Author’s response to reviews

Title: Characterization of the hepatitis B virus DNA detected in urine of chronic hepatitis B patients

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Author’s response to reviews:

February 21, 2018

Dr. Sven Pischke
Editor, BMC Gastroenterology

Dear Dr. Sven Pischke,

We would like to thank the reviewers for their acknowledgement of our work and are very encouraged by the positive comments from reviewers (Rev) #1 and #3. We believe that the concerns raised by Rev #2 have arisen out of an unclear presentation of our original manuscript, and may not of scientific concern. We apologize for not presenting the original manuscript in a format that is clear to read. We have improved the clarity of our manuscript in response to the
concerns raised by Rev #2. If we are correct in our interpretation of Rev #2’s suggested experimentation, then additional experiments may be no need. We hope the improvements we have made based on all three reviewers (as detailed point-by-point in the following section) are acceptable and that you will agree this manuscript can be accepted for publication in BMC Gastroenterology.

We look forward to hearing from you.

Sincerely,

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RESPONSE TO REVIEWERS

General response: We appreciate the constructive comments from all three reviewers and realize that there are some unclear statements as pointed out by the reviewers. We have implemented the feedback with regards to the accuracy and clarity of various statements according to all comments, as described below with our responses. Please note that all changes are highlighted in the revised manuscript and referred to by page number below.

Reviewer 1:

General comments and two minor comments: “The present study addresses an important topic in the prevention of HBV infections and suggests that the risk for HBV infection by urine is low. Previous studies have detected HBV DNA in the urine, however, only short PCR protocols were used. This study here closes the gap of knowledge in this regard. The experiments are well conducted and the conclusions are drawn in the data. I have only minor comments to improve the study: - For tears, it has been shown that HBV DNA is present and infectious in humanized mice. This study as another example of body fluid transmission can be discussed (Komatsu et al. JID 2012).- The high and low viral loads could be indicated in the table.”

Response: We appreciate the positive comments and agree with the addition of the suggested reference (Ref #8 in the revised manuscript, page 5, lines 86, 88). We agree that high and low...
viral loads should be indicated in the table, and have indicated these changes in Table 1, as highlighted.

Reviewer 2:

- The paper has little value. Table 1 needs to be replaced by a summarizing table, table 2 needs to be omitted.

- The data presentation is confusing (Figures 1-2).

- The main conclusion (lack of infectivity by urine HBV particles) is not supported by data. First, HBV particles should be demonstrated by additional methods (Southern Blot, Protein analysis etc.). Second, lack of infectivity from highly viremic patients can easily be tested in a susceptible cell line.

Response: We apologize for any confusion that may have taken away from the value of our study. We believe this could be due in part to an unclear presentation of our study in the original manuscript. We have further revised our manuscript to make our points clear. Despite our disagreement with the value of this study, we appreciate Rev #2’s comments on how to improve our manuscript and have responded to each comment as detailed below:

1) Table 1 needs to be replaced by a summarizing table.

Response: We agree that a summary table helps in understanding of the overall study population, so we have added this as the new Table 2. However, we believe the detailed clinical features of each subject are important for the data interpretation, so we have kept Table 1 with the revisions suggested by Rev #1.

2) Table 2 needs to be omitted.

Response: We agree, and have removed the original Table 2 accordingly.

3) The data presentation is confusing (Figures 1-2).

Response: We apologize for the confusion, and have simplified figures 1 and 2 in the revised manuscript.

4) The main conclusion (lack of infectivity by urine HBV particles) is not supported by data. First, HBV particles should be demonstrated by additional methods (Southern Blot, Protein analysis etc.). Second, lack of infectivity from highly viremic patients can easily be tested in a susceptible cell line.
Response: We appreciate the concerns generated by Rev#2, but would like to point out that the PCR approach is much more sensitive of a detection method than Southern Blot or protein analysis. The limits of detection for our HBV assays are 20 copies per mL urine. If we were unable to detect full-length HBV DNA by PCR assays, we do not believe the proposed methods (Southern blot hybridization or protein analysis) would detect viral content from urine. Full-length viral DNA is a necessary component of an infectious viral particle and also the most sensitive component of the virus to detect.

Rev #2 is correct in that there are susceptible cell lines (such as HepG2-NTCP cells) to use for an infectivity assay with cell culture derived virus, although they require a high MOI (>100). Serum derived virus is difficult to infect NTCP cells for unknown reasons. For samples that are negative for full-length HBV DNA by our HBV PCR assays, the viral titer would be less than 20 copies per mL urine. We feel the cell-culture infectivity will not provide additional information if we do perform the infectivity test. We apologize for not clearly describing why we did not perform an infectivity study using the current available cell culture system, and have included this in the Discussion (page 13, line 267-277 and refs # 27 and 28).

Reviewer 3:

General comments—Overall, the manuscript is well written and concise in its content. The used methods are adequate. However, there are some comments which should be addressed.

Response: We appreciate the encouraging review from Rev #3 and have address each comment as detailed below:

1. the colleagues concluded that there is no hint that infectious HBV is present in the urine samples. As a suggestion, the determination of HBsAg in the urine samples would support this statement.

Response: The limit of detection of HBsAg by the commonly used Abbott kit is 0.015 IU/mL, equivalent to 7.5x10^4 viral particles per mL urine (Locarnini S et al., 2012. Hepatitis B Surface Antigen Quantification: Not What It Seems on the Surface). This is at least 200 times less sensitive than our PCR assays. Thus, we do not think we can detect HBsAg in the urine of samples that were negative to any PCR-detectable full-length HBV DNA. Even if we detected HBsAg in urine, it does not mean it came from infectious viral particles. We hope this reasoning is acceptable for not performing HBsAg in urine.

2. Table 1, there is in the last column shown whether the patient received antiviral treatment or not; however, it would be of interest which antiviral the patient received due to renal side effects of some antivirals (e.g., IFN). This could interfere with the detection of HBV DNA in urine.
Response: We appreciate the suggestion. Since all patients were recruited from the same hospital, all patients that received antiviral treatment received ‘Telbivudine’, which has no known renal side effects. We have now included this information in the footnote of the Table 1 in the revised manuscript.

3. A statically calculation in terms of liver disease progression and identification and number of HBV DNA fragments would show in more detail if the number of detectable fragments is affected. What exactly is "hepatitis" in the "liver disease" column?

Response: Thank you for the suggestion. A statistical analysis Mann Whitney U test was performed to assess if there is an association between the number of HBV fragments detected in 5 regions of the HBV genome by the assays and the liver pathology (51 chronic hepatitis B, 7 cirrhosis). The 2 HCC were not included in this analysis. We found no statistically significant liver disease association (p= 0.170) with the number of HBV fragments detected. This information is now included in the revised manuscript under "Association between urine HBV DNA and clinicopathological variables" section of the results, page 12, line 249-254. We apologize for not clearly stating that “hepatitis” is now revised to “chronic hepatitis B”.

4. Table 2 is not really needed and can be omitted since all data were described in the text giving no specific and additional information.

Response: We agree, and the previous Table 2 has been removed from the revised manuscript.

5. Fig. 1 the grey bars indicating the nucleotide positions are somehow irritating and will give no specific information. Therefore the grey bars could be omitted.

Response: We agree. In order to indicate the accurate location of each assay, we have left the grey bars, but significantly shortened the height of each grey bar so they are not irritating.