Rebuttal letter

- Authors' responses to the comments of editor and reviewers

Editor's comments

(1) I found the authors did not provide the approved grant application form(s). Please upload the approved grant application form(s) or funding agency copy of any approval document(s);

Authors' response: We thank editor to point it out, the approved grant application form has been enclosed in the resubmission package.

(2) I found the authors did not provide the original figures. Please provide the original figure documents. Please prepare and arrange the figures using PowerPoint to ensure that all graphs or arrows or text portions can be reprocessed by the editor;

Authors' response: Following editor's suggestion, the original figures have been arranged using PowerPoint and enclosed in the resubmission package.

(3) I found the authors did not add the PMID and DOI in the reference list. Please provide the PubMed numbers and DOI citation numbers to the reference list and list all authors of the references. Please revise throughout.

Authors' response: We thank editor for pointing it out. The references have been formatted according to the journal's requirement, and PMID and DOI of the references were now added to the reference list.

(2) Editorial office director: I have checked the comments written by the science editor.

Author's response: Thanks for your effort.

(3) Company editor-in-chief: I have reviewed the Peer-Review Report, the full text of the manuscript and the relevant ethics documents, all of which have met the basic

publishing requirements, and the manuscript is conditionally accepted with major revisions. I have sent the manuscript to the author(s) for its revision according to the Peer-Review Report and the Criteria for Manuscript Revision by Authors. Before final acceptance, authors need to correct the issues raised by the editor to meet the publishing requirements.

Authors' response: Thanks for your efforts. We have carefully reviewed the reviewers' comments and responded point-to-point (see below), and we hope all raised issues have been sufficiently addressed and amended in the revised manuscript.

Reviewer's Comments to the Author:

Reviewer #1:

1. An ethical approval is missing in the text.

Author's response: We thank reviewer for pointing it out. The ethical statement now is included in the revised manuscript on the page 11.

2. The authors speculate that the farmer have been infected previously before the onset of LC and HCC by HEV. It would be important to know which HEV genotype was responsible for the positive HEV serology. Therefore if the HEV infection went chronically it could only be by HEV genotype 3 or 4. Therefore HEV genotyping by NAT would be necessary.

Authors' response: We totally agree with reviewer and believe that the knowledge of HEV genotype is important to define chronic HEV infection. Unfortunately, the genotype of the patient was not known since the HEV genotype was not routinely detected in clinical practice in China. That is because chronic HEV is most predominantly caused by the genotype 4 in China since 2000 [1, 2]. And genotype 3 is found much less frequent. Only sporadic cases of chronic HEV have been attributed to other genotypes.

We acknowledge that the lack of the HEV genotype confirmation is a limitation, and have added bit discussion on this issue in the revised manuscript, please kindly refer to line 18-25 on page 9 in the new version of the manuscript. We hope that by doing so we could raise this issue in clinicians and researchers, especially in China, and advocate the routine detection of HEV genotype and RNA in all related clinical departments, research settings, and surveillance centers.

3. No data was given whether the patient survived?

Authors' response: We thank reviewer for pointing it out. Following reviewer's suggestion, we contacted the patient and confirmed that the patient is alive, however, he refused to be followed-up and accept any treatment since September 2018.

Reviewer #2:

1. However, the chronic HEV infection (as illustrated in the first sentence of Discussion) was undefined for the positive HEV-IgG and negative HEV-IgM but without HEV-RNA positive. I can not find the HEV-RNA result of this patients all over the paper.

Authors' response: We appreciate reviewer for this comment. The HEV-RNA result of the patient could be found between line 3-4 of page 7. The HEV RNA is undetectable maybe because that the HEV virus load was too low. However, the immune system of the patient had already responded to the virus invasion, thus the antibody of HEV was detectable. We mentioned this result at the beginning of that section as well in the revised manuscript to emphasize bit this result.

2. The reason of splenectomy in 2016 and Hepatoctomy was carried out after TACE and RFA in 2017 but not before should be demonstrated.

Authors' response: We thank reviewer for this comment. In the revised manuscript, following reviewer's suggestion the reason of splenectomy has been added to the manuscript between line 24 to 29 on page 5.

The hepatectomy was not performed in June 2017 when tumor at multiple sites were detected by imaging examinations, instead TACE and RFA were conducted according to the international guidelines of liver cancer [3, 4]. To make this clear to the reader, we included a brief explanation in the revised manuscript, and please kindly refer to page 6 (line 27-30) and page 7 (line 13-17) in the new version of the manuscript.

3.AFP and PIVKA should be demonstrated in Table 1.

Authors' response: We thank reviewer for pointing this out, and totally agree with reviewer that PIVKA could provide important information for HCC diagnosis.

The result of AFP could be found in Table 1. Unfortunately, since the clinical data was retrospectively collected, we feel regret that we cannot provide the results of PIVKA. The main reason is according to the Chinese guideline for diagnosis of liver cancer, PIVKA is not defined as a primary serum biomarker and not routinely detected in clinical practice[5]. And some studies reported that AFP overperforms PIVKA in discriminating HCCs [6]. Further, the CT and MRI examinations provided strong evidences for diagnosing liver cancer in this case, therefore, no PIVKA test was subscripted by the doctor. We have discussed this issue in line 25 to 30 on page 9.

4. Moreover, some sentence is confusing, for instance, "In addition, no case of repeated HEV infection has been reported, evidence by persistent dual positivity of HEV-IgG and HEV-IgM" .

Authors' response: We appreciate this comment by reviewer. In the revised manuscript, we amended the text between line 12 and 14 on page 5 . The new sentence is written as follow: "In addition, the case with repeated HEV infection has not been reported to our knowledge, which was defined by persistent dual positivity of HEV-IgG and HEV-IgM.".

5. The paper should be carefully revised, for instance, blood pressure should be 122/85 mmHg better than 85/122 mmHg.

Authors' response: We appreciate this comment by reviewer. We changed this sentence in the revised manuscript at line 8 on page 5. And we have improved the manuscript by performing English language polishing throughout of the text.

Reference:

1 Wang Y. Epidemiology, molecular biology and zoonosis of genotype IV hepatitis E in China. . *Chinese journal of Epidemiology* 2003; **24(7)**: 618-622

2 Zhang W, He Y, Wang H, Shen Q, Cui L, Wang X, Shao S, Hua X. Hepatitis E virus genotype diversity in eastern China. *Emerg Infect Dis* 2010; **16**(10): 1630-1632 [PMID: 20875298 DOI: 10.3201/eid1610.100873]

3 Heimbach JK, Kulik LM, Finn RS, Sirlin CB, Abecassis MM, Roberts LR, Zhu AX, Murad MH, Marrero JA. AASLD guidelines for the treatment of hepatocellular carcinoma. *Hepatology* 2018; **67**(1): 358-380 [PMID: 28130846 DOI: 10.1002/hep.29086]

4 EASL Clinical Practice Guidelines: Management of hepatocellular carcinoma. *J Hepatol* 2018; **69**(1): 182-236 [PMID: 29628281 DOI: 10.1016/j.jhep.2018.03.019]

5 Department of Medical Administration NHaHCotPsRoC. [Guidelines for diagnosis and treatment of primary liver cancer in China (2019 edition)]. *Zhonghua gan zang bing za zhi* 2020; **28**(2): 112-128 [PMID: 32164061 DOI: 10.3760/cma.j.issn.1007-3418.2020.02.004]

6 Choi J, Kim GA, Han S, Lee W, Chun S, Lim YS. Longitudinal Assessment of Three Serum Biomarkers to Detect Very Early-Stage Hepatocellular Carcinoma. *Hepatology* 2019; **69**(5): 1983-1994 [PMID: 30153338 DOI: 10.1002/hep.30233]