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).

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 asymptotically, 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.

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.

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.

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.

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?

7. How long were children followed? Maximum 36 months, minimum 12 months?

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.

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.

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-independency 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?

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.

Minor compulsory revisions
Introduction
Page 3, first paragraph: rewrite as "...are different between developed and developing countries."
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"
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".
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"
Page 3, second paragraph: I do not get the meaning of the last statement "Furthermore.... early life". Please be more specific.
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??
Page 4, first paragraph: please delete from "Therefore" to "appreciated"
Page 4, first paragraph: rewrite as "Numerous cross-sectional studies have suggested that in children from developing ... immunity in early life ... high rates of asymptomatic Campylobacter infection and for..."
Page 4, second paragraph: rewrite as "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 4, fourth paragraph: rewrite as "...of children enrolled before 24 months of age was followed up from January ... children were monitored ...
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..."

Page 5-6: section "Definition of events" would better be presented as bullet points and named just "Definitions".

Page 6, third paragraph: rewrite as "Campylobacter infection was defined as being symptomatic if Campylobacter was isolated in a diarrheal stool or 5..."

Page 6, fourth paragraph: rewrite as "Campylobacter infection was defined as being asymptomatic when there were..."

Page 6, fifth paragraph: "spp." not in italics. "Random sample" without hyphen. Also, it is not clear what you mean by random sample, please be more specific.

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.

Page 7, second paragraph: rewrite as "...was the individual child."

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).

Page 7, fifth paragraph: rewrite as "...was performed on newborns enrolled... 28 days from birth." and delete from "The unit" to "of birth".

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.

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)"

Page 8, fifth paragraph: rewrite as "At the time of enrollment ... them were symptomatic and... (30/32) were asymptomatic."

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."

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..."

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 ...".

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 ..."

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..."

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)."

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.

Page 10, fifth paragraph: rewrite as "...isolated in 201 (39.6%) asymptomatic children. The mean age for asymptomatic Campylobacter infection was ... standard deviation ..."

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.

Also, rewrite legend as "...isolation of Campylobacter from diarrheic and
non-diarrheic samples..." and within table delete heading "Campylobacter" and
rewrite as "Number of positive samples"

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

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.
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.

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