Author's response to reviews

Title: Clinical Characteristics of the Autumn-Winter Type Scrub Typhus Cases in South of Shandong Province, Northern China

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Author's response to reviews: see over
RE: MS: 3905916322295992
“Epidemiological and Clinical Characteristics of the Autumn-Winter Type Scrub Typhus Patients in Northern China: by Yunxi Liu et al.

Dear Dr. Todd,

We are pleased to re-submit the enclosed manuscript for your consideration for publication in the *BMC Infectious Disease*. The title of our (revised) manuscript is: “Clinical Characteristics of the Autumn-Winter Type Scrub Typhus Cases in South of Shandong Province, Northern China”.

We greatly appreciate the three reviewers’ constructive comments on our manuscript submitted previously. Following their suggestions, we have thoroughly revised the paper. Enclosed are a point-by-point response to reviewers’ comments and the revised manuscript.

We thank you for considering the manuscript and look forward to hearing from you.

Sincerely,

Yunxi Liu, MD, PhD

Enclosures:
1. A point-by-point response sheet.
2. Revised manuscript (clean version).
Response to reviewers’ comments

Manuscript title: “Clinical Characteristics of the Autumn-Winter Type Scrub Typhus Cases in South of Shandong Province, Northern China”.

General Note:

1. The manuscript has been revised thoroughly based on reviewers’ comments and suggestions. The title of the manuscript is revised to be more specific and to reflect the relevance of the work presented. In particular, the Background and Discussion have been thoroughly revised with broader arguments drawing from additional referenced literature; the methods have been described in detail.
2. The research question is defined and contextualized in the text (Abstract, Background, and Discussion)
3. The results and conclusions have been revised to be more concise and specific based on reviewers’ comments.

Reviewer 1:

[Abstract]

4. Comments: [Methods] What the authors described here is not the “Method”. The authors failed to explained how they designed to obtained the samples and how the samples were collected?

Response: The method is concisely described in Abstract and elaborated the Method section of the main text.

5. C [Results] Need to specific what serological or molecular techniques were used? What were findings? The total number of 480 patients did not indicate that they were occurred yearly. Any statistical results/data to back this statement.

R: Specific serological/molecular techniques used in the study are now clarified in both Abstract and main text. The total 480 confirmed cases spanned the 12-year period and the confusion is clarified. Specific statistical test and results are now added in the abstract and text.

6. C [Results] Their findings were summary of clinical observation/data and without any epidemiological results which clinical data observed in those 5 hospital were different from each other or not

R: Although the study falls in a broad category of ‘epidemiological study/description’, we agree that the study is more focused on clinical observation. Clarifications are made in the revised version. Data observed in the five hospitals were compared and no significant differences were found.
7. C [Conclusion] The authors failed to conclude their results which they observed in different age groups as well as in different occupation groups as exhibited in Table 2-4. Again their findings may only be indicating that there were endemic scrub typhus in those study site

R: The conclusion has been revised to reflect that (1) the autumn-winter type scrub typhus cases in northern China manifested with the four major typical symptoms of scrub typhus: fever, cutaneous rash, eschar and local lymphadenopathy, while the associated complications were less severe than those of the summer type scrub typhus cases in southern China; (2) Infections occurred more frequently in farmers of the youth and midlife; farm work was the main exposure pathway to infection.; (3) Careful examination of skin eschars provides important information to the clinical diagnosis.

[Background]

8. C [Background] Need and explanation as well as a comparison between the “summer-type” and “autumn-winter type”. Is this 2 types were distinguished by the virulent of the strain of O. tsutsugamushi or by clinical manifestations!

R: Done as suggested (in the revised Background). A comparison between the two types of scrub typhus is described in Background section and also discussed in the Discussion section. Please see page 6-7, 14-15 in the revised version.

9. C [Background] It should be written as ……had not been studied or conducted … not rare.

R: Done as suggested.

10. [C] [Background] The authors did not “systematically summarized!

R: We agree that we didn’t systematically summarize epidemiological characteristics. In revised version, as suggested in the revised title, we focused on clinical characteristics and systematically summarized clinical aspects.

[Materials and Methods]

11. [C] Study Sites. No information on populations mention here!

R. We thoroughly revised this section – information on the study site, the cases and study design were specifically introduced (please see page 7 in the revised version).

12. [C] This statements did not belonged here under the “Study Sites”
**R:** We agree with you and thank you. We moved this statement to newly added section “The cases and study design”.

13. How many were tested by PCR? Only 44 (out of 421) which was less than 10% of the positives IFA detection!!!!

**R:** PCR method became available in 2001 and was used for detection and genotyping of O. tsutsugamushi connected with RFLP and nucleotide sequences analysis since then. From the IFA-positive samples collected from 2001 to 2006, 34 out of 45 acute stage blood samples of cases were PCR positive. 10 eschars from 10 cases whose blood samples were IFA-positive as well as PCR-positive were also tested positive by PCR. In addition, 15 isolates were tested positive by PCR.

14. As the Gold standard method for detecting and or diagnosis of the scrub typhus in human patients, ELISA is the techniques of choice. IFA is still in use, however, it required experience slide reader. IIP is more accurate than the IFA. Currently, if blood samples can be collected, PCR techniques (which is accurate, precise & specific) will provide the best diagnostic tool for detecting the O. tsutsugamushi. The classical serological diagnosis of rickettsial diseases is based on a > or equal 4-fold rise in the titer between paired acute and convalescent sera. This study did not followed that guidelines. See Coleman et al. 2002. Am. J. Trop. Med. Hyg. 67(5):497-503

**R:** We agree with you. Although ELISA and IIP are more accurate than the IFA, the two methods were not commercially available in China, IFA was still widely used in China for serodiagnosis of scrub typhus until now. In present study, according to previous reports [see references 5, 12-14 in the revised version], the confirmatory serodiagnosis of scrub typhus was made in case of a fourfold or greater rise in titers between paired acute and convalescent sera, or IgM or IgG titer in a single serum above 1:80, or 1:400. However, a few of cases whose IgM or IgG titer in a single serum under 1:80, or 1:400 might be neglected according to this criterion, especially when their convalescent sera were unavailable. This is a limitation of our paper.

15. Therefore, it would be strongly recommended that samples must be confirmed by more than one tests, in order to obtain the reliable results/data.

**R:** We agree to your comment. The test result might be more convincing if more than one test was used. As Weil-Felix test is neither sensitive nor specific, ELISA and IIP were not commercially available in China, IFA was still widely used for serodiagnosis of scrub typhus until now. PCR method became available by us only after 2001. Most cases (436 out of 480) in our study were tested positive only by one test (IFA). This is a limitation of the study.

16. Therefore, only 223 were presumably found positives of scrub typhus!!!
According to the results of IFA, totally 480 cases were confirmed as scrub typhus: 280 cases were confirmed as scrub typhus based on a 4-fold or more rise; 86 cases were confirmed by having an IgM titer in a single serum sample$\geq 1:80$; and 114 cases were confirmed by having an IgG titer in a single serum sample $\geq 1:400$. A total of 198 cases were identified as non-scrub typhus cases by IFA.

17. Either rewrite this sentence or delete those double-strike-words.

R: We have rewritten this sentence in the revised version according to the reviewer’s suggestion.

[Results]

18. Is this meant that during January to August of every years between 1995-2006, there was no case of scrub typhus reported. Or else? Although the population density of chigger-mites varies during the year due to the changing of the habitat as well as the abundance of their hosts. Cases of scrub typhus in human do occur year-round. Did the authors neglect this fact?

R: Yes, there was no case of scrub typhus reported between January to August at all across the 12-year period of study. Between May 1995 and April 1996 in the study area, Liu et al (1997) systematically observed seasonal variations in population density of chigger-mites and their hosts, revealing a strong seasonal pattern of population dynamics, as commented by the reviewer. However, cases of scrub typhus in human in the study area do not occur year-round, which are confined to September-December based on the 12-year period of study. Clarifications have been made in the revised version. [See: Liu YX, et al: Studies on L. scutellare as a vector of Scrub typhus of the autumn-winter type in Shandong Province. Literature and Information on Preventive Medicine 1997, 3:305–306.]

19. Sex & age should be considered with types of occupations. Presumably, these people were villagers, most of them (children, men and women) would went off to the fields and would/could had chances to get bitten by infected chigger-mites. Therefore, their risk of obtaining the disease might not be significantly different.
   It would be better to find a way to combine Table 2+3+4

R: We agree. In the revised version, we have combined Tables 2, 3, 4 (in previous submission) to one (Table 2 in the revised version) to include age/sex, occupation, and activities.

20. It would be more useful and informative if the authors described the differences between the summer-type VS autumn-winter type. Which clinical symptoms exhibited in patients first. Was there any clinical-symptom format? Matching those outstanding cases which their PCRs were positives along with the IFA results.
In the revised version, we have described the differences between the summer-type vs. the autumn-winter type. The major typical symptoms caused by the two types were very similar but severity of complications was different. In the current study (which was based on hospital-admitted cases), clinical symptoms (reported) didn’t show any particular chronological order in patients. We didn’t observe any particular clinical-symptom format in those who were IFA-positive as well as PCR-positive.

[Discussion]

21. Discussion was too long and redundant. It must be brief and precise to the points which the authors would like to address. Again samples were collected only from the months of October to December, how could the authors discussed about the seasonal variations in case number?

R: Thank you for pointing this out. The Discussion section has been revised to be more concise and redundant sections were deleted. A more precise way to express the ‘sample’ is that cases (or infections) only occurred between September and December with the peak month in October. The seasonal variations in occurrence of cases were discussed in the context of other contributing risk factors.

22. The reference cited here was from 1997, it could not be applying to the year from 1998-2006!!! There was no data on chigger-mite population presented in this manuscript!!! The authors must present data on chigger-mites surveys during the time of study in order to make that statement! Was the authors speculate on it?

R: We agree and clarification has been made in the revision. Liu et al (1997) systematically observed population dynamics of chigger-mites and their hosts, which was cited in the paper. Data on chigger-mite population (as indicated by the chigger index) have been included in the revised manuscript.


23. This incorrect. How the authors determined the rate of infection and assumed that is higher in males than female. The fact was (data from Tables) males were found positives with lab results than females!

R: Thanks for pointing this out. Clarifications were made to correct the inaccuracy.

24. Although, this was discussion by the authors, however, chigger-mites of the vector of scrub typhus always with closed-association with their hosts. In this cases, wherever hosts go, chigger-mites go along. It’s true that there is more
chance to get chigger bites in the field and receive the scrub typhus. One has to prove such area was infested by the host populations, as well.

R: We agree to the reviewer’s comment. In the revised version, we have deleted the long and redundant discussion about this topic.

25. This was clinical reports! The clinical characterization on Table 5 should be modified to showed the chronological symptoms as well as the grouping the main clinical symptoms, i.e., all patients (100%) with fever; 90+% with maculopapular rashes; 80+% with skin eschars, loss of appetite and so on

R: According to the reviewer’s comment, Table 5 (Table 3 in the revised version) has been modified to showed the chronological symptoms as well as grouping the main clinical symptoms.

26. It would be interesting to match the patients with the eschars and the results of the serological test + PCR analysis. Since the eschar is the unique characteristic of the chigger-bite on patients. It could also confirmed that such patients received the O.t from chigger-mites.

R: We agree to the reviewer’s comment. In current paper, ten eschars from 10 cases whose blood samples were IFA-positive as well as PCR-positive were also tested positive by PCR. Partial results have been published in 2006 (Liu YX, et al. Orientia tsutsugamushi in eschars from scrub typhus patients. Emerg Infect Dis 2006, 12: 1109–1112.). This result indicated that patients’ eschars could be used as an alternative, easily acquired and sensitive sample for the diagnosis of O. tsutsugamushi infection. In addition, the result also confirmed that scrub typhus patients received the O. tsutsugamushi from chigger mites.

27. Again the locations of eschars were significant only when they were associated with 1. the dressing/wearing styles of the patients (another words, villager exercise their personal protection or not 2. behaviors of hygiene of patients like bathing or showering daily or not cleaning up after return home from the field. Those factors would be affected and indicated where and how chigger entered and stayed onto the body of patients, that’s where the location of eschars could be found!

R: We agree with you. We have added the content in the Discussion section in the revised version (please see page 16).

Reviewer 2:

Major Compulsory Revisions

28. (Page 7 lines 20-21 and Page 9 lines 11-13) In “Confirmatory diagnosis”, IFA was used for detecting IgM and IgG antibodies against pooled Karp, Kato, and
Gilliam strains of O. tsutsugamushi antigens. However, in Results (Cases of scrub typhus), the genotypes of O. tsutsugamushi were similar, but distinct from Kawasaki strain. Therefore, you need to clarify that these antibodies can also detect other strains as well.

**R:** In the present paper, serotyping of the 480 IFA-positive sera revealed that all belonged to Gilliam types. However, the genotyping results of the 34 IFA-positive blood samples, 10 eschars and 15 isolates indicated that the epidemic genotypes in the study area were similar to Kawasaki strain. The inconsistent results obtained by us may be explained as follows. Previous report found that the sequence homology of scrub typhus antigen 56-kilodalton (Sta56) surface protein gene between Kawasaki and Gilliam strain was the highest among the known O. tsutsugamushi strains, and high cross-immunoreaction existed between the two antigens. Because of the high cross-immunoreaction, antibodies to Kawasaki strain in the sera of cases could be tested positive when Gilliam strain was used as antigen instead of Kawasaki strain (Kawasaki strain was not available as antigen in China). Clarifications were made in the revised version (page 17 in revised version)

29. The distribution map of the cases in the study areas will be highly attracted the readers of the journal. Please add the information.

**R:** Done as suggested.

30. (Page 14 Lines 6-8) This statement “In addition, 5.2% of cases contracted infections from housework suggesting that farmers working in the field may bring infected chiggers into home together with crops or their clothes” was not supported by the data and this is only speculated. Discuss more in detail or delete.

**R:** Thanks for pointing this out. The statement was deleted in the revised version.

31. In Japan, 2 peaks of scrub typhus cases, one was in spring and the other in October (Autumn-winter type) were found from 2000 to 2005. Here, only one peak on October and during the study period, no cases were found in other seasons. Please add the explanation to the discussion. Shuji H, et al. Epidemics of vector-borne diseases observed in infectious disease surveillance in Japan, 2000-2005. Journal of Epidemiology, 17: S48-55, 2007.

**R:** The Japanese study was discussed in the context of the current study, as suggested (see page 13 in the revised version).

**Minor Essential Revisions**

32. (Page 1) Northern China in the topic should change to a smaller scale, for example, south of Shandong province, China.

**R:** Done as suggested (please see the revised title).
33. (Page 7) In “The study site and population”, no population information was mentioned. Additionally, add the description of the weather, especially temperature, and annual precipitation.

R: Done as suggested (please see Page 7 in the revised version). The population information was described in Materials and Methods section. We also added the description of the weather of Feixian, Shandong province in the revised version.

34. (Page 8 line 16) In “data collection”, it stated activities at the time of infection. Please define the time of infection. For example, ? time before the day of onset.

R: We agreed that “Activities at the time of infection” in previous version caused confusion, which it has been revised to “Activities possibly associated with scrub typhus infection within 1-3 weeks prior to onset of symptoms”. Clarification has been made in the revision.

35. (Page 9 Lines 1-2) In Statistical analyses, it stated that Statistical analyses were performed using Chi-square test (or Fisher’s exact test) for categorical percentages. However only Chi-square tests were mentioned in text or tables. Specify where Fisher’s exact test was used or delete. Additionally, change categorical percentages to categorical data.

R: Done as suggested.

36. (Page 10 Line 2) The cases occurred exclusively every year between 11-20 September to 1-10, December changed to between…. and or rom …..to.

R: Done as suggested.

37. (Page 12 line 4, 23-25, 27 ) All x2 change to its correct symbol

R: Done as suggested.

38. (Page 23-25) The results of statistical test were stated in the footnotes of Table 2, 3, and 4, but did not mention in the text. Please explain these test results in the text.

R: Done as suggested.

39. (Page 27) In Table 6, only P values were stated, please add what statistical test was used.

R: Done as suggested.

40. (Page 15 Lines 13-14) In our study, 88.5% of cases had eschars. Stills this is higher than the usually reported figure (?). Please specify the reported figure.
R: Done as suggested. We have provided the reported figures in the revised manuscript (please see page 15, paragraph 2).

41. (Pages 18-21) The format in References was not consistent for each reference and do not followed the format of BMC infectious diseases. For example, reference 9, 10, 11, 13, 16, and 17 used the full names of the authors (reference 9: Huang Yong, Jiang You-Hai). Please double check the format used in the references.

R: Done as suggested. We have checked and revised the references format according to the format of BMC infectious diseases.

Reviewer 3:

Major compulsory revisions

42. The authors showed the epidemiologic and clinical characteristics of autumn-winter type of scrub typhus in China. The authors analyzed many cases and showed the clinical and epidemiologic features of the cases. However, I think that it would be better if the authors compare the autumn-winter type and the other type(s) (in previous reports) of scrub typhus in China and discuss them. In the present manuscript, it seems difficult to find what is new in autumn-winter type and how different autumn-winter type is. So I think comparison of the different types in China make readers understand the suggested specific and important features of the cases more clearly.

R: Thank you for this insightful point. We have thoroughly revised the manuscript in which comparisons between the autumn-winter type and the summer type were made. In the Background section, we have described the differences of epidemic season, host, and vector between the two types. In Discussion section, we also compared the differences of epidemiology, routine laboratory and clinical characteristics between the autumn-winter type cases obtained in north China and the summer type previously reported in south China. Please see page 6-7, 14-15 for the revisions to reflect this.

Discretionary revisions

43. I recommend that the authors should use some figures and maps to show distribution of the cases, vectors and types of infected O.tsutsugamushi. It may be very helpful for readers because all readers are not familiar with the geography of China.

R: Done as suggested.