Reviewer's report

Title: Microarray of Surface-exposed Proteins of Rickettsia heilongjiangensis for Serodiagnosis of Far-eastern Spotted Fever

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Reviewer: Wei-Mei Ching

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Comments for authors:
This manuscript described a recombinant protein microarray containing 11 previously identified SEPs of R. heilongjiangensis were assayed with paired sera from 17 FESF patients and 20 sera from other febrile patients. Four rSEP are identified as potential candidates for serological diagnosis of FESF.

Specific Remarks
1. Page 2, Results and Conclusions. Among the four rSEPs mentioned in the paragraph, rRplA has a very poor specificity (65%). The specificity of the combination assay with rRplA can only be lower than 65% not higher. Because of the low specificity, rRplA should not be included in the assay even though it showed adequate sensitivity.
   
   Among the rest of three rSEPs, please calculate combinations of two rSEPs and finally all three rSEPs and show each combination’s sensitivity and specificity in a new table.

2. Page 3, the authors mentioned a standard practice in the French National Reference Center (FNRC) for rickettsioses diagnosis is IFA followed by PCR. The authors seemed to have done both PCR and IFA but referred IFA as the final confirmation test based on titer. It would be interesting to see whether all samples were PCR positives for both acute and convalescent serum. Furthermore, whether the PCR was performed using serum samples and/or blood?

3. Page 4, the authors stated that pair serum samples were used in this study. It was not clear when the diagnosis of FESF was made and whether subsequent treatment was prescribed to the patients. If a proper diagnosis and treatment was prescribed, how does the treatment affect convalescent serum samples.

4. Page 9, Immunoblotting assay. “In Figure 1, all of these rSEPs were recognized by acute- ” is an incorrect statement. Based on the gel picture and Fig. 3, none of the serum with an IFA titer of 256 (as sample #8 acute-phase does) recognize more than eight proteins.

5. The author stated that there are 5%- 20% of reference sera without antibodies to R. heilongjiangensis reacted positively to individual rSEPs in microarray assay, did similar results observed on western blot? If not observed on western blot, what is the explanation of the discrepancy? If it was also observed on western
blot, what are these proteins? Was the result due to impurity of recombinant protein preparation? How does this kind of “non specific” interaction affect the interpretation of the correlation of IFA titers and number of recognized proteins (Figure 3)?

6. There are 7 samples have IFA titer of 64, 5 samples have IFA titer of 128, and 5 samples have IFA titer of 256. Among these samples, what are the numbers (or percentages) of sample that were recognized by rOmpA-2, rRpsB, and rSdhB? These results may provide supporting evidence for your choice of potential candidates.

7. 11 (line 225) “Considering....” till the end of the sentence (line 229) “As a result...' was not very clearly written, please re-write to ensure the meaning comes across.

8. Line 228 to line 229. The statement for a good specificity cannot be true. RplA’s specificity was only 65%, much less than desired to be clinically useful, plus the specificity for the combination of four antigens could not be higher than 65% (90% as claimed by the author in the Discussion section). The results of combined sensitivity and specificity based on the four antigens need to be presented for all samples (34 for the paired samples and 20 for the control) in a table.

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Needs some language corrections before being published

**Statistical review:** Yes, but I do not feel adequately qualified to assess the statistics.

**Declaration of competing interests:**

'I declare that I have no competing interests'