Author's response to reviews

Title: The prevalence of hepatitis B virus markers in a cohort of students in Bangui, Central African Republic

Authors:

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Author's response to reviews: see over
Dear Ms Roxane Rajabi,

Please find below, responses to comments and suggestions about our manuscript, MS: 8790974033457722

Here, responses to reviewers:

REVIEWER: Maimuna Mendy

Major Compulsory revisions

1) Reviewer’s comment: Although DBS collection for HBsAg and HBV DNA quantification have been validated against serum, Detection of anti-HBc and anti-HBs from dried blood spot collection is a novel technique. The Data on sensitivity and specificity of DBS compared with serum should be presented. If this has been done before which I not aware of, then the publication refering to that work should be referenced in the manuscript.

1) Response: This method was described for the first time on the markers of the virus of hepatitis B by Villa et al. in 1981 (Hepatitis B virus markers on dried blood spots. A new tool for epidemiological research, J Clin Pathol, 1981, 34(7):809). In this paper, they described as well for HBsAg as for all the other HBV markers. Mendy et al. also published a paper on this technique but only for HBsAg, Anti-HBs Ab and alpha-fetoprotein (Mendy and Al, J Viral Hepat, 2005, 12:642 - 647). In our study, we slightly modified the technique of Villa and al. on the level of elute buffer. Thus, instead of using various volumes of elution for the markers, we used the same volume for all markers tested. We validated this on 15 samples of sera and dried blood spots. The comparison between these two blood collection showed a light discrepancy in the case of HBsAg that we corrected by using the confirmatory test. But, in the case of the other markers (Anti-HBc antibody) there is no discrepancy observed.

You will find in the text, the description of the method (Serological Assays section of Methods) as well as the results (second paragraph of Results) that we had introduced.

2) Reviewer’s comment: The authors reported a much lower prevalence of anti-HBc (42% vs 89%) and anti-HBs (3.7% vs 32%) in CAR than what is reported by Pawlotsky et al (ref 8). Suggestions on why there is decrepancies between two studies should be presented and discussed.

2) Response: Pawlotsky et al. had published in 1995 on a targeted population, the young adults were sexually active and were being examined in a Public health clinic for sexually
transmitted disease. Thus, they belong to a population at risk. This study was the first on the prevalence of hepatitis B in Central Africa but was limited by the fact that it addressed only the specific at risk population. Our survey was undertaken on a greater number of individuals with various socio-demographic and economic origins. Our results show for the first time the prevalence of hepatitis B in a healthy population of Bangui and highlight the various risk factors involved in the infection of hepatitis B in Bangui.

We have introduced sentences in the second paragraph of the Background:

- Third sentence beginning at “Previous study on young sexually active adults, examined in a Public health clinic for sexually transmitted disease in CAR, …… anti-HBc antibodies at 89% [8]”
- Fifth sentence “All these studies have been carried out in the hospital environment and did not show the right prevalence of HBV in the population.”
- The last sentence: “Thus, the purpose .......... the prevalence of HBV in the apparently healthy population ……. to propose strategies of prevention”

Minor essential Revisions

3) Reviewer’s comment: Anti-HDV was mentioned as one of the tests that were performed, but there is no mention of the outcome of this test. Either delete anti-HDV from the methods section or show the data in the results section if the samples were tested.

3) Response: Study is currently ongoing on hepatitis delta, so we cannot publish the results in this manuscript. We prefer to suppress anti-HDV in the methods.

4) Reviewer’s comment: The title of table 3 is misleading, calculation of ODDS ratio should be obtained for the risk factors.

4) Response: About Table 3, percent values were replaced by the calculated OR values and CI95% which better describe risk factors.

Discretionary Revisions

Reviewer’s comment: The authors stated that informed consent was sought from parents of younger participants (<18 yrs old) but there is no clarification as to whether the parents were informed of the results as should be the case. This needs clarification.

5) Response: Participants and parents of the younger participants were informed of the obtained results. It is now introduced in the Methods (Ethical approval section of the Methods).
REVIEWER: Ephraim Ayoola

Specific Comments

TITLE

Reviewer’s comment: Title needs to be changed to reflect the content: Example “The prevalence of hepatitis B virus markers in a cohort of students in Bangui….”

Response: We agree with the Reviewer's suggestion and the new Title is: “The prevalence of hepatitis B virus markers in a cohort of students in Bangui, Central African Republic”

ABSTRACT

1. Reviewer’s comment: The abstract should be shortened. For example the third sentence… “. Because…. attributable to HBV” can easily be expunged.

   1) Response: We agree with the Reviewer's suggestion and removed that sentence. We also brought some clarification in the abstract. Thus:

   - Background of the Abstract: “The objective of this study …… of HBV in apparently healthy young people …….. this population in Bangui.”

2. Reviewer’s comment: The conclusion is weak. Furthermore, what is meant by the statement “The results suggest that both vertical and horizontal are responsible for maintaining high prevalence….” The study reported here is a cross-sectional survey and not a study of transmission! The data cannot support such inference.

   2) Response: The conclusion was rewritten to highlight the high prevalence of hepatitis B in the adolescents and the young adults and also to show that this is the first time that kind of survey was performed in Bangui.

3. Reviewer’s comment: What is meant by “familial antecedent”? This has to be well defined in the methodology for it to mean much

   3) Response: Familial antecedent: This relates to a family in which at least one family member is a chronic carrier of hepatitis B virus. It has been defined in the methodology under the Study population section (third paragraph)

BACKGROUND
1. **Reviewer’s comment:** The background is too lengthy and should be shortened

   1) We rewrote the background and brought more clarification to allow better comprehension of the objective of the study.

2. **Reviewer’s comment:** If previous surveys had shown high prevalence the authors need to provide the basis for wanting to re-survey and state it clearly. For example could they want to determine whether the epidemiology had changed and if so why? And if not, why not? (This my suggestion) I am not sure the authors need 10 references (See references 9-19) to convey the content of the second paragraph under this section. I suggest shortening and changing this paragraph to make the basis of study meaningful.

   2) **Response:** The previous survey had been carried out in a non-representative population because Pawlotsky et al (1995) performed their study on the young adults attending the National Center for sexually transmitted diseases. Most young adults who go to this Center belong to the group practicing at risk sexual behavior. So the prevalence of sexually transmitted diseases could be high when such a group is studied. In contrast, an abstract published on the 12th Symposium on viral hepatitis and the liver diseases in Paris (NP Komas et al. Seroprevalence and age of acquisition of HBV infection during childhood in “Complexe pediatrique de Bangui” (Central African Republic) *J Clin Virol* **36** (suppl.2):S199) showed that 48% of the teenagers from 10 to 15 years had already been in contact with hepatitis B.

   All these precisions have been introduced in the second paragraph of the background (see response to reviewer 1).

   Some references were removed as suggested by the reviewer and this paragraph has been rewritten.

3. **Reviewer’s comment:** Since the author used the finger stick (capillary) method instead of venous blood they should establish comparability and concordance from the literature of highlight any limitation in addition to the advantage mentioned.

   3) **Response:** We have created a new table (table 1) to validate the technique of DBS in order to show the effectiveness of this technique compared to the test on sera (see more details on reviewer 1).

4. **Reviewer’s comment:** What is “besides serology”? 
METHODS

1. Reviewer’s comment: From what month to what month was the survey carried out? How did you select the schools? Randomly? How did you determine an expected HBsAg rate of 22.3% in determining the sample size when the reported prevalence was much less by your literature review? (your reference #8)

1) Response: We agree with the reviewer’s comment, so we inserted in the manuscript the duration of this study, which was from 1st to 28th February, 2007. The schools were chosen by geographical area and the selection was made randomly between the public schools and the private schools. The rate of 22.3% was chosen from a study presented in 2006 to Paris to the 12th International Symposium on Viral Hepatitis and Liver Disease, 1-5 July 2006, Paris, France (Komas et al. Seroprevalence and age of acquisition of HBV infection during childhood in “Complexe pediatrique de Bangui” (Central African Republic), J Clin Virol 36 (suppl.2):S199). This work showed that the prevalence in the children from 0 to 15 years was of 22.3%.

All these information have been introduced in the text.

2. Reviewer’s comment: The last two sentences under “methods’ belong better under” results”

2) Response: As suggested by the reviewer, we moved the two last sentences of the section on population studied to put them in the results

3. Reviewer’s comment: Grammatical errors “Individual were insured” instead of assured

3) Response: The grammatical error was corrected by replacing insured by assured

4. Reviewer’s comment: How many students were invited to participate butr declined? What were specifically asked to convey “Familial antecedents” though I am not quite clear what is meant by this!
4) **Response:** No student refused to participate to this study. Family antecedents already explained at the point number 3 of Abstract.

5. **Reviewer’s comment:** The authors tested for HDV but the date was not included in the result

5) **Response:** Study is currently ongoing on hepatitis delta, so we cannot publish the results in this manuscript. We prefer to suppress anti-HDV in the methods.

**Reviewer’s comment:** Statistical analysis:
The authors are better served if they were to limit themselves to reporting positivity of these markers instead of referring to “being in contact” or being infected in the past or in the present since no IgM – anti HBc was tested and no biochemical data was done or needed in a prevalence study.

**Response:** We rewrote this sentence

**RESULTS**

1. **Reviewer’s comment:** Expunge the second line about seroconversion among 6 individuals especially since the other participants could have also changed in their serologic profile. In any case this observation contributes little to the objective of the study

   1) **Response:** We removed the second line of the sentence as suggested by the reviewer.

2. **Reviewer’s comment:** Fig 1 can be reported in prose in the text. Was the difference statistically significant? Were the ages and sexual activities, number of sexual partners etc different between districts? Reference to “socioeconomic conditions or status” invites strict definition of parameters used in stratify these sub – populations. I suggest that the authors limit the analysis to specific variable that they questioned about (paragraphs 2 and 3. ) Reference to “population in high promiscuity… may therefore not be necessary.

   2) **Response:** Figure 1 has been removed.

3. **Reviewer’s comment:** Again how was” familial antecedent” determined? (paragraph 3) In African populations caution is needed in interpreting what is frequently reported as “hepatitis”

   3) **Response:** See point 3 of the abstract

**DISCUSSION**

1. **Reviewer’s comment:** The first paragraph about the study being “representative” of “young educated people” is speculative. For example, what proportion of young population is ”
uneducated”. What about those who had elementary and technical education in the population. I think this paragraph should simply highlight the limitation of a cohort study and that it might not be applicable to the general population etc.

1) **Response:** We have better clarified the first sentence of the discussion.

2. **Reviewer’s comment:** It is important to show the concordance, limitation in terms of comparative sensitivity either from your own data or the literature (Reference to “data not shown” is not helpful)

2) **Response:** From a new table (table 1), we have introduced new information about sensitivity of the DBS and discussed this information.

3. **Reviewer’s comment:** (a) Your second paragraph “The persistence of high prevalence …..” does not “clearly confirms that there is a complete absence of program for fighting against hepatitis B”. For example, if a program started a few years ago whereby children are vaccinated the impact would not be seen in this age category. Also is there no health education program in the schools and Universities?

3) a. **Response:** There is no program to fight against the viral hepatitis B. Nevertheless, a program of vaccination against hepatitis B in young children from 0 to 11 months has just begun (in November 2008 precisely) following our abstract (Komas et al., 2006). Although the vaccination program is set up, unfortunately, there is not yet a program of education in the schools on viral hepatitis. In addition, we modified the sentence to better clarify our idea.

(b) **Reviewer’s comment:** I suggest that the high prevalence and risk factors should be the focus of the discussion. The limitation of the study should be highlighted. The last paragraph on page 9 under discussion is very confusing. One is not quite sure of what the authors are trying to communicate here.

b. **Response:** At the suggestion of the reviewer, we focused our discussion on the risk factors and the high prevalence. We modified the last paragraph highlighted by the reviewer.

(c) **Reviewer’s comment:** Also the reference to "acute infection" is untenable in a crosssectional (non clinical) study that did not test for IgM anti HBC.

c. **Response:** We removed all this part

4. **Reviewer’s comment:** On page 10 reference to “a silent HBV infection’ was made. What does that mean .. What “anomaly”?

4) **Response:** We mentioned “silent HBV infection” with regard to the low level of anti-HBs antibody. The word “anomaly” was related to “silent HBV infection”. To reduce any ambiguity on this sentence, we modified the 4th paragraph.
5. Reviewer’s comment: Paragraph 2 on page 10 states “HBV infection is linked mainly to sexual transmission”. In what populations? State reference, though I suggest that this is not the case in most African populations where HBV infections occur in early childhood.

   5) Response: This paragraph now the last of Discussion has been clarified.

6. Reviewer’s comment: The last paragraph is not helpful or meaningful. The entire last paragraph under discussion contributes little and should be removed.

   6) Response: We have removed this paragraph.

TABLES

1. Reviewer’s comment: Tables 1 and 2 can easily be merged into 1

   1) Response: To better understand the data, we think that it is preferable to keep tables 1 and 2 separate. They have become tables 2 and 3. It is not easy to put together data from these two tables.

2. Reviewer’s comment: Table 3 contains many elements that need either removal or clear definition. For example how was “familial antecedent of HBV “ determined” and validated? Also if the HBV in “public school” was much and significantly higher, it might be interesting to analyze and compare the frequency of some risk factors in the three sub groups of students.

   2) Response: We think that all the elements which are in table 3 contribute to the comprehension of this manuscript.

FIGURE

1. Reviewer’s comment: Reviewer’s comment: Figure 1 can easily be reported in the text

   1) Response: See response number 2 in the Results

REFERENCES

1. Reviewer’s comment: The number of references should be reduced to about 20 that are relevant.

   1) Response: The number of references has been reduced to 26.