG ELISA kit (Siemens, Germany; specificity 98.5% and sensitivity 100% according to the manufacturer) since the persistence of rubella IgG antibodies in infants beyond that age may be suggestive of CRS [14-16]. From five of the children who were IgM and/or IgG positive, enough OF was left for RNA extraction and reverse transcription PCR using previously published primers [17, 18] and to attempt virus isolation [13].”

Comment #4 3. You do comment on the limitations of your study, and on the possibility that testing for other pathogens could be helpful. But isn't it surprising that so few cases were laboratory confirmed, especially among those with confirmed CRS according to the WHO clinical criteria? Could you comment further on this?
Reply from authors: We agree with the reviewer that a low number of clinically-confirmed cases were laboratory confirmed. Besides the involvement of other pathogens, we can only speculate that maybe also the clinical findings were not completely reliable, but the infants were examined by qualified physicians according to their specialty and that of the hospital. In addition, the type of clinical material used, the sample quality or collection may have been suboptimal (lines 186-188).

Comment #5 Discretionary revisions
4. Information on the distribution of defects and age at presentation in the 24% clinically confirmed and 76% clinically suspected would be informative. Maybe a table? Would these infants have had investigations for hearing loss since they were being considered as possible CRS cases?

Reply from authors: In addition to the overall cohort information about age and clinical defects, we have now added the requested data also for the two different groups of infants. No, unfortunately no tests for hearing deficits, which is another common presentation of CRS, were done. We have mentioned this study limitation in the text.

The following information has been added:
(lines 131-135)  “At presentation, 24.5% of the infants were classified as clinically-confirmed CRS cases (median age 4 months; 20 cases had congenital eye defects and 11 infants congenital heart defects) and 75.5% as suspected CRS cases (median age 6 months; 74 cases with congenital eye defects, none had congenital heart defects) according to the WHO case definitions [11]).”
(lines 192-194) “However, this study was subject to some limitations including the short participant recruitment period, the limited number of participating hospitals and the lack of clinical examination of hearing deficits, another common symptom of CRS.”