Author's response to reviews

Title: High prevalence of HCV infection and associated risk factors in a Chinese population: a cross-sectional study We had revised the title as "Use of parenteral caffeinum natrio-benzoicum: an underestimated risk factor for HCV transmission in China"

Authors:

Hongqin Xu (hongqinxu11@163.com)
Ge Yu (fishyuge@163.com)
Haibo Sun (13578711604@163.com)
Juan Lv (15943021175@163.com)
Moli Wang (lndlwm1@163.com)
Fei Kong (kongfei531@163.com)
Mingyuan Zhang (mingyuanzhang@hotmail.com)
Xiumei Chi (xiumeichi@hotmail.com)
Xiaomei Wang (xiaomeiwang2010@aliyun.com)
Ruihong Wu (wuruihong1984@163.com)
Xiuju Gao (leona.1985@163.com)
Jing Jiang (yupan20000@163.com)
Yu Pan (yupan20000@163.com)
Junqi Niu (junqiniu@aliyun.com)

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Author's response to reviews: see over
Dear Prof. Proel Vargas;

Thank you very much for the decision letter and your valuable advice pertaining to our manuscript (No. 1754695755157592) titled “High prevalence of HCV infection and associated risk factors in a Chinese population: a cross-sectional study”. We also wish to extend our gratitude towards the reviewers for their constructive feedback and suggestions.

Please find enclosed with this letter, a revised version of the manuscript addressing the concerns and incorporating the recommendations of reviewers. The response to individual comments is also reproduced below for your reference. Further, due care has been taken to correct the typographical and grammatical errors and improve the quality and flow of the text.

We hope that the revised manuscript will qualify the criteria for publication in your esteemed journal.

Look forward to hearing from you soon.

Yours sincerely,

Yu Pan
Response to Reviewer 1

Major points

Comment 1: The title should be changed and more reflects the main finding of the authors. For example: “use of parenteral caffeinum natrio benzoicum: a underestimated risk factor of HCV transmission in China”

Response: Thank you for your suggestion. We have changed the title as per the advice, which now reads as “Use of parenteral caffeinum natrio-benzoicum: an underestimated risk factor of HCV transmission in China”.

Comment2: The authors should provide more information about this drug, caffeinum natrio-benzoicum and its use worldwide”

Response:
Several modifications have been effected in the “Introduction ”section of the revised manuscript (Page 3, Lines 58-68).
“Caffeinum natrio-benzoicum (CNB), also known as caffeine and sodium benzoate, is used as a psychotropic drug. Caffeine has an excitatory effect on the nervous system, while sodium benzoate assists in solubilization and absorption of caffeine. In the 1970s, certain population groups in north, northeast and northwest regions of China started abusing CNB through oral or nasal inhalation (snorting). Around the same time, residents in Fuyu developed the habit of recreational use of parenteral CNB during community celebrations. Similar recreational use of CNB has not been reported from any other part of the world. Parenteral abuse of caffeinum natrio-benzoicum during celebrations has earlier been implicated as a risk factor for acquisition of HCV and hepatitis B virus (HBV) infection in China [2]. Caffeinum natrio-benzoicum was
listed as one of psychotropic drugs and got more strict regulations by the Chinese government in 1988.”

**Comment3**: (Methods): The authors should clarify whether this study was a population-based study. How were participants selected? This is unclear. And what were the inclusion and exclusion criteria?”

**Response:**

Yes, our study was a population-based study. The following additional information has been added in the Methods section in the revised manuscript. (Page 4, Lines 76-92)

“**Study population and recruitment**

The target population was permanent residents in Fuyu City. In September 2012, we conducted a cross-sectional survey using non-random, convenience sampling in Fuyu City, Jilin Province, China. In the first stage, five survey locations (Township Health Center) were obtained from all the 5 towns in Fuyu City (Desheng, Gengxin, Wanfa, Dalinzi and Gongpengzi). In the second stage, more than 100 village committee members and rural doctors in all the 5 towns in Fuyu City were recruited and trained together on making publicity for the survey procedures for 2 days. Than they make publicity in their village, which is necessary for the investigators in survey site used cluster sampling. At the same time, our outreach workers passed out recruitment cards and posted flyers in villages. In the third stage, our research team procedure the survey for 5-8 days at each survey location. Current study participants were also encouraged to inform their peers about the study.

Individuals were eligible to participate, if they were current residents of Fuyu city, and consented to undergo laboratory investigations for HCV, HBV and HIV. After screening for eligibility and obtaining informed consent, each subject was made to complete a questionnaire. Participants
who did not complete the questionnaire were excluded from the study. Subjects were asked to return 4-6 weeks later to receive the laboratory test results. Those subjects who were diagnosed with HCV and/or HBV infection were referred to a medical center for further treatment and care.”

Comment 4 “2) Methods: The authors should give figures: Five survey locations (rural counties) were shown to have a higher prevalence of Hepatitis B during the epidemiological investigation of Hepatitis C virus infection” in Fuyu City of Jilin Province in the First Hospital of Jilin University. Higher? compared to what? Please clarify

Response:
As the study is not designed to draw a comparison of HCV and HBV infection between different areas in the method section, the sentence has been deleted in the revised manuscript.

Comment 5 “3- The authors should describe the method for HCV genotyping.”

Response:
The following additional information has been included in the “Methods” section (Page 5, Lines 110-112):

“HCV genotyping was performed by multicolor fluorescence polymerase chain reaction (PCR) using an HCV-RNA genotyping kit (BioAssay Science & Technology Co. Ltd., Beijing, China).”

Comment 6 “2) Methods

4- In the ethical considerations, the authors should add more information and should state somewhere that data were anonymously recorded and how they were recorded.

Response:
The following additional information has been added in the sub-section “Ethical considerations” in the “Methods” section (Page 6-7, Lines 127-136).
“Written informed consent was obtained from all participants before initiating any study-specific procedure. Due care was taken to incorporate specific protocols aimed at maintaining data confidentiality, as well as to protect the subjects against potential ethical violation. Data collection was usually conducted at the township Health Center. At the time of filling of questionnaire by the participants, presence of both interviewee and interviewer was made mandatory. All necessary precautions for obtaining data confidentially were taken (the questionnaire was applied only in the presence of the interviewee and interviewer). Those agreeing to take part in the study were asked to have their blood collected at the university hospital laboratory for further evaluation of anti-HCV and anti-HBV activity, always in the presence of the researcher.”

**Comment 7** “Results: In the results: 3228 participated to the study but 9 were not included, the authors should provide reasons why these participants were excluded.

**Response:**

A sentence had been added in the Results of the revised manuscript (Page 7, Lines 139-140) to address this issue.

“Data pertaining to 9 participants was excluded due to incomplete or unreliable information.”

**Comment 8** A table should describe the clinical and biological/virological characteristics of the HCV positive participants.

**Response:**

Thank you for your comment. Considering that the major objective of this manuscript was demonstrating the most important risk factors for HCV transmission in Fuyu city, and space lacks for a detailed description of it in text, so we have added this table as an additional table in the revised manuscript as below:
**Additional Table.1 Clinical and virological characteristics of the HCV positive participants**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Median (P25,P75)</th>
<th>N (%)</th>
<th>reference ranges</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>54.5(48.5, 60.2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>797(58.8%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>558(41.2%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HCV Genotype *</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1a</td>
<td>426(58.0%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2b</td>
<td>295(40.2%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1a/2b</td>
<td>13(1.8%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HCV load (log(_{10}) IU/ml)</td>
<td>5.76(2.44,6.77)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver function</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>35(25,58)</td>
<td>8-40</td>
<td></td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>37(22,67.8)</td>
<td>8-50</td>
<td></td>
</tr>
<tr>
<td>ALP(U/L)</td>
<td>77(64,94.8)</td>
<td>15-112</td>
<td></td>
</tr>
<tr>
<td>GGT(U/L)</td>
<td>39(22,80)</td>
<td>5-54</td>
<td></td>
</tr>
<tr>
<td>Total Protein(g/l)</td>
<td>77(73.3,81)</td>
<td>60-83</td>
<td></td>
</tr>
<tr>
<td>Albumin (g/L)</td>
<td>44.9(42.6,47.4)</td>
<td>35-55</td>
<td></td>
</tr>
<tr>
<td>total bilirubin(μmol/L)</td>
<td>13.6(9.8,18.5)</td>
<td>6.80-30.00</td>
<td></td>
</tr>
<tr>
<td>Direct Bilirubin(μmol/L)</td>
<td>4.3(3.2,5.7)</td>
<td>0-8.60</td>
<td></td>
</tr>
<tr>
<td>Cholinesterase (U/L)</td>
<td>7999(6488,9620)</td>
<td>4300-12000.00</td>
<td></td>
</tr>
<tr>
<td>Platelet (10(^9)/L)</td>
<td>185(145,226)</td>
<td>100-300</td>
<td></td>
</tr>
</tbody>
</table>

*HCV-RNA genotyping was performed on 873 subjects while 139 was not typeable due to low HCV-RNA levels.

ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, glutamyl transpeptidase; ALP, alkaline phosphatase."
Comment 9 “Why the authors did not assess HIV serology or do they have the results. It would be very informative to get the results.

Response:

Thanks for your thoughtful suggestion. Actually, we did assess HIV serology in our study (Page X, Lines X-Y). and our results showed that there were negative HIV results in our samples. The following sentence has been added in the “Results” section (Page 7, Lines 151-152):

“None of the study subjects tested positive for HIV infection.”

Comment 10 “6- The authors should give the median viral load in the results.

Response:

Necessary information had been added in the revised manuscript and Additional file.1 (Page 7, Lines 148-150)

“Hepatitis C virus load range from 0 IU/ml to 111700000 IU/ml and the median viral load was 577000 IU/ml among HCV positive participants.”

Comment 11 “7- How do the authors explain that HBV infection, ear piercing and tattoo protect against HCV infection?

This statement deserves a reference, in particular since HBV transmission is mainly observed from mother-to-child transmission in China”

Response:

Thanks for raising this critical issue. As the confidence intervals (CI) cross 1(0.62-1.02), HBV infection should not be reported as significantly associated with HCV infection. We had revised this mistake in the revised manuscript and tried to explain that earing piercing and tattoo protect
against HCV infection as below:

The following additional information has been added in the “Discussion” section (Page 11, Lines 218-226):

“In addition to syringe-sharing, sharing of injecting paraphernalia is a well-recognized risk factor for HCV infection [16]. However, in the present study ear piercing and tattooing were significantly associated with a decreased risk of HCV infection on univariate analysis. Since the older ages and male gender are risk factors for HCV infection in our study, this outcome could have resulted from the influence of age and sex (to that there were Females and youngsters are more likely to be involved in these practices). After adjusting for ages, gender and other potential confounders, the association between the history of ear piercing or tattooing was not significant on multivariate analysis. As PCNBSS is reported popular in older (>50 years) male group, all above can deem that PCNBSS was the independent risk factor of HCV transmission in Fuyu city.”

Comment12 “In the discussion, the authors should discuss whether the parenteral Caffeine and sodium benzoate was previously reported in China and in other countries. If it is still used.

Response:

Thank you for your comment, the abuse of parenteral Caffeine and sodium benzoate was previously reported in China, but not in the other countries. The information had been added in the “introduction” section (Page3-4, Line 63-68).

“Around the same time, residents in Fuyu developed the habit of recreational use of parenteral CNB during community celebrations. Similar recreational use of CNB has not been reported from any other part of the world. Parenteral abuse of caffeinum natrio-benzoicum during celebrations has earlier been implicated as a risk factor for acquisition of HCV and hepatitis B virus (HBV) infection in China [2].”
“As parenteral caffeine and sodium benzoate is still being used as a prescription drug in China, the potential for abuse of the drug needs to be highlighted.” The information has been added in the “Discussion” section (Page 12, Line 257-259).

**Comment13.** Conclusion “Fuyu has a higher HCV prevalence and we observed: compared to ??”

**Response:**
Correction has been made in the revised manuscript (Page 12, Line 259-262) as below:

**Conclusion:** The prevalence of HCV infection is likely to be high among residents in Fuyu and we observed that genotypes 1b and 2a dominated in the city. Our finding support the transmission of HCV was mainly due to PCNBSS which become endemic in Fuyu city during 1970s-1980s. More attention should be paid to routine screening for HCV in high-risk areas.”

**Replies to Reviewer2**

**Major points**

**Comment1:** The study does not contain a detailed discussion of the limitations of the study methodology used. What biases might be expected from convenience sampling in this population? What are the limitations of relying upon self-reported risk factors? What data on potential confounding variables are missing?”

**Response:**
A statement regarding limitations of this study has been added in the “Discussion” in the revised manuscript (Page 12, Lines 251-255)
“Use of a non-random convenience sample, and, self-reporting of at-risk behaviors by subjects, are key limitations of our study. The study sample may not be representative of the general population in Fuyu. Similarly self-reporting by subjects could have been affected by recall bias.”

However, to minimize recall bias, we used other short questions for substance use and injection behavior recall (such as when and where did you take the PCNBSS).

Comment2. Line 174-175: Where is the data that supports the conclusion that “The rate and geographical pattern of prevalence of antibodies to HCV in Fuyu and surrounding villages confirmed the risk factor (PCNBSS) in our study”? It appears that no data on geographical pattern were presented?

Response:
Appropriate reference has been included in the revised manuscript (Reference No.11)
(Page 9, Line 193):
“In our earlier study conducted in Changchun ling, a village near Fuyu City, where the custom of PCNBSS abuse during ceremonies was not practiced, the prevalence of HCV infection was found to be only 3.9% [11]. These contrasting findings strongly implicate PCNBSS as a factor responsible for the wide variability in the prevalence of HCV infection in two geographically contiguous areas.”

Comment3. Line 201-205 The lack of association between ear piercing and tattooing and HCV in the multivariate analysis is ascribed to the fact that those so-exposed were young and female. How would this protect them from HCV? Is it proposed that the study was underpowered in this group to detect an increase in HCV? The logic here needs explanation.

Response: The following additional information has been added in the “Discussion” section (Page 11, Lines 218-225):
“However, in the present study ear piercing and tattooing were significantly associated with a decreased risk of HCV infection on univariate analysis. Since the older ages and male gender are risk factors for HCV infection in our study, this outcome could have resulted from the influence of age and sex (to that there were Females and youngsters are more likely to be involved in these practices). After adjusting for ages, gender and other potential confounders, the association between the history of ear piercing or tattooing was not significant on multivariate analysis. As PCNBSS is reported popular in older (>50 years) male group, all above can deem that PCNBSS was the independent risk factor of HCV transmission in Fuyu city.”

Comment4. Line 215-217 How would participants being unaware of their HCV status change their self-reported behaviours, with regard to a total population which presumably was also largely unaware of its HCV status?

Response:

There are language errors in this part and we had corrected in the revised manuscript (Page 11, Line 237-244) as below:

“Some HCV positive subjects were not aware of their seropositive status before their enrollment in this study. It is likely that in these subjects the risk behaviors for HCV transmission may have been influenced by their new found seropositive status, possibly contributing to minimize the occur of risk behaviors such as sharing toothbrush with families. Furthermore, these HCV positive subjects are willing to get earlier treatment to prevent from development of chronic liver disease and hepatocellular carcinoma. Therefore, there is a pressing need for one-time screening for HCV infection to populations who had risk behaviors.”

Comment5. Line 235-238 How does individual risk factors being common point to high HCV prevalence being due to PCNBSS? Why does the final conclusion recommend healthcare
assurance quality measures when no mention has been made of them to this point, and how might they be expected to improve the situation? The logical flow in the conclusion generally seems flawed.

**Response:** We had modified the “conclusion” section in the revised manuscript as below: (Page 13, Line 261-264).

“The prevalence of HCV infection is likely to be high among residents in Fuyu and we observed that genotypes 1b and 2a dominated in the city. Our finding support the transmission of HCV was mainly due to PCNBSS which become endemic in Fuyu city during 1970s-1980s. More attention should be paid to routine screening for HCV in high-risk areas.”

**Minor Essential Revisions:**

**Comment6.** Abstract. Methods. Line 29-33. No mention is made of a survey tool, to assess risk factors.

**Response:** The following additional information has been included in the abstract (Page 2, Lines 28-31):

“Recruitment of study subjects involved a cross-sectional survey using non-random, convenience sampling. Information on demographic variables, risk factors for HCV infection, clinical manifestations, behavioral practices and family history was collected by administering a questionnaire.”

**Comment7.** Abstract Line 41 (and many instances throughout paper). P-values <0.001 could just be reported assuch, not reported exactly with E- numbers.

**Response:**

Thank you for your suggestion. The necessary revisions have been effected in the revised manuscript.
Comment 8. Line 61-63. This statement of the previous implication of PCNBSS as a risk factor for HCV needs a reference.

Response:
Appropriate reference has been included in the revised manuscript (Reference No.2) (Page 3, Lines 66)

Comment 9. Line 136-137. HBV infection is reported as significantly associated with HCV positivity on bivariate analysis, but the confidence intervals cross 1 (0.60-1.02), suggesting it is in fact not significant. This needs revision.

Response:
Thank you very much for you modification. Necessary corrections have been made in the revised manuscript (Page 8, Line 161-163).

“Factors negatively associated with HCV infection were not farming occupation (OR=0.23, 95%CI: 0.16-0.33), ear piercing (OR=0.54, 95%CI: 0.47-0.62), blood donation (OR=0.35, 95%CI: 0.20–0.59), and tattoo (OR=0.34, 95%CI: 0.22–0.53).”

Comment 10. Line 135 “occupation” is listed as associated with HCV positivity but it is not made clear in the text what occupations were associated with HCV (it is however clear in the table).

Response:
The necessary information has been added (Page 8, Line 161)

“Factors negatively associated with HCV infection were not farming occupation (OR=0.23, 95%CI: 0.16-0.33), ear piercing (OR=0.54, 95%CI: 0.47-0.62), blood donation (OR=0.35, 95%CI: 0.20–0.59), and tattoo (OR=0.34, 95%CI: 0.22–0.53).”
Comment 11. Multiple language errors, too numerous to mention here. Examples include “who’s” (line 36), “to be independently variables” (line 41), “got more strict regulations” (line 60), “elicit” (line 82), no verb in sentence on line 118 etc.

Response:

Thank you for your comment. We had revised these errors as below:

Examples include “whose” (line 35), “to be independently associated with” (line 42), “Caffeinum natrio-benzoicum was listed as one of psychotropic drugs and the abuse of it has been forbidden from 1988 in China.” (line 67), “race” (line 94), “Out of 3219 study subjects, 2,182(67.8%) were aged between 40–60 years.”(Line 140-141) etc.

Due care has been taken to correct the errors and improve the use of language. The revised manuscript has been meticulously proofread.
Discretionary Revisions

Comment 12. Consider changing the title to include a reference to PCNBSS as the key risk factor in this population, or at least the fact that a novel risk factors has been found. eg. “High prevalence of HCV infection, associated with a novel risk factor, in a Chinese population: a cross-sectional survey.”

Response:
The title has been changed as suggested:

“Use of parenteral Caffeine and sodium benzoate: a underestimated risk factor of HCV transmission in China”

Comment 13. Consider rewording “teeth repair” to “prior dental surgery”.

Response:
The necessary change has been effected in the revised manuscript.

Comment 14. More background on PCNBSS, and any prior research on its relationship to bloodborne virus exposure would add interest and value to the study.

Response:
The following additional information has been included in the “Introduction” section (Page 3-4, Lines 58-67):

“Caffeinum natrio-benzoicum (CNB), also known as caffeine and sodium benzoate, is used as a psychotropic drug. Caffeine has an excitatory effect on the nervous system, while sodium benzoate assists in solubilization and absorption of caffeine. In the 1970s, certain population groups in north, northeast and northwest regions of China started abusing CNB through oral or nasal inhalation (snorting). Around the same time, residents in Fuyu developed the habit of
recreational use of parenteral CNB during community celebrations. Similar recreational use of CNB has not been reported from any other part of the world. Parenteral abuse of caffeinum natrio-benzoicum during celebrations has earlier been implicated as a risk factor for acquisition of HCV and hepatitis B virus (HBV) infection in China [2]. Caffeinum natrio-benzoicum was listed as one of psychotropic drugs and the abuse of it has been forbidden from 1988 in China.”

Comment 15. What next? Based on your assessment of the validity of the manuscript, what do you advise should be the next step?

Response:

The following additional information has been included in the “Discussion” section.

(Page 12, Lines 245-250):

“PCNBSS appears to be the most important risk factor for HCV infection in Fuyu. Other factors also contribute to the transmission of HCV in the city. Ongoing research is required to assess the emerging risk of transmission. Most of the HCV infected subjects in this study were males. Further research is needed to understand how familial transmission occurs with regard to spouses and children. Given the high risk of infection transmission among family members of HCV-infected individuals, awareness raising activities are urgently needed.”

 Replies to Reviewer3

Major Compulsory Revisions

Comment 1. At methods, it is informed that a survey using non-random convenience sampling was used. It is not clear how the recruitment was done and the inclusion and the inclusion and exclusion criteria. These characteristics should be included in the text.
Response: Thanks for your thoughtful suggestion. The following additional information has been added in the “Methods” section in the revised manuscript (Page 4-5, Lines 74-91):

"Study population and recruitment

The target population was permanent residents in Fuyu City. In September 2012, we conducted a cross-sectional survey using non-random, convenience sampling in Fuyu City, Jilin Province, China. In the first stage, five survey locations (Township Health Center) were obtained from all the 5 towns in Fuyu City (Desheng, Gengxin, Wanfa, Dalinzi and Gongpengzi). In the second stage, more than 100 village committee members and rural doctors in all the 5 towns in Fuyu City were recruited and trained together on making publicity for the survey procedures for 2 days. Than they make publicity in their village, which is necessary for the investigators in survey site used cluster sampling. At the same time, our outreach workers passed out recruitment cards and posted flyers in villages. In the third stage, our research team procedure the survey for 3-5 days at each survey location. Current study participants were also encouraged to inform their peers about the study.

Individuals were eligible to participate, if they were current residents of Fuyu city, and consented to undergo laboratory investigations for HCV, HBV and HIV. After screening for eligibility and obtaining informed consent, each subject was made to complete a questionnaire. Participants who did not complete the questionnaire were excluded from the study. Subjects were asked to return 4-6 weeks later to receive the laboratory test results. Those subjects who were diagnosed with HCV and/or HBV infection were referred to a medical center for further treatment and care.”

Comment 2. At methods, authors informed that used an enzyme-linked immune sorbent assay (ELISA), however they used Abbott ARCHITECT i2000SR (Abbott Laboratories, Abbott Park, IL, USA), explained this.
Response:

The following corrections have been made in the “Methods” section”(Page 5, Line 102-105)

Blood samples were tested for Hepatitis B surface antigen (HBsAg), and antibodies against HBsAg (anti-HBsAg), Hepatitis B core antigen (anti-HBc), Hepatitis C (Anti-HCV)and HIV, using Abbott ARCHITECT i2000SR Immunoassay System (Abbott Laboratories; Abbott Park, IL, USA) at the clinical laboratory of the First Hospital of Jilin University.”

Comment 3. At methods, it is informed that anti-HCV positive results were confirmed by recombinant immune blot assay (CHIRON RIBA HCV 3.0 SIA, Ortho Clinical Diagnostics, Johnson&Johnson, USA) in individuals who subsequently tested negative for HCV-RNA. However it is not clear if all samples were submitted to PCR or RIBA? Had all anti-HCV reactive samples tested positive to HCV-RNA? The number of anti-HCV reactive samples is relatively high compared to other areas of China.

Response:

The following revision has been made in the “Methods” section (Page 5-6, Line 102-111):

“Blood samples were tested for Hepatitis B surface antigen (HBsAg), and antibodies against HBsAg (anti-HBsAg), Hepatitis B core antigen (anti-HBc), Hepatitis C (Anti-HCV)and HIV, using Abbott ARCHITECT i2000SR Immunoassay System (Abbott Laboratories; Abbott Park, IL, USA) at the clinical laboratory of the First Hospital of Jilin University. All samples that were anti-HCV positive were confirmed by HCV-RNA test (COBAS AmpliPrep/TaqMan, Roche Diagnostics Ltd, Rotkreuz, Switzerland). Anti-HCV positive results were confirmed by recombinant immune blot assay (CHIRON RIBA HCV 3.0 SIA, Ortho Clinical Diagnostics, Johnson&Johnson, USA) in individuals who subsequently tested negative for HCV-RNA. HCV
genotyping was performed by multicolor fluorescence polymerase chain reaction (PCR) using an HCV-RNA genotyping kit (BioAssay Science & Technology Co. Ltd., Beijing, China)."

**Comment 4.** Discussion should be revised in order to discuss the other risk factors found in the study. Why blood donation was a significant risk factor?

**Response:** The following additional information has been added in the “Discussion” section (Page 10, Lines 207-216):

"In the multivariate model, a history of blood transfusion and prior dental surgery were significant risk factors associated with HCV infection, which suggests that unsafe medical practices may have contributed to HCV transmission. Since limited resources are available for prevention of HCV infection, the focus should be directed on ensuring implementation of safe practices in healthcare settings. To prevent nosocomial infection, mandatory screening of blood donors for HCV should be enforced. The Chinese government has prohibited the use of paid blood donors since 1998, which has improved blood safety [15]. This transition in the blood donor recruitment method has been associated with a gradual decline in the prevalence of anti-HCV among blood donors, which is also reflected in the low prevalence of HCV infection in the blood donors participating in our study."

**Comment 5.** In addition, the limitations of the study should be included.

**Response:** Thank you for your comment. The following statement regarding limitations of this study has been added in the “Discussion” section in the revised manuscript (Page 12, Lines 251-257):

“Use of a non-random convenience sample and self-reporting of at-risk behaviors by subjects are key limitations of our study. The study sample may not be representative of the general
population in Fuyu. Similarly self-reporting by subjects could have been affected by recall bias.
In order to minimize recall bias, questions related to substance use and injection behavior recall (such as when and where did you take the PCNBSS) were kept in the questionnaire. Despite these limitations, the survey demonstrated a high prevalence of HCV infections in Fuyu city where PCNBSS was most important risk factors for HCV transmission.”

**Comment6.** English grammar should be revised.

**Response:** Due care has been taken to correct the errors and improve the use of language. The revised manuscript has been meticulously proofread.