Reviewer’s report

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

Version: 2 Date: 19 November 2008

Reviewer: Volker Thiel

Reviewer’s report:

The manuscript “Experimental infection of Balb/c nude mice with Hepatitis E virus” by Fen Huang et al., describes that Balb/c nude mice can readily get infected by HEV and may serve as a small animal model to study HEV replication in vivo. The authors showed that HEV can be detected by RT-PCR and IFA in a number of organs of experimentally infected mice. Furthermore, they present evidence that HEV can be transmitted to contact-exposed mice and that HEV may possibly induce an antibody response.

Overall, the proposed mouse model for HEV may be interesting but the presented data are too preliminary to consider this model as useful. The authors failed to provide a comprehensive and convincing analysis of HEV replication and immune responses in infected mice.

Major concerns:

1. The presented data are based on a total number of 12 mice that were divided into three groups (3 mice each): uninfected control – HEV infected – contact-exposed mice. In none of the experiments it is clear at which time points the analyses were done and how many samples/mice were used. Therefore, there are also no statistics provided to support the main conclusions. Some experiments show only little effect of virus infection (as compared to non-infected mice) which makes it even more difficult to draw conclusions.

2. RT-PCR analysis: Please provide more information on when and where HEV is detectable. To get an idea on how HEV can replicate and spread in Balb/c nude mice it is inevitable to analyze HEV replication by RT-PCR frequently at several time points post infection. When is the virus first detectable, when is maximal spread and when is the virus cleared?

3. Figure 1 and corresponding results section: it is not clear what the putative differences in OD450 measurements between serum from infected mice and non-infected mice mean. Assuming that each measurement has only been done once, this experiment is not convincing. Furthermore, the figure legend describes the experiment (this should be in the results section) and not relevant facts to describe the figure.

4. Figure 2 and corresponding results section: When was the analysis done, how many mice were analyzed, are the presented data the mean of several experiments and if yes provide statistics and error bars. How are kinetics of liver enzyme values at different time points post infection?
5. Figure 3 and corresponding results section: Please provide evidence that the used antibody is specifically recognizing HEV antigens – show control panels. Are presented data derived from an experimentally infected mouse of from a mouse of the contact-exposed group? At which time point has the analysis been performed?

6. Figure 4: Again no control panels and no indication when the analysis was done.

7. General comment: the manuscript needs spell check.

**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:** Yes, and I have assessed the statistics in my report.