Reviewer’s report

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

Version: 3  Date: 9 January 2009

Reviewer: Khin SA Myint

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Major Revisions:
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.)

Minor Revisions:
1. Page 3, Line 22, “Although cell culture systems……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”.
2. Page 5, Line 7, Group No. 1 – clarify whether the mice was inoculated with a placebo (e.g. PBS).
3. Page 5, Line 10, “labeled” can be omitted.
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?
5. Page 5, Line 21, “Serum was separated from” can be omitted.
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.
7. Page 6, Line 6, Suggest changing the “Activities of liver enzymes” to “Serum liver chemistry profile”.
8. Page 7, Line 5, Suggest changing the “whose recombinant protein is completely conservalional in four genotypes (I-IV) to “designed to detect capsid proteins of HEV genotypes (1-4)”.
9. Page 7, Line 10, delete “s” from “positives”.
10. Page 7, Line 18, “gross microscopic lesions” – should be deleted.
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”.
12. Page 8, Line 8, “ELISA was used to detect...” can be omitted.
13. Page 8, Line 15, “The activities of liver enzymes” could be replaced with “liver enzyme profile”.
14. Page 8, Line 15, “The sera was separated…” can be omitted.

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.

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

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

18. Page 10, Discussion, Suggest rewriting the first sentence as follows for clarity “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.

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

20. 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”.

21. 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.

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

Level of interest: An article of importance in its field

Quality of written English: Needs some language corrections before being published

Statistical review: Yes, but I do not feel adequately qualified to assess the statistics.

Declaration of competing interests:
I declare that I have no competing interests.