Author's response to reviews

Title: Experimental infection of Balb/c nude mice with Hepatitis E virus

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Author's response to reviews: see over
Dear Editors,

We would like to resubmit the revised manuscript for your consideration, and we have read the referee’s comments very carefully and we are very appreciating your considerate suggests. The manuscript according to the three reviewers’ suggestions had been great improved and edited by professional editing service a second time. Revised manuscript conforms to the journal style. The reviewers’ suggests we answered point-by-point as follows:

To Saleem Kamili’s questions:

Background:

Question: The Background is written incoherently with extensive redundancies and all stressing on the zoonosis of HEV infection. Till date the only conclusive molecular evidence that HEV infection is transmitted from animals to humans has been published from Japan; viral genomic sequences of amplicons from the deer or boar meat were identical from the patients who had developed HEV infection after ingesting this meat. The authors create an impression in the readers’ mind as if HEV is frequently transmitted from animals to humans “Hepatitis E has become a widespread zoonosis”. This is simply not true. Similarly, statements like “Rodents are considered to be a potential reservoir for human transmission…” is not correct. Till date, to the best of my knowledge, HEV RNA has not been detected in rats anywhere. The Introduction of the manuscript starts with an incorrect statement like “Hepatitis E (HE) is an acute fulminant hepatitis in adults”. Hepatitis E is an acute self-limiting disease in adults! Please rewrite the Background and make a case why there is a need to
develop a small animal model for HEV infection.

Answer: We had rewritten the Background according to your suggestions, and added the necessary of development a small animal model for HEV infection in page 3, line 20 to page 4, line 1 (red colour). The sentence “Hepatitis E has become a widespread zoonosis…..” had been replaced by “Hepatitis E has widely been paid attention for…. ” in page 3, line18 (red colour); “Rodents are considered to be a potential reservoir for human transmission…” had been replaced by “Rodents are considered to be a potential reservoir of HEV…” in page 3, line 11 (red colour); and the sentence “Hepatitis E (HE) is an acute fulminant hepatitis in adults” had been replaced by “Hepatitis E (HE) is an acute self-limiting disease in adults…..” in page 3, line 7 (red colour). HEV RNA was detected in experimental infected rat has been reported by Maneerat Y et al., 1996 (Reference 11).

Methods:

ELISA determination:

Question: Please delete “serum was prepared from blood……20 min”. This is too commonplace to be included in the methods.

Answer: we had deleted the sentence.

RT-PCR detection

Question: Please replace the text “A reverse transcription analysis was conducted…….” with a simple statement like “reverse transcription was performed using…….” The oC is missing from all the temperatures shown in the RT-PCR protocol.
Answer: we had replaced the text “A reverse transcription analysis was conducted……” with “Reverse transcription was performed using…..” in page 6, line 9 (red color) and we added the °C in the RT-PCR protocol.

Results:

Question: Please describe what were the criteria of “apparent clinical disease” in mice.

Why was not the PCR for negative strand of HEV RNA performed on the tissues harvested from the infected mice?

Answer: we describe some symptoms “such as acute hepatitis with icteric viral hepatitis or diarrhea in humans….“ in page 7, line 17-18 (red color). We didn’t detect the negative strand of HEV RNA in these tissues.

Question: The activities of liver enzymes: Please delete the first sentence.

Answer: we had deleted the first sentence.

Discussion:

Question: The authors need to clearly what they mean by clinical signs of infection in mice. Viral antigen and HEV RNA detection in various tissues are not “typical symptoms” but are evidences of infection and viral replication.

Answer: we deleted the sentence and added the related information in the first sentence of Discussion with “as evidenced by viral antigen expression in liver and other extra-hepatic tissues, fecal viral shedding, hepatic lesions, and the presence of anti-HEV antibodies.” in page 10, line 6-8 (blue color).

References

Question: References #11 and #34 are the same references. Please make necessary
corrections in the citation and the bibliography.

Answer: we checked the References and deleted the #34 reference.

To Khin SA Myint’s questions:

Major Revisions:

Questions: Table 1 did not specify whether RNA detection was in feces or serum. The authors need to improve the table with appropriate Column Headers (Group, HEV RNA, Anti-HEV IgG, HEV antigen, etc.)

Answer: we added the related information in Table 1 and improved the table in page 20.

Minor Revisions:

Question 1. Page 3, line 22, “Although cell culture system…. HEV pathogenicity remain unresolved” could be replaced by “Although cell culture systems for propagating hepatitis E have been developed, the availability of laboratory animal models is still of critical importance for studying HEV pathogenicity”.

Answer: We had replaced the sentence in page 3, line 20- page 4, line 1 (red color).

Question 2. Page 5, Line 7, Group No. 1 – clarify whether the mice was inoculated with a placebo (e.g. PBS).

Answer: We injected Group No.1 with sterilized PBS and added the information in page 5, line 5-6 (blue color).

Question 3. Page 5, Line 10, “labeled” can be omitted.

Answer: we deleted the word.
Question 4. Page 5, Line 11, Each nude mice was humanely euthanized at either 4, 7, 14 or 21 dpi– did this include group 3 as well?

Answer: Yes, we humanely euthanized each nude mouse at either 4, 7, 14 or 21 dpi include group 3.

Question 5. Page 5, Line 21, “Serum was separated from” can be omitted.

Answer: we deleted these words.

Question 6. Page 5, Line 21, Suggest changing “HEV IgG was determined ….and negative controls” to “HEV IgG was determined using a commercial ELISA kit (KHB, Shanghai, China) based on recombinant HEV fusion proteins according to the manufacturer’s directions.

Answer: we changed the sentence in page 5, line 20-21 (blue color).

Question 7. Page 6, Line 6, Suggest changing the “Activities of liver enzymes” to “Serum liver chemistry profile”.

Answer: we changed the sentence in page 6, line 4 (blue color).

Question 8. Page 7, Line 5, Suggest changing the “whose recombinant protein is completely conservational in four genotypes (I-IV) to “designed to detect capsid proteins of HEV genotypes (1-4)”.

Answer: we changed the sentence in page 7, line 4 (blue color).

Question 9. Page 7, Line 10, delete “s” from “positives”.

Answer: we deleted the “s”.

Question 10. Page 7, Line 18, “gross microscopic lesions” – should be deleted.

Answer: we deleted the sentence.
Question 11. Page 8, Line 5, However “HEV RNA…” could be replaced by “HEV RNA was not detected in any of the tissues, sera or feces in the negative controls”.

Answer: we changed the sentence in page 8, line 3-4 (blue color).

Question 12. Page 8, Line 8, “ELISA was used to detect….” can be omitted.

Answer: we deleted the sentence.

Question 13. Page 8, Line 15, “The activities of liver enzymes” could be replaced with “liver enzyme profile”.

Answer: we changed the sentence in page 8, line 12 (blue color).

Question 14. Page 8, Line 15, “The sera was separated…” can be omitted.

Answer: we deleted the sentence.

Question 15. Page 9, Line 7, Suggest adding “using appropriate controls” after the sentence and delete “using a positive HEV infected rat liver as a positive control in the following statement (line 17). The using of mouse antibodies to detect rat tissues does not sound right.

Answer: we added the sentence of “using appropriate controls” in page 9, line 4 (blue color) and delete the sentence you mentioned.

Question 16. Page 9, Line 13, “This indicates that HEV replication….’ should be deleted as it is a topic for discussion.

Answer: we deleted the sentence.

Question 17. Page 9, Line 16, suggest deleting “of HEV antigens”.

Answer: we deleted the words.

Question 18. Page 10, Discussion, Suggest rewriting the first sentence as follows for
The results presented here strongly suggested that nude mice are susceptible to swine HEV as evidenced by viral antigen expression in liver and other extra-hepatic tissues, fecal viral shedding, hepatic lesions, and the presence of anti-HEV antibodies.

Answer: we changed the sentence in page 10, line 6-8 (blue color).

Question19. Page 10, line 17, In addition, HEV antigens were observed…” could be deleted as it was discussed again on Page 11.

Answer: we deleted the sentence.

Question20. HEV replication can not be concluded by antigen staining methods. Suggest changing “the main replication sites” on Line 8 to “heavily infected tissues”. Suggest deleting “indicating that these intestinal locations are minor replication sites”. And add a sentence on “This would indicate that HEV was probably replicating in these tissues but this need to be demonstrated with in situ hybridization”.

Answer: we changed the sentence in page 10, line 22 and deleted the sentence you mentioned, added the sentence you suggested in page 10, line 22-page 11, line 1 (blue color).

Question21. Page 12, Line 14, Suggest changing to “Therefore the nude mouse could be considered as a promising animal model for HEV studies, especially to evaluate candidate vaccines or antiviral treatments.

Answer: we changed the sentence in page 12, line 7-9 (blue color).

Discretionary Revisions:

1. Page 5, “Experimental infection of nude mice with HEV” – suggest replacing with
“Experimental Design”.

Answer: we changed as you suggested in page 5, line 3 (blue color).

2. Page 7, Line 14-16, Suggest replacing the sentences “Tissues preserved in ……” with “Tissues for histologic examination were fixed in 10% neutral buffered formalin, routinely processed, sectioned at a thickness of 7µm, and stained with hematoxylin and eosin.

Answer: we changed the sentence in page 7, line 12-14, and added the two words “neutral buffered” before “formalin for histopathologic examination …. ” in page 5, line 17 (blue color).

To Volker Thiel’s questions:

Minor Essential Revisions:

Question1. It still needs to get clearly stated if the entire study in done with a total number of 12 mice. If so, data from each time point (4d, 7d, 14d, 21d) are resulting from one mouse per group. This has to be stated clearly or otherwise clarified. Whenever experiments are stated to be performed in triplicates (e.g. page 8/9) does this mean the same sample was measure three times, or were samples derived from three different mice? Another example is in figure legend 2 “…represents the average of three experiments…”, are these indeed three experiments or just three measurements?

Answer: the total number of nude mice is 12, and each time point (4d, 7d, 14d, 21d) are resulting from one mouse per group. The performed in triplicates means same
sample was measured three times, and the “…represents the average of three experiments…” also means three measurements.

Question2. Results, page 8, “The activity of liver enzymes”: The statement “The level of AST was significantly increased” suggests that significance was calculated. If so please indicate the p value in the figure or delete the term significant.

Answer: we delete the word.

Question3. Discussion, page 11: Please change the term “fluorescent particles of HEV” and “fluorescent HEV antigen particles”. Particles imply virus particles which are not visible by immunofluorescence imaging. It is rather the HEV ORF2 gene product produced in infected cells that is visualized after IFA.

Answer: we delete the word of “particles”.

Question4. Discussion, page 11: Please correct ALP activities to ALKP activities.

Answer: we changed all “ALKP” to “ALP”.

We believe that the revised manuscript is substantially improved. So, we wish to be considered for publication. Thank you very much for your attention again. We are look forward to receiving your letter. Best Regards.

Sincerely yours,

Xiuguo Hua