Author’s response to reviews

Title: Prevalence and risk factors of hepatitis B and C virus infections in an impoverished urban community in Dhaka, Bangladesh

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Cover letter with a point-by-point response to comments

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Prevalence and risk factors of hepatitis B and C virus infections in an urban community in Dhaka, Bangladesh Hasan Ashraf, Nur H Alam, Christian Rothermundt, Abdullah Brooks, Pradip Bardhan, Lokman Hossain, Mohammed A Salam, Mohammed S Hassan, Christoph Beglinger and Niklaus Gyr

Dear Sir,

Thank you very much for considering our manuscript entitled “Prevalence and risk factors of hepatitis B and C virus infections in an urban community in Dhaka, Bangladesh” for publication in your journal after submission of a revised version. We have tried to address the comments in a revised version of the manuscript and provided the cover letter by giving a point-to-point response to the concerns raised by the two reviewers. We have also highlighted by underlining all the changes made when revising the manuscript to make it easier for the Editors to
give a prompt decision on our manuscript.

The point-by-point response to the concerns is given below:

Reviewer No. 1

1. Comment: The study population is community-based, mostly asymptomatic chronic HBV or HCV-infected population. There was no any discrimination between new/recent infection and chronic infection. Some of the adult participants might have been infected perinatally or during childhood. In this case, the risk factors elucidated in this study may not actual risk factors of HBV infection in the past. Authors should present their own idea about this point in Discussion section, not just mentioned that it is a kind of the shortcomings of this study.

Response: We discussed this as an important limitation of our study (page 13) admitting that we did not perform some diagnostic tests for HBV to reduce the laboratory costs (page 8), and study cost constraints (page 13), such as determination of anti-HBc IgM, the presence of which indicates acute infection; and the determination of anti-HBs that differentiates susceptible persons from those immune persons, which can be due either natural infection or hepatitis B vaccination (page 13). However, we have planned to perform the above mentioned laboratory tests in a future follow-up study from our stored blood samples as well as by collecting additional blood samples from the study subjects, and are currently looking for adequate funds for the new project.

2. Comment: In Abstract section, “the use of disposable needles and syringes” was not presented in the result, why did it appear in the conclusion? In addition, this data were not presented in the main text just discussed a little in Discussion section. I suggest delete it.

Response: In Abstract section, “the use of disposable needles and syringes” for injecting drugs was deleted (page 3). It was also deleted from the conclusion section (page 14).

3. Comment: There are some confusing data presented in this paper. In Discussion section, it was mentioned that the seropositivity for both HBsAg and anti-HBc in school children had been reported to be less than 0.8%. However, in this study, the seropositivity for both HBsAg and anti-HBc in “children under 5” were around 10%. Pls clarify this point.

Response: Both the data mentioned in the Discussion that the seropositivity for both HBsAg and anti-HBc in school children had been reported to be less than 0.8% (page 11) as well as that found in this study that the seropositivity for both HBsAg and anti-HBc in “children under 5” were around 10% (Table 1, page 21) are correct. The only clarification about the above point can be given by the fact that the two populations mentioned were totally different. The school children
came from the upper and middle socio-economic status of the Dhaka city and the under 5 study children came from an impoverished urban area (page 5) of Dhaka with all the potential reported risk factors for acquiring infectious diseases, such as overcrowding, low income, poor sanitation, as mentioned in the manuscript (page 6). The higher rates among our under 5 study children could be attributed to the general lack of proper health care because of deprived socio-economic status (monthly household income of US$50) and less public health awareness about the transmission of HBV infection as well as lack of hepatitis B vaccination in the community, as also discussed in the manuscript (pages 11, 12). The reverse is true for the school children, such as taking proper health care because of better socio-economic status, increased public health awareness about the transmission of HBV infection, as well as receiving hepatitis B vaccination by their own initiative by most of the school children, as it is widely available in the Dhaka city on payment.

4. Comment: The idea discussed in Discussion section (page 12 lines 13-16) is not correct. HBsAg is the most reliable biological biomarker of HBV infection. Anti-HBc antibody only indicates that the subjects have ever been infected, it may be either recovered or persistent infection. Pls check.

Response: We fully agree with the reviewer that HBsAg is the most reliable biological biomarker of HBV infection (pages 4, 13) and the anti-HBc antibody is an important marker for surveying the burden of HBV infection as it persists even after resolution of infection, and thus identifies both past and current HBV infection (page 4) and only indicates that the subjects have ever been infected, it may be either recovered or persistent infection, as also mentioned in the manuscript as detection of anti-HBc indicates exposure to HBV, which may be acute, chronic, or resolved (page 11). We have modified the sentence as “Therefore, estimation of the prevalence of anti-HBc, in addition to the estimation of the prevalence of HBsAg which is the most reliable biological biomarker of HBV infection, is much more informative about indicator of HBV disease burden among the population” (page 13) in the revised version of the manuscript.

5. Comment: Serological test (page 7 lines 1-2 from the bottom and page 8 lines 1-12) should be moved to Discussion section. It should not appear there.

Response: The serological tests were moved to the Discussion section, as suggested by the reviewer (pages 10, 11).

Minor changes

1. Comment: “eventually guide” should be “guide eventually”. Pls check split-infinities throughout the text.

Response: The “eventually guide” was changed to “guide eventually” (page 5).
The split-infinities throughout the text were checked.

2. Comment: Pls explain “ICDDR,B” at its first appearance.

Response: “ICDDR,B” was explained as “International Centre for Diarrhoeal Disease Research, Bangladesh” at its first appearance in page 5.

3. Comment:….14 patients (0.7%) were seropositive for HBsAg alone. The same is true for anti-HBc… (page 9 lines 13.14) and Abstract section. Pls correct grammatical errors and unsuitable English expression throughout the text.

Response: The statement was changed to “14 participants (0.7%) were seropositive for HBsAg” (page 9). The same was done for anti-HBc (page 9) and Abstract section (page 2). The grammatical errors and unsuitable English expression were corrected throughout the text.

4. Comment: It is not actual prospective study. Pls clarify this in Discussion.

Response: We accept that it was not an actual prospective study. This is actually a population-based cross sectional survey of the prevalence of HBsAg, anti-HBc and anti-HCV (page 6).

Reviewer No. 2

1. Comment: There are significant scientific flaws regarding logics for conducting this study. In the Introduction, the authors have mentioned that there is lack of prevalence study about HBV among general population of Bangladesh, and most of the studies have been conducted in selected groups of population (they have provided reference 5-9 to validate their claims). This is not true and the authors are also aware of this. They have provided reference 4 that described HBV prevalence among general population of Bangladesh. I am confused why Reference 4 was used to validate a global impact of HBV, which is not the case. Also, Mahtab et al. Studied HBV prevalence among 1018 apparent healthy population of Bangladesh (Mahtab et al. Hepatobiliary Pancreat Dis Int 2008; 7: 595-600). As prevalence study of HBV has been conducted in Bangladesh, the authors of this study should cite these references. Subsequently, they should explain the rationality of the present study. In that section, they are supposed to state the novelty of their study (I believe that there are some excellence in their study design). In this part, they should mention why further studies were needed to develop insights about HBV and HCV prevalence in Bangladesh. The limitations of already published studies should be clearly mentioned. I am not sure why the authors decided to skip already published studies about HBV and HCV prevalence at Bangladesh in general population.
Response: The significant scientific flaws regarding logics for conducting this study have been corrected in the modified version of the manuscript (page 4). We are really sorry for the misunderstanding about the authors comments regarding the mention of the lack of prevalence study about HBV among general population of Bangladesh, and most of the studies have been conducted in selected groups of population. Now, the authors have modified the statement as “there is paucity of information on the prevalence of HBV infections among general population and majority of the previous studies were conducted in selected group of people” (page 4). As there was confusion about why Reference 4 was used to validate a global impact of HBV, it was removed (page 4). As Mahtab et al. studied the HBV prevalence among 1018 apparent healthy population of Bangladesh, it is also cited in the reference section (Reference 9, page 17). As prevalence study of HBV has been conducted among the general population in Bangladesh, and published recently, the authors also cited the reference in the modified version of the manuscript (Reference 9, page 17). Subsequently, we modified the rationality of the present study in the introduction section (page 4). In that section, the authors also stated the novelty of their study (page 4). In addition, we also decided to estimate the prevalence of anti-HCV in the same population, as there is also lack of its information (page 5). We also tried to identify the possible risk factors for acquiring the infections (page 5). The limitations of already published studies are clearly mentioned in the background section of the manuscript (page 4). The authors already cited the published study about HBV and HCV prevalence at Bangladesh in general population (Reference 9, page 17).

2. Comment: The authors should provide the rationale for conducting the study at Kamalpur area of Dhaka. If this is due to presence of urban field site of ICDDR,B, please explain why this field site was selected. Also, the activities of ICDDR,B at Kamalapur that may influence prevalence of HBV or HCV at that area should be mentioned. This is needed to develop insights if these activities up or down regulate HBV and HCV prevalence at Kamalpur. The title of the manuscript is about HBV and HCV prevalence at an urban area of Dhaka. This site may not be a proper place for epidemiological study as stipulated in Title. Study of HBV and HCV prevalence at an urban study location of ICDDR,B may be a biased location because the people of this area may have altered KAP (knowledge, attitude and performance) regarding infectious diseases due to prolonged presence of ICDDR,B team and health education provided by ICDDR,B. This point must be properly addressed.

Response: The authors provided the rationale for conducting the study at Kamalpur area of Dhaka. It is definitely due to the presence of an urban field site of ICDDR,B. The field site selected at Kamalpur is divided into seven geographical strata and 450 clusters, each with about 100 household (page 6). We had specific identification number of all the households (HID) as well as person identification number (PID) of all the individuals living in these households.
at Kamalapur. Randomization of the households and study subjects was done by a statistician not related to the study and finally identification of the study subjects by the research assistants and study health workers were only possible because of the availability of already existing data of the Kamalapur field site. It would not be possible to perform this type of study so easily and conveniently, if other site was selected for the study. We fully agree with the reviewer that the activities of ICDDR,B at Kamalapur might influenced the prevalence of HBV or HCV at that area, which is mentioned as an important limitation of our study in the discussion section of the modified version of the manuscript (page 13). The title of the manuscript is about HBV and HCV prevalence at an impoverished urban community in Dhaka, which was modified in the modified version (page 1). As this site might not be a proper place for epidemiological study as stipulated in Title, we have changed the title to “Prevalence and risk factors of hepatitis B and C virus infections in an impoverished urban community in Dhaka, Bangladesh” (page 1). We fully agree with the reviewer that study of HBV and HCV prevalence at an urban study location of ICDDR,B might be a biased location because the people of this area may have altered KAP (knowledge, attitude and performance) regarding infectious diseases due to prolonged presence of ICDDR,B team and health education provided by ICDDR,B. This point is properly addressed in the discussion section of the manuscript (page 13).

3. Comment: As mentioned in Reference 26 and 27, the study area is an improvised urban slum area. The authors should alter the title of their paper accordingly, and should add that their study checked HBV and HCV prevalence in an improvised urban area of Dhaka. An urban area and an urban slum area may exhibit different epidemiological spectrum of prevalence of different infectious diseases including HBV and HCV prevalence.

Response: The authors have altered the title of their paper to “Prevalence and risk factors of hepatitis B and C virus infections in an impoverished urban community in Dhaka, Bangladesh” (page 1).

4. Comment: Fourteen subjects were expressing HBsAg, but negative for anti-HBc. Please explain the mechanism underlying this. This makes it necessary to show the sensitivity and specificity of estimation of HBsAg and anti-HBc. The authors only mentioned that assessment of anti-HBc and HBsAg were done by ELISA method using commercial kit from Italy. What is Diasorin? Is it the name of the company or name of the city? Both are needed. What is the lowest limit of estimation of HBsAg and anti-HBc by these kits? HBV DNA should be checked in these cases. Also, there should be discussion about these cases after checking sensitivity and specificity of anti-HBc and HBsAg.

Response: Fourteen (0.7%) study subjects were expressing HBsAg, but negative for anti-HBc. Although rare, the serological profile of HBsAg positive, but negative anti-HBc is not that exceptional, as discussed in the manuscript (page 11). In addition to the diagnostic kits efficacy, immunosuppressive state of the subject may also contribute to such profile (Reference 29, page 20). The authors
clearly mentioned that assessment of anti-HBc and HBsAg were done by ELISA method using commercial kit from Italy and the name of the manufacturer is DiaSorin S. A., Italy (page 7). The sensitivity of HBsAg is 100%, the specificity is 99.7%, and the limit of detection is 0.05PEI units/ml (page 10). Similarly, the sensitivity of anti-HBc is 100%, the specificity is 99.83%, and the limit of detection is <0.5 PEI units/ml (page 11). Checking of HBV DNA was not possible because of the high cost, as the current charge for each test is about 110 US$ in Dhaka and also it is done primarily for treatment purpose. The test can be done but with fresh sample.

5. Comment: As mentioned by the authors, this study was conducted to develop insights about prevalence of HBV and HCV and also to develop control or preventive strategies (Page 5, last 2 lines of Introduction). They have suggested that a national strategy may be developed on the basis of this study. However, they have failed to attain these objectives in their study. The research physicians shared the result with the participants. Four-hundred ninety two subjects were anti-HBc-positive, but HBsAg-negative. It is required to assess how many of these subjects were positive for anti-HBs. The presence or absence of anti-HBs would determine the management strategies of these subjects. Taken together, data of anti-HBs of anti-HBc-positive subjects are essential.

Response: The authors of this study mentioned that the study was conducted to develop insights about prevalence of HBV and HCV and also to develop control or preventive strategies. They also suggested that a national strategy might be developed on the basis of this study. We also accept that we failed to attain all these objectives in our study. The research physicians shared the results with the participants and infected individuals were provided with appropriate information on the prevention of spread of these infections to others and referred to the nearest public health care facilities (page 7). Although the data of anti-HBs of anti-HBc-positive subjects are essential, we mentioned that determination of anti-HBs was not possible because of the study cost constraints, and it is one of the important limitations of our study (page 13). However, we have planned to perform the above mentioned tests in a future follow-up study from our stored blood samples as well as by collecting additional blood samples from the study subjects, and are currently looking for adequate funds for the new project.

Minor essential revisions

6. Comment: Provide more information about the followings

Comment A: Animal bites; type of animals

Response: We took the history of animal bites, such as dog, cat, rat bites and tried to identify animal bites as a potential risk factor for HBV-seropositivity. In both the bivariate and multiple logistic regression analysis (Tables 2, 3, pages 22, 23), animal bites were found to be a significant risk factor for HBV-seropositivity.
Comment B: Visiting unregistered health workers; do they give parenteral injections?

Response: Visiting unregistered health workers included visiting medicine sales men at the medicine shops/pharmacies, visiting unqualified medical practitioners like Kabirajs (traditional healers), homeopaths, quacks etc. Yes, all the above mentioned unregistered health workers in Bangladesh frequently give parenteral injections than necessary to satisfy their poor clients, who also have a fascination for injections. The above mentioned unregistered health workers usually give injections without taking any strict aseptic precaution as well as without using disposable needles and syringes. Till date, some of them re-use non-disposable glass syringes to save costs. Therefore, in both the bivariate and multiple logistic regression analysis (Tables 2, 3, pages 22, 23), visiting unregistered health-care providers were found to be a significant risk factor for HBV-seropositivity.

Comment C: Receiving treatment for STD; nature of treatment

Response: Receiving treatment for STD means receiving treatment mainly for syphilis and gonorrhea, both of which are still treated with multiple doses of injectable antibiotics such as penicillin. Most of the STD patients were also treated by the above mentioned unregistered health workers by using parenteral injections to satisfy their poor clients, without taking any strict aseptic precaution as well as without using disposable needles and syringes. Still some of them use non-disposable re-usable glass syringes for treating their STD clients to save costs. In only bivariate analysis (Tables 2, page 22), receiving treatment for STD was found to be a significant risk factor for HBV-seropositivity, but not by multiple logistic regression analysis (Table 3, page 23).

7. Comment: The authors should discuss how these factors may influence prevalence of HBV and HCV infection.

Response: The animal bites, such as dog, cat, and rat bites might cause transmission of HBV and HCV to the human host by direct contamination of blood through saliva of the infected animals.

Visiting unregistered health workers, who frequently use parenteral injections to satisfy their poor clients, without taking any strict aseptic precaution as well as without using disposable needles and syringes, and rather using re-usable glass syringes, thus spreading the infections to their clients by the use of infected needles and syringes.

Similarly, those individuals receiving treatment for STD usually receive treatment mainly for syphilis and gonorrhea, both of which are treated with multiple doses of parenteral injections given by the above mentioned unregistered health workers, usually without taking any strict aseptic precaution, thus spreading the infections to their STD clients.
Finally, we would very much appreciate your final consideration in accepting the manuscript for publication in your journal.

Thank you again and looking forward to hear from you soon.

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