Format for ANSWERING REVIEWERS

March 5th, 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: WJH Crossan et al resubmission).

Title: Hepatitis E Virus in patients with acute severe liver injury

Authors : Claire Crossan, Kenneth J Simpson, Darren Craig, Chris Bellamy, Janice Davidson, Harry R Dalton, Linda Scobie.

Thank you for the opportunity to revise our manuscript in accordance with the reveiwers' suggestions.   We have made comments below referring to changes in the manuscript as indicated. Changes are highlighted in the resubmitted manuscript for clarification.   We hope that the modifications are acceptable to the reviewers and the editor and look forward to the outcome.

Reviewer 02528832 comments to the authors:
This is a well written manuscript, dealing with the prevalence of hepatitis E among a series of patients subjected to liver transplantation, in whom the etiology of liver failure was masked by coexisting cirrhosis and/or drug overdose. Authors correctly stress the importance of excluding HEV infection in Western countries, irrespective of the travel story. However, the series is quite short; there are other series analysing prevalence of HEV in Western countries which include hundreds of patients, and should be cited. There are some few spelling errors (i.e, caeroloplasmin (patient 1), choliangitis (end of the discussion) among others

We thank the reviewer for their helpful comments, but we wish to clarify that, of the patients described in this paper presenting with acute disease, only two had been subject to liver transplantation at the time of testing.   The remaining 78 (Table 2), had a number of aetiologies resulting in an acute condition, so severe they were referred to the SLTU for treatment.   This study is not on individuals receiving transplants but on those with acute disease, supporting the need for HEV testing.   We assume that the studies that the reviewer is referring to are on transplantation and not specifically acute disease of which there are a lot fewer, these are already cited in the text.   We hope this response is accurate and satisfactory for the editor and the reviewer.
Those spelling errors highlighted, as well as others, have been corrected.

We have expanded the methods section to include the details requested by the reviewer. Assays used for antibody detection are commercial and already indicated to be the most sensitive for use as indicated in the literature (Bendall et al , 2010 - more recent literature refers to the assay used as Axiom). In addition, the use of the qPCR assay described has also performed sufficiently in comparative tests using the WHO standard (Mokhtari et al 2013). All patients were tested for HEV RNA. We apologise if this was not made clear and have amended the manuscript accordingly.

Recent publications regarding HEV have now been cited. In particular the Drebber reference which was published after our initial submission.

The references have been removed from the abstract and the core tip section as advised.

The other causes of ALI were already referred to in the cohort description in the methods section. In addition, a comment has been made in the results – page 9.

The sample storage conditions and the timepoint of sampling have been indicated in the methods section. Samples were taken at time of presentation and stored at -80 before testing.

The accession numbers for the HEV reference strains used to determine genotype are given in Figure 1 and in the legend for clarity.

The presence of HEV antibodies for patient 2 has now been included in the results section, as suggested.

In the discussion we have now described the methods of transmission of genotype 3 in developed countries and have explained that we are unable to determine the exact source of infection for the patients presented in this paper.

Figure 1 has been redone for clarity including the addition of further HEV sequences to try to elude to route of infection, i.e., genotypes 3c, 3e and 3f, were included for alignment.

The symbols $\chi \pm \sigma$ have been replaced with mean$\pm$SD in table 1. This was a syntax error.

The term transaminitis has been removed from the manuscript and replaced with a suitable alternative.

Reviewer 01221188 comments to the authors:

This paper is described very well, but minor revisions are needed to accept. Minor revisions. 1. In the abstract section, a reference should not be used. 2. In Core Tip, what is " transaminitis" ? the elevation of transaminases ? Is this a formal medical term? 3. The diagnostic criteria of hepatitis E virus infection should be described in the text. 4. Were all of 80 patients examined for HEV-IgG, IgM and HEV-RNA ? Were there any patients who were negative for HEV-IgG but positive–IgM? Were there any patients who were negative for HEV-IgG-IgM but positive–HEV-RNA? The authors should clarify the prevalence of HEV-IgM,-IgG, and HEV-RNA in 80 patients. 5. In the cases, the authors should clarify the date when the blood sample were taken for HEV-related examination. 6. In briefly, the method of conventional PCR, cloning, and phylogenetic analysis should be written in the text. 7. In figure legend 1, the accession number of nucleotides "6332-6476" should be clarified. 8. In Table 1, what is "χ±σ"in the age ? mean, median ± SD ? 9. In Table 1, what is the definition of "Liver Failure" ?

1 and 2 As for the previous reviewers; the reference from the abstract and core tip has been removed and the term transamintis replaced with the correct terminology.

3 We have added a description of the diagnostic criteria for HEV infection used in the UK to the methods section page 8.

4 All samples were tested for HEV RNA in addition to antibody testing. The methodology has been clarified on page 8.   In addition, the virus/antibody combination results have been added to the results text on page 9.

5 The sample storage conditions and the timepoint of sampling have been indicated in the methods section.   Samples were taken at time of presentation and stored at -80 before testing.

6 The methods section has been expanded to include the details requested by the reviewer.

7 In the Figure 1 legend, the relevant accession number has now been included.

8 As above; χ±σ have been replaced with mean±SD.

9 A definition of liver failure has now been added to the legend of table 1.

Sincerely yours,

Claire Crossan – on behalf of all authors