November 1, 2016

Dear Editor,
Please find enclosed the edited manuscript in Word format (file name: 30224-article.docx)
Title: Clinical features of acute hepatitis E super-infections on chronic hepatitis B
Authors: Chong Chen, Shuye Zhang, Dandan Zhang, Xinyan Li, Yuling Zhang, Weixia Li, Jingjing Yan, Min Wang, Jingna Xun, Chuan Lu, Yun Ling, Yuxian Huang, Liang Chen

Name of Journal: World Journal of Gastroenterology

ESPS Manuscript NO: 30224

Thank you for reviewing our manuscript and the reviewers' valuable suggestions on this work. We have submitted the new version in accordance with your recommendations and the amendments are highlighted in the revised manuscript for easy identification. Reviewers' specific concerns were carefully addressed in the following response letter. To improve the manuscript, we have also requested the language editing service which made this article more readable and understandable for the readers. In addition, all the required documents listed as "30224-docu category" have been properly prepared. We hope now that the revision is acceptable and look forward to hearing from you soon.

Please note that some minor changes are made on the locked manuscript (provided by the language editing company - *Filipodia*). For clarification, we have provided the unlocked manuscript "30224-Unlocked revised manuscript" as the revised manuscript. These minor changes were listed below:

1) Shuye Zhang's name in the authorship has been moved to the second place, after Chong Chen;
2) The corresponding author's telephone is changed to "+86(21)37990333-8357";
3) For the grant support section, the 12th 5-Year National Science and Technology Major Projects (No. 2013zx10005002 to LC) should be removed.
4) In the introduction section, we added one sentence to state the reason of HEV infection in response to the reviewer (code.03664231). The sentence is as follows:
   "On the other hand, the meat industry in China is in the stage of rapid development, rapidly replacing the traditional family farms, which can lead to increased viral spreading between domestic animals and contamined meats will results in more human infections. "

Authors' request: We do not know the WJG's no permission for co-first or co-corresponding authorships until the final stages of the revision process. We certainly respect this policy and have made changes accordingly, and hope that our work can be accepted and published soon. However, we also feel strongly obligated to request the co-first authorship for both Dr. Chong Chen and Dr. Shuye Zhang, considering their equally important contributions to this work, and the importance for their future career development. Please take our request into serious account and this will be very helpful for the authors contributing to this work.

Thank you again for all the efforts in processing this manuscript and considering to publish it in the World Journal of Gastroenterology.

Yours sincerely,

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Answers to Specific Comments:

Reviewer code. 03664123

1. *What were the methods and kits used for the laboratory data collected in this study? It is better to describe the laboratory settings and methods used in the hospital with regard to the collected data.*

   Reply: Thank you for the suggestion. We have already added this section to the revised manuscript. Detailed information is as follows:
   Anti-HEV IgM level was determined in serum from patients by use of enzyme-linked immunosorbent assay (ELISA) (MP Biomedicals Asia Pacific, Singapore). Liver function was assessed by measuring standard serum markers using a fully-automated biochemical analyzer (7600 Series; Hitachi, Tokyo, Japan). HBV DNA levels were quantified by real-time PCR (ABI 7500; Applied Biosystems Inc., Foster City, CA, United States), with lower detection limit of 500 copies/mL. HBV serological markers were detected by ELISA (ARCHITECT i2000 SR; Abbott, Wiesbaden, Germany). The routine blood panel was detected with an automated hematology analyzer (XT-2000i; Sysmex, Kobe, Japan). Prothrombin time (PT) was measured by an automatic coagulometer (STA-R; Diagnostica Stago, Asnieres-sur-Seine, France). The PT-international normalized ratio (INR)

was calculated.

Reviewer code.03664231

1. *In the introduction section, authors should state why infection with HEV, which was previously thought limited to developing countries with poor sanitation, is now gradually recognized to be actually prevalent in developed countries as well, where the virus mainly causes sporadic symptomatic infections transmitted zoonotically, like in china.*

   Reply: Thank you for the notice. With the improvement of hygiene in China, the outbreaks of acute hepatitis E (caused by Genotype 1 HEV in China) is rarely seen anymore, however the number of sporadic HEV cases have not been reduced. Consistently, recent epidemiological study confirmed a high HEV prevalence. Genomic sequencing of the clinical isolates was found closely-related to swine HEV (mainly Genotype 4 HEV in China), strongly supporting the zoonotic transmission routes. Therefore, the increased incidence of sporadic HEV infection may reflect the increased zoonotic HEV exposure. Just like in the developed countries, the meat industry in China is in the stage of rapid development, rapidly replacing the traditional family farms, which can lead to increased viral spreading between domestic animals. Eventually, consumption of the contaminated meat products may cause increased clinical cases (*Current opinion in virology 2015, 10:34-41*).
   The corresponding content for this point has been added in the revised manuscript.

2. *Authors not provide future research direction of this topic. It will be interesting to for this HEV-HBV super-infection in immunosuppressed patient like HIV infected individuals.*

   Reply: Thank you for this comment. Indeed, certain populations, like HIV/AIDS patients, immunosuppressed patients and pregnant women may all differ largely from each other after HEV infection (*Kenfak-Foguuena A, et al. Emerg Infect Dis 2011;17:1074-8*). The data regarding these subgroup of patients are still scarce and more studies are needed. However, relatively few number of HIV infected patients and their complicated disease history preclude our further analysis here. Instead, we are planning to analyze a large cohort of HEV-infected pregnant women in an upcoming study.

3. *Page 7, lines 2-7, the authors should clarify whether the participants were selected.*

   Reply: Thank you for the comment. We have added this part to the

material and methods, as well as Figure 1.
From September 2009 to September 2014, 635 acute HEV-infected patients (showing clinical acute hepatitis symptomology and anti-HEV immunoglobulin M positivity) were admitted to the Shanghai Public Health Clinical Center, representing the regional tertiary hospital for infectious diseases in Shanghai city, China. Four hundred and seven of those patients were excluded from study due to pregnancy, other viral hepatitis etiology (e.g. hepatitis A, C or D virus) or infection with Epstein-Barr virus, herpes simplex virus, or human immunodeficiency virus; the remaining 228 HEV-HBV co-infection patients were included in this retrospective study (Figure 1).

4. *Page 9, lines 6 and 12, the authors mentioned these expressions: previous comorbidities and previous associated comorbidities. Did the two expressions mean the same thing? Could the authors list the different items?*

   Reply: Thank you for your comment. We claimed that the two expressions were equal. Extrahepatic underlying diseases include diabetes, hypertension, chronic respiratory diseases, chronic kidney diseases and extrahepatic tumors, which may affect the disease progression. Therefore, we evaluated the relevance of these comorbidities in our co-infected patients.

5. *In the material and methods section, it is important to mention how biochemical (Transaminase enzymes, bilirubin,…), hematological, HBV serological markers and HBV viral load were analyzed; If not it will be important to mention the different items of the clinical database of the hospital.*

   Reply: The reviewer's concern is important. We have added this section to the revised manuscript. Detailed content is as follows:
   Anti-HEV IgM level was determined in serum from patients by use of enzyme-linked immunosorbent assay (ELISA) (MP Biomedicals Asia Pacific, Singapore). Liver function was assessed by measuring standard serum markers using a fully-automated biochemical analyzer (7600 Series; Hitachi, Tokyo, Japan). HBV DNA levels were quantified by real-time PCR (ABI 7500; Applied Biosystems Inc., Foster City, CA, United States), with lower detection limit of 500 copies/mL. HBV serological markers were detected by ELISA (ARCHITECT i2000 SR; Abbott, Wiesbaden, Germany). The routine blood panel was detected with an automated hematology analyzer (XT-2000i; Sysmex, Kobe, Japan). Prothrombin time (PT) was measured by an automatic coagulometer (STA-R; Diagnostica Stago, Asnieres-sur-Seine, France). The PT-international normalized ratio (INR) was calculated.

6. *The result section is presented with comment as it was a discussion. The results should be presented without comment. Too many tables and only tables without figures seem to make the results too heavy and difficult to apprehend. This affects a great job like this. How to present the results should be examined.*

   Reply: Thanks for the suggestion. We have taken your advice and revised the results section. To make our results straightforward, we clarified them by adding one figure, which is presented in the revision.

7. *In Table 1 is « Past medical conditions » means previous comorbidities or previous associated comorbities?*

   Reply: Thank you for bringing this to our notice. 'Past medical conditions' in supplementary table 1 means pre-existing comorbidities. To keep consistency, we have already replaced it.

8. *Page 5, Line 19-22, in this part of introduction, authors present a short conclusion of this work. It should present in abstract and conclusion section.*

   Reply: Thank you for the advice. We have removed this short conclusion from the introduction section.

9. *In Table 1, the Baseline characteristics of the patients should be in the same line with death and survival.*

   Thanks for your helpful reminding. We have taken your advice and revised it.

Reviewer code. 01564209

1. *In the Material and Methods no information about molecular, serological and biochemical tests have been described. The used tests for the study are important and should be described.*

   Reply: Thank you for giving this precious opinion. We have already added this section to the revised manuscript. Detailed content is as follows:
   Anti-HEV IgM level was determined in serum from patients by use of enzyme-linked immunosorbent assay (ELISA) (MP Biomedicals Asia Pacific, Singapore). Liver function was assessed by measuring standard serum markers using a fully-automated biochemical analyzer (7600 Series; Hitachi, Tokyo, Japan). HBV DNA levels were quantified by real-time PCR (ABI 7500; Applied Biosystems Inc., Foster City, CA, United States), with lower detection limit of 500 copies/mL. HBV serological markers were detected by ELISA (ARCHITECT i2000 SR; Abbott, Wiesbaden, Germany). The routine blood panel was detected with an automated hematology

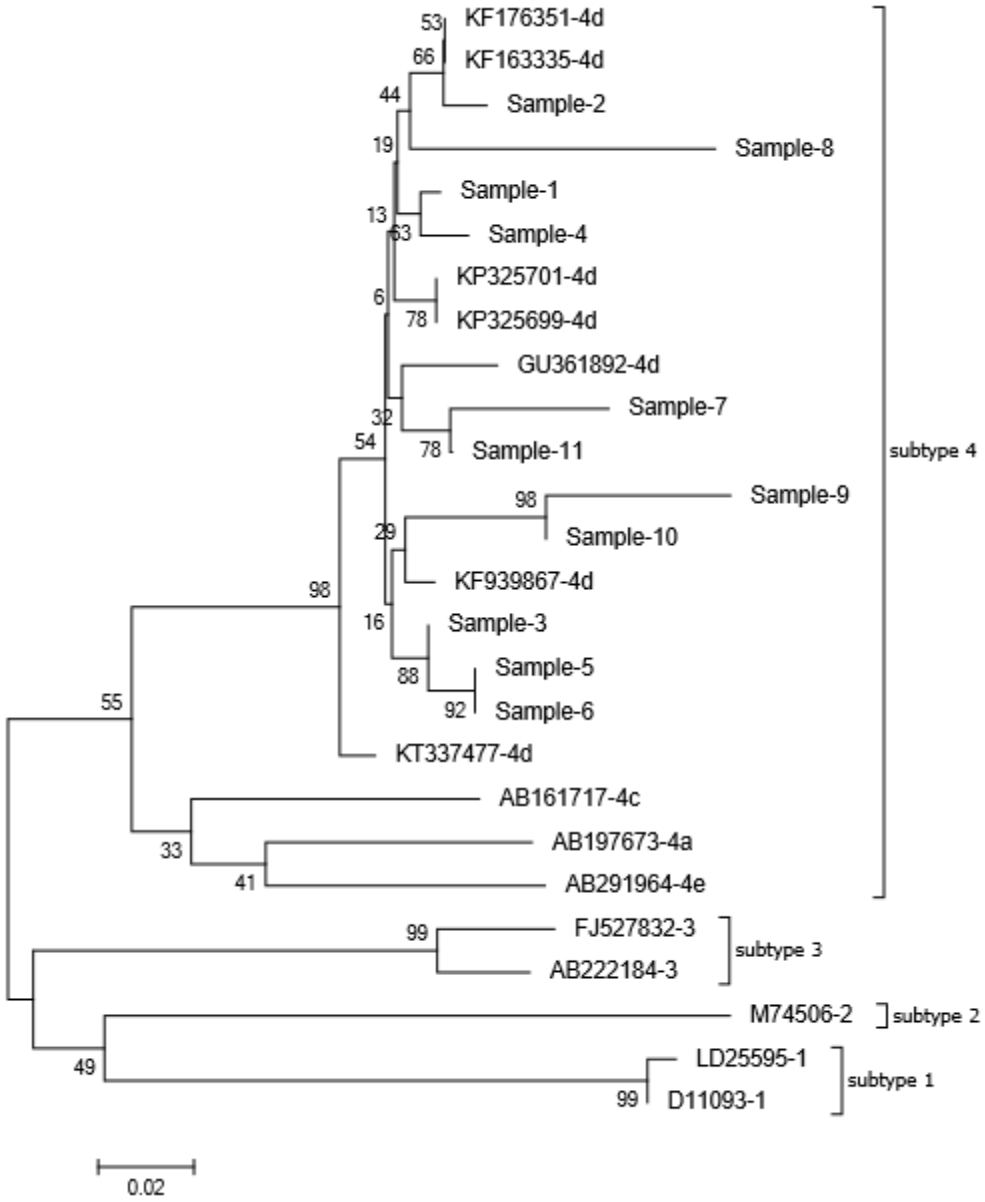
analyzer (XT-2000i; Sysmex, Kobe, Japan). Prothrombin time (PT) was measured by an automatic coagulometer (STA-R; Diagnostica Stago, Asnieres-sur-Seine, France). The PT-international normalized ratio (INR) was calculated.

2. *Were other co-infections excluded such as HCV, HDV, HIV?*

Reply: Thanks for the question. HCV, HDV and HIV were all excluded. For clarification, we have added the "patients selection protocol" to the material and methods.
From September 2009 to September 2014, 635 acute HEV-infected patients (showing clinical acute hepatitis symptoms and anti-HEV immunoglobulin M positivity) were admitted to the Shanghai Public Health Clinical Center, representing the regional tertiary hospital for infectious diseases in Shanghai city, China. Four hundred and seven of those patients were excluded from study due to pregnancy, other viral hepatitis etiology (e.g. hepatitis A, C or D virus) or infection with Epstein-Barr virus, herpes simplex virus, or human immunodeficiency virus; the remaining 228 HEV-HBV co-infection patients were included in this retrospective study (Figure 1).

3. *Which HBV genotypes were identified? Which HEV genotype?*

Reply: Thank you for your comment. HBV genotype was not detected since HBV genotype examination is not a clinical routine in China. Many previous studies have reported that genotype B and C are dominant genotypes in East Asia (*Liu CJ, et al, Semin Liver Dis. 2013 May;33(2):97-102*). Epidemiology study of HBV genotypes in China also showed that most common HBV genotypes were B (41%) and C (53%), while genotypes A and D were rare (*Zeng G, et al, J Viral Hepat. 2005 Nov;12(6):609-17*). So we speculate that the HBV genotypes of most our patients should belong to genotype B and C.
Regarding the HEV genotypes, recently we amplified a viral ORF2-ORF3 overlapping segment from 11 HEV-IgM+ HEV-RNA+ samples collected during the last several months. The PCR products was cloned in T/A vector and sent for Sanger-Sequencing. The 11 sequences and corresponding sequences from some reference HEV genomes were compared to construct the genetic tree using the neighbor-joining method and evaluated by the interior branch test method with MEGA4 software (www.megasoftware.net). The tree is as follows, which clearly indicated our samples all belonged to genotype 4.



Although epidemiology study of HEV genotypes showed genotype 1, 2 and 4 were found in China. However, the prevalent HEV genotype in eastern China including Shanghai belong to genotype 4 (*Nassim Kamar, Lancet 2012; 379:2477–88*). So, we believe this data strongly indicate that most our cases have genotype 4 HEV infections.

4. *Which treatment option for HBV therapy was used?*

Reply: Thank you for the comment. Some of our patients had take Nucleos/tide analogues (NUCs) as treatments before the admission. After admission, only NUCs and not IFN were used for HBV therapy. As we know, IFN is contraindicated in patients with decompensated cirrhosis due to the risk of severe sepsis and worsening liver failure and IFN is also not recommended in patients with compensated cirrhosis and evidence of

portal hypertension (*Konerman MA, et al, Clin Liver Dis. 2016 Nov;20(4):645-665.*). Beyond that, IFN has a finite duration, with poor tolerance and is administered by subcutaneous injections with adverse events (including flu-like symptoms, neurocognitive disturbances and haematological toxicity) (*Vallet-Pichard A, et al, Therapy Adv Gastroenterol. 2014 Jul;7(4):148-55*). So, we are afraid if there is any risk for the HBV-HEV co-infected patients and reserved its use.

5. *Was there also treatment option (IFN and/or Ribavirin) for HEV?*

   Reply: Thank you for the comment. Indeed, immunosuppressed patients with chronic hepatitis E might benefit from this antiviral treatment. But for the normal care of acute HEV infection, this is not mandatory and usually the disease is self-limiting (*World Health Organization, WHO*). Therefore, IFN and/or Ribavirin were not used for treating these patients.

6. *Introduction section: Describe a summary of the findings of the authors at the end of this chapter. This is better for discussion section.*

   Reply: Thanks for your suggestion. We have removed this summary from the introduction section.

7. *Results section: Was there any control group in comparison to the HBV/HEV group? If describing the risk factors, like HBeAg status, alcohol usage, gender, kidney diseases, these factors will also have an pivotal impact on mono HBV-infection. For that, the statement that HEV superinfection will worsen the clinical outcome of CHB is not really supported by the presented data.*

   Reply: Thank you for the question. We agree with reviewer that it may be better to have either mono-HBV or mono-HEV group for more comparisons. However, the studies of HBV-HEV coinfection versus mono-infections had been reported before. Previous studies already showed that co-infected patients had worse outcomes than mono-infected ones *(Shalimar, et al, J Gastroenterol Hepatol. 2016; 31(4):856-64)* (*Cheng SH, et al, World J Gastroenterol. 2013; 19(35):5904-9)*.
   Here, rather than asking whether HEV superinfection will worsen the CHB, our aim is to find whether the underlying CHB status affected the short-term outcome during HEV super-infections. Certainly the CHB status will affect the patients' outcome in a long-run if there is no HEV superinfection, however, these will be the long-term outcomes. However, under the context of acute HEV superinfection in this study, we are focusing on the short-term outcomes which are certainly different from the long-term outcome during CHB.
   Therefore, we were investigating the involvement of CHB-related cirrhosis,

immunological phases, HBV serum markers, HBV viral load and anti-viral treatments in short-term outcomes in the context of acute-HEV infections rather long-term outcomes in the context of chronic-HBV infections. Risk factors like alcohol usage, gender, kidney diseases, previous diabetes and chronic kidney diseases might play roles in both context, and these risk factors were analyzed in the multivariate logistic regression analysis to adjust for the confounding factors. So we think that the lack of mono HBV- or HEV- infections group did not prevent us to draw conclusions.

8. *There is a recent report of Hoan et al (EBioMedicine 2015; 2080–2086) showing also the impact of HEV superinfection on CHB. This paper should be also discussed in the discussion section.*

   Reply: Thanks for the recommendation. We have carefully read this paper. This study revealed high prevalence of HEV infection between large cohort HBV patients and healthy individuals, and showed that HEV superinfection affected the outcome and progression of HBV-related liver diseases. We have cited this important paper in the revised manuscript.

In summary, we greatly appreciate the reviewers' helpful comments and suggestions. We have revised the manuscript according to all the points raised by the reviewers. We hope that it is now suitable for publication. Thank you!