Author's response to reviews

**Title:** Community-acquired diarrhea among children and adults in urban setting in Senegal: clinical, epidemiological and microbiological aspects

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**Author's response to reviews:** see over
Reviewer's report

Title: Community-acquired diarrhea among children and adults in urban setting in Senegal: clinical, epidemiological and microbiological aspects

Version: 1 Date: 27 May 2013

Reviewer: Ralf Krumkamp

Reviewer's report:

Major Compulsory Revisions

1. With your study design you cannot show which pathogen is the cause for childhood diarrhoea. You would need a healthy control group to compare asymptomatic infections. Please remove this statement from your paper (abstract, results and discussion).

We take in account your comment and delete any sentence related to causality in our article.

2. You highlight that you compare children and adults, however in your analysis you compare <5 with >5 years. Adults are defined as 19+ (MeSH definition). In case you like to make statements for adults, you have to use this age stratum as well. In general it seems advisable to use more age-strata, because infections and symptoms differ substantially amongst age clusters.

According to your remark and in order to compare with other published data, we analyzed our data according to three age-groups: younger than 5 years, from 6 to 15 years old, and older than 15 years.

3. Methods: How did you select study participants? Were this all patients with diarrhoea, a convenience or random sample?

The recruitment of the study participants was done at outpatient departments of eight of the main health centers of Dakar region. A prospective enrollment was done every morning from 8 to 12:00am, 4 days a week. All patients with diarrhea were asked for study participation. Their participation was voluntary, so no randomization was done among these selected patients. We are aware that our sample was not representative, but the principal objective of the study was to determine the etiology of diarrhea.

4. Methods: It seems that you have missing data, please highlight how you tackled this in the analyses.

We are aware of the limits of our study. We analyzed only data from the study sample.

5. Lab methods: Please define unusual bacteria and describe how you interpreted these results for your study more clearly. Such bacteria and E. coli tend to be gut flora – you would need healthy controls to see whether they are pathogenic.

A definition of unusual bacteria was introduced in Lab methods section p5 lines 97-100.

6. Lab methods: Were the stool specimens filtered/ concentrated? Not clear weather you applied two methods – please describe more clearly.

The stool specimens were concentrated and description of the methods was presented in Lab methods section p5 lines 97-100.
7. Statistical methods: You compare groups; please describe how the groups are established – what is the denominator (e.g. all patients with particular infection against those without this infection)? In case you do comparisons (e.g. virus vs. no virus) please always highlight your comparison group.

We compared different sub-groups: patients with confirmed viral infection against those without confirmed viral infection (independently of other confirmed bacterial or parasitic infection). We did the same with bacterial and parasitic infection. Thus, we added a definition of confirmed infection in the Methods section, Statistical methods p6 lines 133-144.

8. Never show only the p-value, this measure alone has less meaning. If you compare two groups always show the effect. This is much more relevant as a null-hypotheses test.

We made the modifications in the text.

9. Co-infections: Co-infections are described. Would it be interesting to compare observed proportions (pa&b) and expected proportions (pa*pb) to see whether some combined infection are more frequently observed.

We calculated the expected proportion of co-infections which was superior to the observed proportion.

10. Results p 8: Patients with viral infections: 94.9%+50.7%=145.6%?!

11. Results p 8: Don’t understand the comparison in the next sentence either: “Among patients less than …”

In order to explain the comparison, we added another table.

12. Results last paragraph: (i) Why not together with the other co-infection results? (ii) Don’t understand comparison: Co-infections more frequent than single bacterial infection? I doubt this. (iii) I don’t understand how co-infections were compared with clinical and epi characteristics – please present more details.

As we didn’t find any significant association with co-infections, the last paragraph of Results section was removed from the article.

13. Discussion p 9: “For all these patients, the origin …”, this statement cannot be made from your study, because you did not investigated this study question. You can highlight that other studies found an association.

According to the information given by the patients with *Schistosoma mansoni* infection, we just mentioned a hypothesis of the origin of their contamination. So we modified the sentence: “For these patients who reported a previous stay in the North of Senegal, the origin of the contamination was probably linked to their occupation (farmers) in the Senegal River Basin, which is known to be an endemic area for bilharzias [17, ], but no further investigation was carried out to confirm this hypothesis”.

14. Discussion p 9: “In Dakar and its suburb, the rainy season …”, please provide citation.

A reference was added in the article (Discussion section, p9)

15. Discussion p 10: “Our study also confirmed that rotavirus and other viruses …”, as mentioned above, this statement cannot be made with your study design.

We changed the verb “to confirm” by “to suggest”

16. Discussion p 10: “The prevalence in our patients less then 5 [sic] years …”, please show the prevalence from the cited studies.
Prevalences from the cited studies are now mentioned in the text. Discussion section p line 9.

17. Discussion p 10: “Although this 22-month prospective study…”, is a very strong statement. For doing so you should compare your study with others systematically. I would remove this statement, especially because you did not use a control group, which other studies did.
   We removed the beginning of the sentence “Although this 22-month prospective study…” and replace it by “Our study has several limitations, including the study design, lack of random sample and limited sample size.” p 10 line 221-222.

18. Discussion p 10: Limited sample size is not the reason why you did not find association between risk factors. This is due to the study design.
   We modified the paragraph p 10 lines 223-228 in order to discuss the limitations of our study by relevance order.

19. Discussion p 10: “… 19% of patient were excluded …”, again results shown in the discussion, which are not presented in the results section.
   According to the comment, we added the following sentence “Among the eligible study participants, 44 patients (19%) were excluded because they had reported previous intake of antibiotic or antiparasitic drugs” in the Results section p 6, line 128-130.

Minor Essential Revisions

20. Please stick to the correct tenses throughout the article sections. Please revise your English (e.g. proper use of “respectively”).
   We took in account the comment of the reviewer and made the modification in the text.

21. Abstract, Results: Why are the organisms shown within the brackets? Are they the most frequent ones? What is the denominator for the %?
   The organisms shown within the brackets in the abstract are the main species detected (e.g. among all parasitic identified, Ascaris lumbricoides was the most frequently identified (38/115, 33%). We changed the sentences in the text.

22. Always provide both frequency and the corresponding per cent.
23. Number below twelve should be written using letters.
24. In case you like to report p-values, you don’t have to show more precision than “<0.001”, smaller values have no practical relevance.
25. Please check writing of the organisms (italic, sometimes with sp. or spp or spp. and sometimes without spp. – please harmonise)
26. Abstract, discussion: You do not calculate the prevalence, just the proportion of infections in your group.
   We took in account all the previous comments of the reviewer and made the modifications in the text.

27. Please carefully reread the paper you are citing. Reither et al. for instance does include children >5 years in their study.
   We removed this reference.

28. Page 5: Include citation for R software
   A reference was added in the article.
29. Results, p 6: You don’t have to show the sex-ratio if you present the percentages – both measure the same.

30. Results, p 6: Present median age (IQR) as it is left-skewed

31. Please use the words “observed” and “reported” properly. They seem to be used likewise.

We took in account all the previous comments of the reviewer and made the modifications in the text.

32. Results: Try to avoid interpretation of data in the results section (e.g. blood in adults stool). Interpretation should be done along with further data in the discussion section (e.g. considering specimen distribution within age-groups as well).

We removed this sentence from the results section.

33. Results p 6: “Presence of other diarrhoea cases was …”, do you mean “other cases in the family” or do you compare the two age-groups?

According to the comments of the reviewer and because of the study design, we decided to delete all the sentences referring to data about potential exposure and risk factors.

34. Results p 7-8: Only present numbers in brackets, not parts of the sentences, e.g. better write (40.8% vs. 28.4%, p=0.07).

35. Discussion p 8: “… isolated from 81% …”, not 80%?

We took in account all the previous comments of the reviewer and made the modifications in the text

36. Discussion p 9: Seasonality of roundworm only presented in discussion, yet not in results.

37. Figure 2 is not cited in the results

38. Figure 2 description: “detected” instead of “isolated”, because you used PCR and microscopy as well.

Finally, we decided to remove the figure 2 because this figure doesn’t add any additional value.

Discretionary Revisions

39. Background: change “source of poverty” to “socio-economic status”

The sentence was changed to give more information about the context and the rational of the study (Background section p 3, line 55-59)

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Needs some language corrections before being published

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:

I declare that I have no competing interests.
Reviewer’s report

*Title:* Community-acquired diarrhea among children and adults in urban setting in Senegal: clinical, epidemiological and microbiological aspects

*Version:* 1 *Date:* 10 June 2013

*Reviewer:* Estelle Kanyala

**Reviewer’s report:**
The manuscript describes clinical, epidemiological and microbiological aspects of diarrhea in the urban region of Dakar and two suburban areas close to Dakar.

I. **Major concerns**
The authors acquired interesting data and performed detailed analysis of the data, which is worthwhile to be published, but the presentation of the data may be considerably improved.
The spelling and grammar of the whole manuscript must be thoroughly reworked. Some parts of the manuscript were copied from the protocol without changing the orthography. Before resubmitting the manuscript must be checked by a native speaker.
The methodology of this study is not clearly described. The authors should restructure the whole section and describe the different procedures in different paragraphs to make it more comprehensible.

II. **Minor comments**
The aim of the study not clearly defined and not consistent with the rest of the manuscript:
In the abstract and in the background section the authors formulate the aim to describe the epidemiology and etiology of community acquired diarrheal infections, in children and adults living in urban settings in Senegal. In the manuscript they describe clinical, epidemiological and microbiological aspects of diarrheal infection in urban and suburban areas. Line numbers should be added to the manuscript

Line numbers were added to the manuscript to facilitate the follow-up of the modifications and corrections

II.1. **Abstract**
The abstract contains several orthographic mistakes (eg. line 2: adult (adults), line 3: setting (settings), line 6: virus (viral), parasites (parasitic), line 7 over 5 (children up to five years of age….older than five years of age)).
We made a careful review of the manuscript and hope that all corrections were done.

II.2. **Methods**
II.2.1. Study area, target population and samples collection
- Paragraph 1
This section is not detailed enough. It needs a clearer structure using subheadings for each technique.
Why did the authors choose specially those areas? What motivated their choice?
Why did they not include a control group without diarrhea?
The choice of the study area was based (i) on relevance of the scientific question about diarrhea and urbanization in Africa and (ii) operational and logistical issues (in particular laboratory issues).

- Paragraph 2
How many subjects were included in the study? What is the sample size?
We decided to conduct a prospective study in to determine the etiology of diarrhea in urban setting. No sample size was estimated.

The authors should describe the criteria more clearly a subject had to meet to be included in study (how, when and where the subjects were recruited). The recruitment of the study participants was done at outpatient departments of eight of the main health centers of Dakar region. A prospective enrollment was done every morning from 8 to 12:00am, 4 days a week. All patients with diarrhea were asked for study participation. Their participation was voluntary, so no randomization was done among these selected patients. We are aware that our sample was not representative, but the principal objective of the study was to determine the etiology of diarrhea.

Clinical examination may be described in more detail. What exactly has been investigated (fever, weight……)? According to the comment, we added the sentences about the clinical data recorded and the questionnaire used during the study.

II.2.2.Laboratory methods
The author should give references for the applied laboratory methods or describe the methods in more detail. Which PCR methods with which primers were used? This section was completed p 5 lines 97-104.

II.3. Results
In Table 1 there is a foot note saying that only 185 samples were tested for enteric viruses. This should be explained in detail in the methods section and clearly stated in the results section.
Page 6, line 4: The number of patients included in the study was 223, so the authors must use 223 to calculate the different percentages.
We took in account all the previous comments of the reviewer and made the modifications in the text.

Page 6, line 4: Author must not include patients which lived outside the study area.
The patients, who were not resident in the study area, were present in the study area during the incubation period.

Page 6, line 8: Dehydration should be defined in the methods section
Page 6, line 19: The questionnaire should be described in the methods section.

Page 7 line 1: I cannot find the described information in Table 1
Page 7 line 7: I cannot find that numbers in Table 2.

Page 7. Line 7: I doubt that Citrobacter, Klebsiella, Enterobacter cloacae are causes of diarrhea. I assume the authors could have isolated much higher numbers of these and related enterobacteria, if they had used corresponding methods. The analysis of the data is significantly hampered by the lack of a control group.
Page 7, line 12: The rates of infections with different helminthes are interesting. But again these helminthes (and Trichomonas and Giardia) are probably not a true cause of diarrhea and again a control group would be desirable.
The different areas may be described in more detail and analyzed separately.
We are aware that we didn’t have a control group. That’s we decided to analyze only bacteria and parasites which are known to be responsible for diarrhea. Thus we added a
Definition of confirmed infection in the Methods section p5 lines 93-96, Statistical methods p5 lines 110-114.

II.4. Discussion
Page 8, line 19 - 21 may be deleted.
Page 8 line 23: The authors should discuss that most of the parasites are probably not a true course of diarrhea.
Page 8, line 24: This age group does not exist in the study. Please consider the real age group (children up to five years of age instead less than 5).
Page 9 line 3: see comment page 8 line 23: The authors should discuss that most of the parasites are probably not a true course of diarrhea.
We took in account all the previous comments of the reviewer and made the modifications in the text.

Page 9 line 8: The authors may discuss reasons for the higher rate of infections of Schistosomiasis in older age groups.
According to the information given by the patients with Schistosoma mansoni infection, we just mentioned a hypothesis of the origin of their contamination (Discussion section p9 lines 204-210)

Page 9 line 22: see comment page 8 line 23. The authors should discuss that most of the bacteria are probably not a true course of diarrhea. They should add that the significance cannot be determined because of the lack of a control group.

Page 10, line 1: The high prevalence of Rota Virus in children (and the low rate in adults) is an interesting finding that should be discussed in more detail. How is the situation in other African countries? May that disease be prevented by a vaccine?
The situation in Senegal is similar to those observed in other African countries where data about Rotavirus infections is available. Currently, Rotavirus vaccines exist, but they are not already included in the national routine immunization program of African countries.

Page 10 line 1: The relevance of the further viruses may also be discussed. To me the comparably low rate of Noro-Virus seems interesting. Did the authors determine the Enteroviruses in more details. Why did they decide to investigate the samples for Enterovirus?
Only detection of Enterovirus was done by RT-PCR; no further molecular analyses (including sub-typing) were conducted until now.

Page 10 line 15 and 16 may be deleted
Page 10 line 26: and children are more prone develop diarrhea.
We took in account all the previous comments of the reviewer and made the modifications in the text.

Level of interest: An article of importance in its field
Quality of written English: Needs some language corrections before being published
Statistical review: No, the manuscript does not need to be seen by a statistician.
Declaration of competing interests:
I declare that I have no competing interests