Reviewer's report

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

Version: 2 Date: 18 November 2008

Reviewer: Khin SA Myint

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Consistent and reproducible experimental hepatitis E has been confirmed only in cynomolgus macaques, rhesus macaques, tamarins and pigs. An alternative species that is easier to handle and that could be used in larger numbers is desired for defining the pathogenesis of hepatitis E and to evaluate candidate vaccines. The MS by Huang et al reported the transmission of swine HEV in nude mice as evidence by liver enzyme elevation, fecal virus excretion, and seroconversion.

Major Revisions:
1. The authors' statement that there is no efficient cell culture system to propagate hepatitis E virus (BACKGROUND) is not totally true (References –Takahashi et al, 2007; Tanaka et al, 2007; Lorenzo et al, 2008).
2. Inoculation route is mentioned as intradermal in the abstract and intravenous under Materials and Methods. Please clarify.
3. The manuscript needs a good proof reading for language.
4. When were blood and fecal samples collected (dpi)? Were the serum samples tested for HEV RNA and if so were they viremic?

Minor Revisions:
1. Nude mice may serve as a useful model for studying the replication mechanism...(ABSTRACT). Suggest changing to studying pathogenesis of HEV.
2. [As we all know, pigs, rodents, cats….in order to protect ourselves against HEV] (BACKGROUND). Suggest deleting these statements.
3. Materials and Methods/Experimental infection of nude mice with HEV – The experimental design is confusing. Please clarify the number of mice use for each group/sub-group.
4. Materials and Methods – Describe the weight (in range) of the animals used. [Only seronegative nude mice were used for inoculation] implied that there were seropositive ones. Suggest changing to [Nude mice confirmed seronegative for HEV infection by ELISA were included in the study].
5. Materials and Methods/Virus – It was not clear how the infectivity titer of swine HEV was determined for nude mice.
6. Materials and Methods/ELISA determination – how was seroconversion defined?
7. Materials and Methods/RT-PCR detection - Invitrogen was mispelled.

8. Materials and Methods/RT-PCR detection - [A reverse transcription nested PCR (RT-nPCR) analysis – suggest deleting [nested PCR (RT-nPCR)].

9. Materials and Methods/RT-PCR detection – [The expected product of RT-PCR was 348 bp] Spell out bp for the first time.

10. Materials and Methods/Immunofluorescence – The authors should describe a brief procedure of IHC since it is one of the key assays in this MS.

11. Materials and Methods/Immunofluorescence – [All specimens were tested twice under code with labeled positive and negative controls]. Suggest changing to [All specimens were tested in duplicate with appropriate controls]. Specify the controls used.

12. Results [There weren’t apparent clinical signs in all the experimental groups]. Suggest changing to [Evidence of apparent clinical disease was not found in any of the experimental groups].

13. Results/HEV RNA – Suggest adding HEV RNA was not detected in negative control tissues.

14. Results/Immunofluorescence – The statement [A large number of HEV antigens were detected in the cytoplasm of hepatocytes] is confusing. Suggest changing to [HEV antigen was diffusely or consistently detected in the cytoplasm].

15. Results/Immunofluorescence – Clarify the sentence [the number of HEV antigens in spleen was extremely large]. Suggest to add [No signal was observed in any of the negative control tissues].

16. Results/Immunofluorescence – Explain briefly where HEV antigen was located in extrahepatic tissues.

17. Results/Histopathology – Briefly mention any histopathological changes in extrahepatic tissues (apart from spleen) of the infected group. Were any changes seen in the negative control group?

18. Study results (biochemical, histologic, virologic, serologic data) can be summarized in a table.

19. Study results - It would be interesting to note whether the findings (virological, serological and histological) are different between the oral and the IV inoculation groups.

20. Discussion –The statement [The inoculated nude mice show subclinical signs] is contradictory to the one mentioned under results [There were no apparent clinical signs].

21. Discussion – Error in stating [This may imply that in jejunum and ileum the HEV particles are too limited to be detectable by RT-nPCR] as PCR is more sensitive than IHC.

22. Figure 3 (IFA of infected tissues) – some positive signals are not convincing. Suggest using pictures with good resolution.
Discretionary Revisions:
1. Materials and Methods/Experimental infection – suggest replacing [nude mice was killed] with humanely euthanized.

Level of interest: An article whose findings are important to those with closely related research interests

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.