Author's response to reviews

Title: Intestinal infection with Campylobacter in a rural cohort in Moramanga, Madagascar

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Author's response to reviews:

Madagascar, 12nd November 2013

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To
Philippa Harris
Executive Editor
BMC-series Journals
BioMed Central

MS: 1688584495961192
TITLE Intestinal infection with Campylobacter in a rural cohort in Moramanga, Madagascar

Dear Sir,
Thank you for your correspondence dated October 3rd 2013 about our manuscript referenced above. We were very pleased to read the generally very positive comments of the reviewers. They addressed a number of helpful and insightful remarks and we were able to deal with these without trouble. Besides the responses to the specific points judiciously spotted by the reviewers, we valued to
i) make language corrections, ii) rewrite the study rationale and the different sections of manuscript for an improved organization according to comments of reviewers, iii) consider potential confounders for the assessment of the association between infection and age. All changes made were put in highlight in the manuscript. The paper is now composed by 3677 words, 3 tables, 1 figure and 24 references.

We now feel that we have been able to fully answer all of the reviewer’s comments and hope that you will find the significantly improved manuscript suitable for publication in BMC infectious disease. Below we address the specific comments in turn.

We look forward to hearing from you in due course.

Sincerely yours.

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Confidential Comments to Editors
Reviewer’s Report
Reviewer: Anne E Deckert
Major Compulsory Revisions
1. Methods: Data Analysis: Last paragraph: Please discuss the autocorrelation potentially resulting from taking data from the same individual over time, ie. Samples taken from the same child closer together in time may be more similar than samples taken farther apart in time.

- An autocorrelation from data from the same individual over time may exist. This autocorrelation was already accounted with the individual random effect even if time is not explicitly represented. In addition, children had had the same visit date (twice per week and every two months)

2. Methods: Was the presence of other bacterial or parasitic pathogens investigated in these samples? If not, please justify. If they were, please describe role of co-infections in results and discussion.
- This study has been focused on Campylobacter because the precedent transversal study published in Plos One have showed the role of these bacterial agents. Because of financial constraint for this work we have limited the search to Campylobacter. However, for each children, two faecal samples were collected and kept frozen at -80°C for future biological analysis by PCR (virological, bacteriological and parasitological) to explore the question in relation with co-infections

3. Potential risk factor information was gathered at the time of recruitment (Table 1).

Was the role of these risk factors in primo infection, symptomatic/non-symptomatic primo infection, etc investigated? This would significantly strengthen this manuscript.

- We have already incorporated these potential risk factors in the mixed regression logistic analysis in the assessment of the association between infection, symptomatic infection and age group.

4. It appears that changes in risk factor information over the time period of the study were not collected. Please confirm.

- We collected demographic, socio-economic and household data only at the time of enrolment. Nutritional status data was collected at the time of enrolment, during the twice weekly (if the child had diarrhoea) and the bi-monthly surveillance.

5. Was the isolation rate from diarrheal and non-diarrheal samples different for C. jejuni than C. coli? Was there a difference in the percentage of C.jejuni cases with more than one infection compared to C. coli cases?

- The isolation rate of C.jeuni and C.coli from diarrhoeal and non-diarrhoeal samples did not differ significantly (p=0.16). We found that children infected with C.jejuni were likely to be reinfected than those infected with C.coli (p=0.04): 8.5% of reinfected children had C.coli whereas 25.8% of them had C.jejuni.

6. Discussion: The isolation rate of Campylobacter, and the annual incidence rate of symptom producing infection are compared to Reference 9, please also compare to reference 5 (Calva) and 6 (Rao).

- We have already compared the annual incidence rate of symptomatic infection to the study from Egypt (Rao) and Mexique (Calva). For the isolation rate of Campylobacter, we have only compared with result from Egypt (Rao), because data from Mexique was not available.
7. Discussion: The authors discuss the role of maternal immunity, please also discuss the hypothesis that repeated exposures may be required to achieve immunity and that different strains or overwhelming challenge may overcome immunity.
- We have already discussed about the impact of repeated exposures, different strains and overwhelming challenge in the immunity.
8. Discussion: It is stated that these results bring into question the role of maternal immunity, please elaborate.
- We have already elaborated the discussion about the role of maternal immunity

Minor Essential Revisions

Abstract:
1. Under Background: Second sentence: “A previous study conducted in Madagascar has supported this finding.”
- We made these change
2. Under Background: Fourth sentence: Campylobacter is endemic and seems to lead to …”
- We made these change
3. Under Background: Last sentence: “We conducted this study to obtain evidence for this hypothesis in a rural setting….”
- We made these change
4. Under Results: Indicate the complete time period i.e. “Between January 2010 and May 31 2012, 508 children were included in the cohort.”
- We made these change
5. Under Results: Third sentence: “; the highest isolation rate was found in samples from children aged 6 to 11 months and 12 to 17 months, 13.4% and 15.2% respectively.”
- We made these change
6. Under Results: Last sentence: “… whereas this period was at 137 days for…”
- We made these change

Introduction:
1. Paragraph 2: “The question of symptomless excretion is not clear and may be linked to
convalescents carriers….”
- We rephrased this sentence

2. Paragraph 2: You state that studies are more often cross-sectional but only cohort studies are referenced. Either remove the comment on cross-sectional studies or include some of these cross-sectional studies in the references.
- We removed the word cross-sectional

Methods:
1. Under Surveillance Activities: Fourth sentence: “… according to the Ministry of Health guidelines.”
- We made these change

2. Under Definition of Events: “… symptom-free if Campylobacter was isolated in a diarrhoeal stools or within 5 days after a day with diarrhoea.”
- We made these change

3. Under Definition of Events: “The days at risk include the 3 days period…”
- We made these change

4. Under Microbiological Analysis: Fourth sentence: How many isolates were randomly selected for PCR?
- We selected randomly 271 isolates for PCR

5. Under Data analysis: First paragraph, Last sentence: Khi2 should be changed to chi2.
This change should be made throughout the manuscript
- We made these changes

6. Under Data analysis: Second paragraph, First sentence: “… we assessed the strength of association between Campylobacter infection and age group at the time of recruitment..”
- We made these change

7. Under Data analysis: Third paragraph, First sentence: “..the associations between Campylobacter infections and symptom-producing Campylobacter infection with age group were assessed…”
- We made these change
8. Under Pathogenicity of Campylobacter: Last sentence: “The unit of analysis was each children enrolled....”
- We removed this sentence

9. Ethics
- We made these change

Results
1. First paragraph; First sentence: “and 152 children included after their 28 days of birth age”
- We rephrased this sentence

2. First paragraph; Fourth sentence: “and 68 (13.4%) dropped out of the study before the completing 2-year because “
- We made these change

3. Second paragraph; First sentence: “At the recruitment”
- We changed by “at the time of enrollment”

4. Second paragraph; Second sentence: “By age groups, the prevalence of Campylobacter infection at the recruitment was “
- We made this change

5. Second paragraph; Third sentence: “Campylobacter infection at the time of enrollment differs”
- We made these change

6. Second paragraph; Fourth sentence: “The age-specific odds-ratios for the association between infection at the time of enrollment and age were”
- We made this change

7. Third paragraph: First sentence: “3424 stool tests for Campylobacter pathogen”
- We made this change

8. Third paragraph: Second last sentence: “; 190 (70.1%) were Campylobacter jejuni, 64 (23.6%) were Campylobacter coli”
- We rephrased this sentence

9. Fifth paragraph; First sentence: “fever or to children with a blood or mucus in
the stool, so we administered antimicrobial treatments”
- We rephrased this sentence

10. Sixth paragraph: Second sentence: “, for those having 6-11 months of age and 5.7”
- We rephrased this sentence

11. Seventh paragraph: First sentence: “was 0.05 episodes/children.
- We made this change

12. Seventh paragraph: Third sentence: “the mean age for symptom-producing Campylobacter infection symptom-producing”
- We used the word symptomatic Campylobacter infection instead of symptom-producing Campylobacter infection throughout the manuscript

13. Eighth paragraph: First sentence: Campylobacter was isolated without symptoms in”
- We rephrased this sentence

14. Eighth paragraph: Second sentence: The mean age for symptom-free Campylobacter infection symptom-free”
- We used the word asymptomatic Campylobacter infection instead of symptom-free Campylobacter infection throughout the manuscript

15. Tenth paragraph: First sentence: “a recurrent infection episode occurred more rapidly than in multi-infected children: 25% of the former group had their second infection episode 63 days after the first episode, in the latter group this period was at 317 days “
- We made this change

16. For clarity please re-order the paragraphs in the Results section: Paragraph 1 (At the start of the study) remains unchanged, Paragraph 2 (At the recruitment) becomes paragraph 3, Paragraph 3 (Over the 2-year period) becomes paragraph 2, Paragraph 4 (We observed 475) becomes paragraph 6, Paragraph 5 (Antimicrobial treatments were) – unchanged, Paragraph 6 (During the follow-up) becomes paragraph 4,
Paragraph 7, 8
9 and 10 remain unchanged.
- We re-ordered the paragraphs in the Results section
Discussion:
1. First paragraph: Break second sentence into 2 sentences: “association between age and Campylobacter infection [8,9]. Infection increased up to 6 months of age”
- We made these change
2. Second paragraph: First sentence: “primo-infected children compared to those with multi-infection”
- We made these change
3. Second paragraph: Fourth sentence: “thereafter microbial intestinal colonization seems to play a part in the maturation of the immune”
- We made these change
4. Third paragraph: Fourth sentence: “Results about the pathogenicity of Campylobacter were not consistent in difference studies reported from the developing”
- We made these change
5. Third paragraph: Fifth sentence: “infection may depends on bacterial”
- We made these change
6. Third paragraph: Seventh sentence: “cohort children brings into question about the role”
- We made these change
7. Third paragraph: Ninth sentence: “A previous study conducted in the Central African Republic suggested a protective role of antibodies from mothers. Children who”
- We made these change
Conclusion
1. First sentence: “In this study, we provided evidence of the high”
- We made these change
2. Third sentence: “highlights the need for strengthened awareness in diarrheal disease prevention in infants.”
- We made these change
Table 1:
1. Title: “at the start of the study a 28 month”
- We made these change

Table 3:
1. Title: “with age group at the time of enrollment and during follow-up”
- We made these change

Figure 1:
1. The legend shows that primo infected children have a longer period until recurrence and multi-infected children a shorter period which differs from the text, this would appear to be reversed?
- We have already made the change as it was reversed

2. Discretionary Revisions
1. 

Reviewer's report
Title: Intestinal infection with Campylobacter in a rural cohort in Moramanga, Madagascar
Version: 1 Date: 30 September 2013
Reviewer: Wilfrid van Pelt
Reviewer's report:
General comments
This study aims at assessing whether children frequently infected with Campylobacter early in life develop protective immunity against Campylobacter infection later on. The rationale for this hypothesis is based on two observations from previously conducted research: 1) many Campylobacter infections are asymptomatic among children in developing countries where campylobacteriosis is hyperendemic; 2) as children in these countries become older, their illness-to-infection ratio decreases. The study has been conducted in two areas of the middle-east region of Madagascar.

Although carrying out this study has clearly required a lot of efforts in gathering valuable data on the relationship between Campylobacter infection, symptoms and age in young children, I found it very hard to read and understand. The English standard is very low and requires somewhat extensive editing. Furthermore, the rationale, study design, presentation and interpretation of results are not always reported in an intelligible fashion, making it difficult to identify the methodological and interpretational issues to be amended.

Based on these observations, I have made a number of specific comments listed below for the authors to consider. These comments need to be addressed before deciding on acceptance or rejection of the manuscript.

Major compulsory revisions

English writing
1. Although I have tried to edit the English in my revision, I cannot make an exhaustive list of the many confusing paragraphs, inappropriate wordings, typos and other inaccuracies. I therefore recommend the authors to have their manuscript carefully edited, preferably by a native English speaker. Moreover, the authors should be consistent with the same spelling throughout the manuscript (British or America English, but not a mixture of both).
- We have already edited the manuscript by a native English speaker

Study rationale
2. The rationale of the study is somewhat ambiguous and should be more clearly defined in the abstract and introduction. The authors hypothesize that protective immunity against Campylobacter infection in children may explain why many Campylobacter infections occur asymptotically, and why the proportion of asymptomatic infections increases with age. The most important finding of this study is that the many Campylobacter infections in children living in Madagascar
occur asymptomatically, and that the proportion of asymptomatic infections increase as this child population becomes older. Therefore, other than the indication which we already knew from literature and on which the authors based their hypothesis, the article provides no new data. The authors should be more clear about how their results add to assessing whether the hypothesis of protective immunity against Campylobacter is true and why this hypothesis is relevant to address.

- We accounted these remarks about the study rationale in the abstract and introduction, about how our results add to assessing our hypothesis and why our hypothesis is relevant to address.

3. The authors explain their results entirely with immunity developed over time due to repeated exposures to campylobacters. The study, however, does not account for potential confounders such as age-varying risk behaviors in children. Moreover, the authors have collected a large amount of epidemiologically relevant variables that are not included in any of the analyses, but only presented descriptively in Table 1. I wonder why the authors did not include these variables in the analysis, perhaps as covariates in the regression models? I will further stress this point later in comment #9.

- We have already added these potential confounding variables in the mixed regression models, we found that there was no changes in the results.

Study definitions

4. The authors should be more clear and consequent in definitions and terminology, especially in the abstract and introduction, where the reader has yet to read the methodology section. For instance, in the abstract is not clear how asymptomatic (Campylobacter infection?), symptomatic infection (Campylobacter symptom-producing infection?), primo-infected (first time ever infected, or infection + episode??) and multi-infected children were defined? I urge the authors to not only be clear on the terminology used, but also to use them consistently throughout the manuscript.

- These remarks and suggestions were taken into account

5. Asymptomatic Campylobacter infection was defined as fives symptom-free days after isolation of Campylobacter in the feces. Although this definition excludes pre-symptomatic Campylobacter infection, it does not exclude post-symptomatic infection. The authors are therefore invited to comment on how this might have influenced the results.
We made changes on the definition of asymptomatic and symptomatic 
Campylobacter infection 

Study design 

6. The study design is very difficult to grasp. It is not clear how 
children/homes/parent-child pairs were enrolled. Were they selected from 
population registries? On voluntary basis? After ad-hoc sensitization campaigns? 
etc. Did they need to visit a clinical site or were children visited at their homes? 
- The study was conducted in the Health and Demographic Surveillance Site of 
Moramanga; the 2 villages were the pilot area of the HDSS. An initial census and 
socio-economic interview have already been conducted in the 2 villages. All 
children less than two years living in the 2 villages were eligible for enrolment, 
those whom parents agreed to participate to the study was enrolled. Children 
were visited at their homes during the twice weekly visits, every two months 
parents were asked to bring the child and his fecal specimen in the village health 
care centre (if the village had a health care centre) or in the village office. 
7. How long were children followed? Maximum 36 months, minimum 12 months? 
- The maximum duration of follow-up was 36 months, we did not limit the 
minimum duration of follow-up 
8. The authors seem to have multiple surveillance schemes. A daily surveillance 
performed by mothers on their children experiencing a diarrheal episode, a 
two-weekly surveillance performed by physicians and CHW’s on the children, a 
cross-sectional surveillance of children regardless of symptoms. I would suggest 
to make several paragraphs with their own headings, one for each data collection 
scheme. Each paragraph should discuss how this surveillance scheme 
contributes to reaching the study objective, how (often) data were collected, what 
the outcome/exposure variables were etc. As this is a longitudinal study, authors 
could also make a timeline showing why/when/how different surveillance 
schemes were conducted. 
- We made these changes in the methods section 

Data analysis 

9. The authors have studied the relationship between symptomatic 
Campylobacter infection and age using mixed effects logistic regression models. 
The authors should be aware that the association between symptomatic 
Campylobacter infection and age might be confounded by many other effects 
that are currently not incorporated in their analyses. If the authors really want to 
assess the effect between symptomatic infection and age and consequently the 
possible effect of protective immunity, they should include (at least) variables
such as season of sampling, individual characteristics of the child (other than age) and characteristics of the environment. As far as I can tell, the authors have this information, as they report to have collected it at enrollment of every child (Table 1). Without including these additional characteristics, it is difficult to assess whether protective immunity, rather than mere decreased exposure to Campylobacter, is responsible for the decline in symptomatic infections later in life. If the authors also looked at enteropathogens other than Campylobacter, they could include these in the analysis as these enteropathogens may also cause gastroenteritis.

- We included these potential confounding variables in the analysis. We did not look at enteropathogens other than Campylobacter as we had a financial constraint.

10. The authors added a random effect to account for the dependency in data from children that were repeatedly sampled. This is fine. However, I can see that there are other issues about non-independence of data that are not accounted for by the analysis. For example, I can imagine that in the cohort there were different children related with one another, e.g. children belonging to the same family or living in the same households. This leads to clustering of observations made on these children, as these children are clearly more similar with each other than with the others because of closer genetics (which in turn might influence immunity), more similar food consumption patterns, level of exposure to campylobacters, closer interpersonal contacts, etc. As far as I have understood correctly, the sample design does not account for this issue. If the authors have such information (I think so), then it should be incorporated in the models, perhaps as an additional (hierarchical) random effect. Moreover, what about the cluster effect of the two study regions?

- We have already incorporated the household as random effect in the model, and we found the same results. The model incorporated each children observations, household and villages as random effect. We found respectively for children, villages and household a variance at 0.03, 0.04 and 10^-8

Discussion

11. The authors state that their data showed evidence of the effects of immunity on Campylobacter infection, given the age-related decrease of infections and the shortening period of recurrence of infection in primo- compare to multi-infected children. Based on the analyses performed, I do not think that this conclusion is consistent. The authors are recommended to reconsider this statement, e.g. that
their results provide an indication that the hypothesis regarding protective immunity might hold true for children living in Madagascar.
- We accounted this remark and we rephrased our statement about effects of immunity on Campylobacter infection.

Minor compulsory revisions

Introduction
Page 3, first paragraph: rewrite as "...are different between developed and developing countries."
- We made this change in the introduction, page 3, first paragraph
Page 3, first paragraph: rewrite as "...Campylobacter infection is endemic, and is one of ... from both diarrheic and healthy children [1]" and delete from "'s stools " to "common"
- We made this change in the introduction, page 3, first paragraph
Page 3, first paragraph: rewrite as "...second most commonly isolated enteropathogen after parasites in <5 year-old diarrheic children, showing a prevalence of 9.7%." and delete from "But" to "subjects".
- We made this change in the introduction, page 3, first paragraph except the sentence from “But” to “subjects”. It was retained as we want to mention the absence of association between diarrhoea and infection with Campylobacter in the previous study, whereas in the present study we found an association between diarrhoea and Campylobacter infection in the birth cohort.
Page 3, second paragraph: rewrite as "Campylobacter isolation rates are highest during the first two years of life and appear to..." and delete from "Another" to "age-related"
- We made this change in the introduction, page 3, second paragraph
Page 3, second paragraph: I do not get the meaning of the last statement "Furthermore.... early life". Please be more specific.
- This sentence was removed
Page 4, first paragraph: rewrite as "Asymptomatic shedding of campylobacters may be due to convalescent carriers that are still excreting the bacterium after cessation of diarrheal symptoms". What the authors mean with "healthy children here?". Do you mean children with mounted immunity??
- We removed “healthy children” and also this sentence in this paragraph. We rephrased and put it in the second paragraph of the introduction.
Page 4, first paragraph: please delete from "Therefore" to "appreciated"
- We made this change in the introduction, page 4, first paragraph
Numerous cross-sectional studies have suggested that in children from developing ... immunity in early life ... high rates of asymptomatic Campylobacter infection and for...

To obtain evidence supporting this hypothesis we have ... less than two years ... an area where diarrheal infections are endemic” and delete from "to determine" to "infection".

Methods

Page 4, third paragraph: rewrite as "...in the low-income rural areas of ... Ampitambe, Moramanga, middle-east region ... as they have already been investigated ... in 2008." Please also reference this statement, if possible.

Page 4, third paragraph: please rephrase from "The highest" to "10%"; it is confusing.

Page 4, third paragraph: rewrite as "...inhabitants is mostly..." and delete "In these areas". Are the 1006 households "registered households”? "...lack of basic sanitary ... free-range ... at home; thus, the risk for faecal contamination of the environment is likely to be high.”

Page 5, first paragraph: rewrite as "...followed up in their new homes, whereas those who moved outside the study area went out from the study.". To whom the interview was made? Parents or children themselves? "...or new arrivals of children."

Page 5, second paragraph: rewrite as "From the day of enrollment in the study until 36 months of age, each child was ... recent diarrheal episodes in each of the
children during the prior 3-4 days. This was made by recording daily frequency of bowel movements, consistency of feces and presence of blood or mucus in the feces." Why the range 3-4 days and not an exact limit of days? "...and anthropometric examinations, took a fecal specimen for cultural examination of Campylobacter, and provided ... Ministry of Health ... mothers who annotated the number of bowel movements and consistency of their children's feces from the first..."

- We made these changes. We changed the range of 3-4 days as we asked about recent diarrhoeal episodes since the last visit

Page 5-6: section "Definition of events" would better be presented as bullet points and named just "Definitions".
- We made these change in the definitions

Page 6, third paragraph: rewrite as "Campylobacter infection was defined as being symptomatic if Campylobacter was isolated in a diarrheal stool or 5..."
- We made these change in the third paragraph of definitions

Page 6, fourth paragraph: rewrite as "Campylobacter infection was defined as being asymptomatic when there were..."
- We made these change in the fourth paragraph of definitions.

Page 6, fifth paragraph: "spp." not in italics. "Random sample" without hyphen. Also, it is not clear what do you mean by random sample, please be more specific.
- We removed the hyphen. As we had a financial constraint, we did the species differentiation only in a sample of Campylobacter isolates selected at random.

Page 7, first paragraph: rewrite as "...symptomatic Campylobacter infection ... Chi-squared test..." and "...24 to 29 months" (not 23 to 29 months).

Page 7, second paragraph: rewrite as "If the Chi-squared test...age at enrollment..." and find another wording for "we estimated age odds-ratios" , it makes no sense by itself.
- We made the change in methods section, statistical analysis. We removed the sentence with age odds ratios

Page 7, second paragraph: rewrite as "...was the individual child."
- Change is made

Page 7, please rewrite completely from "During follow-up" to "between children". It is very confusingly written and important information is missing (see comment n. 8).
We rewrite these sentences and we accounted remarks in comment n.8.
Page 7, fifth paragraph: rewrite as "...was performed on newborns enrolled... 28 days from birth." and delete from "The unit" to "of birth".
We made these changes
Page 8, first paragraph: rewrite as "...recurrent episodes of Campylobacter infection in..." Where these infections those asymptomatic or symptomatic? "...Kaplan-Meier ... as the interval between the time of the first episode and that of the second one." Episodes of diarrhea? Please be as specific as possible.
We made the change. Here we assess the recurrence of all Campylobacter infection (symptomatic and asymptomatic). Time to-event was estimated as the interval between the time of the first episode of infection and that of the second one
Results
Page 8, fourth paragraph: rewrite as "...after turning 28 days of age were subsequently ... standard deviation" and give lower range limit in months too. "...of the children at the start of the study are shown in ... (male/female ratio = 0.96)"
These changes were made
Page 8, fifth paragraph: rewrite as "At the time of enrollment ... them were symptomatic and... (30/32) were asymptomatic."
We made these change
Page 9, first paragraph: rewrite as "...at the time of enrollment was 4.6% ... in children aged 12 months or older." " The frequency of (symptomatic???) Campylobacter infection at the time of enrollment differed significantly among age groups (Chi-squared test, p<0.001). The age-specific odds ratios for the association with (symptomatic???) Campylobacter infection and the time of enrollment were 8.6 (95% Confidence Interval [CI]: 3.0-24.1)... for the age groups of 6-11 months ... 18-23 months, respectively."
These changes were made. Campylobacter infection means all infection (symptomatic and asymptomatic)
Page 9, second paragraph: rewrite as "...period, 3424 stool samples were tested for Campylobacter presence. Of these samples, 2965 (87%) were collected from non-diarrheic children during the cross-sectional survey conducted the 2-month interval." "...(319/3424): 8.9% (41/459) in diarrheic samples and 9.4% (278/2965) in non-diarrheic samples." and also please rephrase completely from "The 319" to "Campylobacter infection", I do not understand what do you mean here. "...of
all children. Speciation was performed on 271 out of the 319 Campylobacter isolates: 190 (70.1%) consisted of C. jejuni, 64 (23.6%) of C. coli and 17 (6.3%) of other species. "... from diarrheic and non-diarrheic samples..."
- These changes were made
Page 9, third paragraph: rewrite as "...diarrheal episodes with median ... an annual incidence rate of 0.7 episodes/child ... collect stool samples for ... at least one diarrheal episode during ..."
- We made these changes
Page 10, first paragraph: rewrite as "...under 12 months of age. The annual incidence ... 0.8 episodes/child ... 0.7-0.9 episodes/child ... 0.5 episodes/child ... 0-0.6 episodes/child ..."
- We made these changes
Page 10, second paragraph: rewrite as "... to children with blood or mucus in the feces; thus, antimicrobial treatment was given to 18.5% ... of diarrheal episodes because of fever and 17.8% of them because of blood or mucus in feces (3.1% for blood and 14.8% for mucus in feces). There were..."
- We made these changes
Page 10, third paragraph: rewrite as "...the odds of being infected ... aged between 6 and 18 months ... , the odds ratio was 5 ... for children aged 6-11 months and ... for those aged 12-17 months (Table 3)."
- We made these changes
Page 10, fourth paragraph: rewrite as "...symptomatic Campylobacter infections ... was 0.05 episodes/child. ... duration of symptomatic Campylobacter ... The mean age for symptomatic Campylobacter infection was 13.8 ... standard deviation ... excreted Campylobacter ... Symptomatic Campylobacter infections did ..." Also, where this "Wald-z-statistics test" came from?? It is not mentioned in the Methods. Please be more specific.
- Changes were made and we added detail about Wald-z –statistic test in the methods section, statistical analysis
Page 10, fifth paragraph: rewrite as "...isolated in 201 (39.6%) asymptomatic children. The mean age for asymptomatic Campylobacter infection was ... standard deviation ..."
- We made these changes
Page 11, first paragraph: rewrite as "There was a statistically significant association ... the odds ratio for shedding Campylobacter for the first time ..."
Discussion
Page 11, third paragraph: rewrite as "...this is the first cohort study about child's diarrhea in Madagascar." and rephrase from "Our perspective" to "after".

Page 12, first paragraph: rewrite as "...than those who did..."

Page 15: I do not understand how Table 1 helps meet the study objectives. The Information provided in this table is not used in any formal analysis as far as I can tell.

Page 17, Table 2: please provide incidence rates in addition to the presented at-risk days and number of episodes. This makes it a lot easier to compare the occurrence of Campylobacter between symptomatic and asymptomatic children.

Perhaps you could combine Table 2 and 3.

Throughout the manuscript
"Chi-squared", not "Khi2"
The word "Campylobacter" must always be in italics.
"odds ratios" or odds ratio" without hyphen.
Change the wording "symptom-producing Campylobacter infection" to "symptomatic Campylobacter infection"
"standard deviation" without hyphen.

All the changes suggested should also be made in the abstract, accordingly.

The interquartile range (IQR) is by definition equal to the difference between the
upper and lower quartiles (IQR = Q3 # Q1), so it must be expressed by a single value. If the authors want to report the actual range, then they should call it as 25th and 75th percentile.
- We used the IQR and we changed the values
Level of interest: An article of importance in its field
Quality of written English: Not suitable for publication unless extensively edited
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