Author’s response to reviews

Title: Detection of SFTS Virus RNA and Antibodies in Severe Fever with Thrombocytopenia Syndrome Surveillance Cases in Endemic Areas of China

Authors:

Xiaoxia Huang (huangxiaoxia2012@163.com)
Shujun Ding (dsj_jn@126.com)
Xiaolin Jiang (jxl198607@126.com)
Bo Pang (myangel1985@126.com)
Quanfu Zhang (zhqf1960@163.com)
Chuan Li (lcehfcdc@163.com)
Aqian Li (liaq@ivdc.chinacdc.cn)
Jiandong Li (ldong121@126.com)
Mifang Liang (mifangl@163.com)
Shiwen Wang (wangsw@ivdc.chinacdc.cn)
Dexin Li (lidx@chinacdc.cn)

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Author’s response to reviews:

Dear Editor:

Greeting from Beijing, hope you have a successful year in 2019.

We were pleased to have an opportunity to revise our paper entitled, “Detection of SFTS Virus RNA and Antibodies in Severe Fever with Thrombocytopenia Syndrome Monitoring Cases in Endemic Areas of China” (INFD-D-18-01831R1). In revised the paper, we have carefully considered all comments and suggestions from editors and reviewers. As instructed, we have attempted to explain changes made in reaction to all comments succinctly. We reply to each comment in the point-by-point fashion.
Your and the reviewers’ comments were very helpful, and we are appreciative of such constructive feedback on our original submission. After addressing the issues raised, we do feel the quality of the paper is much improved and hope you agree. If you have any further questions, please do not hesitate to contact us. Thank you again!

Response to editor Comments:

Please copyedit your submission. We suggest that you ask a native English-speaking colleague to help you with this. If this is not possible, please consider using a professional service.

Reponses: Thank you very much for giving the opportunity to revise our paper. We found someone to help us to improve the language. If there still have any problems, please do not hesitate to contact us, and we will use professional service for this.

Response to Fei Deng (Reviewer 1)

1. The result in the Abstract mentioned "Group A" and "Group B" for the first time, which makes me confused. A brief description is necessary in Abstract to clarify how the two groups were divided.

Reponses: Thank you for your comments. We revised those sentences. Please see line 13-16.

Line 13-16: The SFTSV RNA positive rate peaked (52.2%) in samples collected ≤7days after onset, then showed a decreasing trend. The detection rate of SFTSV specific IgM antibody was 30.5% (46/151) and was highest in samples collected among 8-14days (43.3%, 26/60).

2. line 27  A reference is needed.


3. line 31  delete "for many times".
4. In line 40, the description "The detection rate of RNA and antibody was different." is too simple to explain the dissinilarity between qRT-PCR and ELISA.

Responses: Thank you very much. Here we just wanted use one sentence to summarize. So we changed this sentence to “More information should be known about the detection of SFTSV RNA and antibodies (especially IgM antibody) in the early stage after disease onset” (Line41-42, page4).

5. line 65 please provide the primer and probe sequences.


Line 68-69: The primers and probes [11] located in L, M, and S segments of SFTSV which were showed in previous study.

6. line 74 is the cut-off value too low?

Responses: Actually, we followed the instructions of the ELISA detection kit. The cut-off value was described in the instruction.

7. line 84 Is there a approval number issued by the ethics committee?

Responses: Yes. The number is IVDC2018-006.

8. line 93 had fever.

Responses: We corrected “were fever” to “had fever”. Thank you very much.
9. Table 1 listed the general information of participants, however, it is not clear enough for me to get full understand of the enrolled population. For example, most of them were farmers accounting for 81.3%, but information for the occupation of rest persons was not mentioned. The title of the last volume does not suit for all parameters, such as "sample collection days after onset", "leukocyte count" and "platelet count", which would make the values to be misunderstood or difficult to be understood. Dividing this table into two parts is suggested including personal information and clinical parameters, both with the corresponding headers.

Reponses: Thank you very much for your suggestions. The total enrolled participants were 374. But we did not get all these 374 cases’ information such as age, occupation, gender, and so on. So when we described the percentage and other values, we wrote the observed numbers.

We moved and added personal information and sample collection information to the paragraph before Table 1. As the same time, we deleted this information from Table 1. And we added one column (Median and interquartile range) to put the Leukocyte count and platelet count information.

Please see this in line 97-114, page 7, 8.

10. Both RNA and IgM could be detected on the day of disease onset. But the detailed sampling days for all samples were not described.

Reponses: In this study we divided the samples into 3 groups (group A, B, C) according to the sampling days. We described the sampling days of these three groups in line 99-102. If any more detailed sampling data is needed, we are willing to provide. Thank you very much.

11. It is better using a table to show the RNA, IgM and IgG detection results of three groups. The temporal change of viral RNA, IgM and IgG could be illustrated by using a bar chart or something like that for better presentation of these results.

Reponses: Thank you for your suggestions. We added table 2 in page 9 to describe the detection results of SFTSV RNA, IgM and IgG antibody. Fig. 1 and 2 showed the temporal change of these detections.

12. The "Comparison of SFTSV RNA and IgM antibody detection" part needs to be deeply improved. There were 3 groups as divided, but why the comparison analyses were performed using ≤14d and ≥15d groups. At least a sentence should describe the reason or background. Table 2 also confused me. How many samples were collected ≤14d in total? How many were IgM positive and negative in the 14d? 41 positive and 95 negative? Those do not fit the total
number. And dose the table mean there are 41 IgM positive samples including 15 RNA positive and 26 negative? If so, why not use the viral RNA positive and negative as subtotal to analyse the percent of IgM results. The results and significance of McMemar test and Kappar values in this table were not explained. Anyway, please rebuild the table to better illustrate the comparison results and significance.

Responses: Thank you for your suggestions. We explained the reason for the comparison analyses performed using ≤14d and ≥15d groups in the discussion part (please see that in line 164-167, page 11).

Line 164-167: For SFTSV RNA detection, the serum collected during the acute phase (within two weeks after disease onset) of disease were often recommended, so here we compared the SFTSV RNA detection and IgM antibody detection results in two groups (≤14d and ≥15d), respectively.

Table 3 was like a fourfold table. Totally, 151 serum were conducted IgM detection. Of that, 136 samples including 41 IgM positive and 95 IgM negative were collected ≤14d; 15 samples including 5 IgM positive and 10 IgM negative were collected ≥15d.

We rebuilt this table, please see that in page 10.

13. Has the specificity of the IgM and IgG ELISA kit been evaluated before? Please provide the reference and explain how it was done.

Responses: The antibody detection ELISA kit was commercial kit obtained from Zhongshan bio-tech CO., LTD. It has high specificity. We strictly followed its instructions to conduct the experiments.

14. The authors mentioned that the detection rate of IgM antibody was higher in the second week. So a figure or/and table showing the detection rates changing along with sampling days after illness onset is strongly recommended.

Responses: Thank you for your suggestions. Please see figure 2 in the material provided to the journal.

15. line 168 SFTSV RNA detection.

Responses: Sorry, we cannot clearly get the meaning of this comment. Would you like to write more detailed information? Thank you very much.
16. According to the results in this study, what is the potential reason of the inconsistency of the viral RNA and IgM detection results? Why there were IgM negative but RNA positive cases? Were the sera from the 9 cases collected very early after illness onset?

Reponses: We think there may be some reasons including sampling days, personal immunocompetence, sensitivity of detection methods, together affecting the inconsistency of the viral RNA and IgM detection results. Of that, we think that the sampling days of serum is the mainly potential reason. 9 IgM negative but RNA positive cases’ sampling days were 1 for 4 days, 2 for 5 days, 2 for 7 days, 2 for 8 days, 2 for 11 days. We think the reasons for this phenomenon is also related with the above reasons to some extent.

17. There were both IgM negative and viral RNA negative cases (86 cases in table 2). So they are not confirmed SFTS cases just with SFTS-like symptoms. Other infection which could induce symptoms similar to SFTS could be suspected. I don't think these cases can be included in this study.

Reponses: Thank you very much for your suggestions. The cases included in this study were monitoring SFTS cases which were suspected SFTS cases or clinically diagnosed SFTS cases required making further laboratory detection. In this study, we want to get more information of the SFTSV RNA and antibody detection situation during the real disease monitoring, so here we included the monitoring cases. Although among these monitoring cases, there will some were excluded finally after laboratory detection.

Response to Weiwei - Chen (Reviewer 2)

SFTS is a newly identified severe infectious disease, and SFTS monitoring have been carried out since 2010. The diagnostic and laboratory detection methods of SFTS have been established for many years, and there are hundreds of corresponding research papers. This study focused on SFTS monitoring case in Shandong province, and detected the SFTSV RNA and antibody by Real time RT-PCR and ELISA respectively. The design and experimental method of this study lacked innovation, so it is not recommended to publish.

Reponses:

Thank you very much for your feedback. SFTSV has been identified for a period of time, but with the development of monitoring, SFTSV antibody and RNA detection in SFTS monitoring cases in Henan province showed some differences. At the beginning of our study, Shandong as a SFTS high-risk province did not carry out related research. And we think different provinces
may have different SFTS monitoring situation, so we carried out this study in Shandong province. We think this work will benefit national SFTS monitoring through providing background information of SFTS cases laboratory detection, and it is a very meaningful study to SFTS control.

Sincerely,

Xiaoxia Huang, Ph.D.
Associate Professor,
National Institute for Viral Disease Control and Prevention,
Chinese Center for Disease Control and Prevention (China CDC)