Author's response to reviews

Title: Characterisation and analysis of thioredoxin peroxidase as a potential antigen for the serodiagnosis of sarcoptic mange in rabbits by dot-ELISA

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Author's response to reviews: see over
Dear Editors,

Thank you for your comments. Our manuscript entitled, “Characterisation and analysis of thioredoxin peroxidase as a potential antigen for the serodiagnosis of sarcoptic mange in rabbits by dot-ELISA” has been revised according to the reviewers' suggestions. We have highlighted the changes to our manuscript in red font. We have also added information about the committee that approved the animal studies into the methods section of our manuscript and had this manuscript copyedited by a professional English editing service that specializes in scientific papers.

Please find our responses to the revisions proposed by the reviewer’s comments attached, and thank you for your attention to our paper.
Thanks so much for your comments. We have answered each of the questions below.

1. My main concern is about the specificity of the new method. It seems that it works both for *S. scabiei* and *P. cuniculi*, and maybe for other Psoroptes species, not tested in the present study.

Response: Thanks for your advice. Considering that *P. cuniculi* is one of easily susceptible Psoroptes species in rabbits, we chose *P. cuniculi*-infected serum to detect the potential specificity and possible cross-reactivity of the new method developed in the present study.

2. The scientific name is not “S. Scabiei” but “S. scabiei”. Please correct this mistake in whole paper.

Response: We are sorry for this mistake. We have replaced “S. Scabiei” with “S. scabiei” throughout the revised manuscript.

3. We use “scabies” when we speak about Sarcoptes affecting humans, but we should use “mange” when we speak about Sarcoptes affecting animals, which is the case of this paper.

Response: Thank you for pointing out these mistakes. We have replaced the term “scabies” with “mange” when referring to Sarcoptes that affects animals in the revised manuscript.

4. The sentence “may be suitable for development of as a diagnostic tool in humans
in the developing world” should be modified. The other ELISA-based methods for the diagnosis of mange in animals were not effective in humans, for some reasons, and scabies is not only a problem in the developing world but it is a worldwide problem.

Response: We have removed the relevant content. It is well-known that scabies is a parasite that has spread worldwide. Importantly, a lack of advanced technology and diagnostic methodology still exists for this parasite in developing countries. In this study, we aimed to emphasize that our assay can offer assistance to these regions. Finally, the sentence in question has been rewritten as follows: “This technique is a rapid and convenient method that can be used worldwide for the clinical diagnosis of sarcoptic mange in rabbits, and is especially useful in developing regions.”

5. Introduction: Please give more details about other mange/scabies diagnosis methods.

Response: Thanks for your suggestion. We have added details regarding the diagnostic methods for mange and scabies into the Introduction section.

6. Methods: what do authors mean with “Mites were starved prior to treatment.”?

Response: We apologise for any confusion. The mites were not fed before the start of the experiment to avoid any contamination from the host RNA. This meant that we did not use mites that were collected from rabbits to extract total RNA immediately. We have altered this sentence to, “The mites were unfed before the start of the
7. Do authors have the sequences encoding the open reading frame (ORF) of SsTPx? Why they did not submit them to the genbank.

Response: We have previously submitted the sequence of SsTPx (GenBank accession number: KC693033) to GenBank. However, we accidentally omitted mentioning this important information in our first manuscript. We have added the SsTPx GenBank accession number into the Results section of our revised manuscript.

8. Figures are not clear: Fig.2: two colours in the same gel? Fig.3: body parts are not clear.

Response: We have incorporated the two figures together. One figure is the gel of the purified protein (left side) and the other is the Western blotting band on PVDF membranes (right side). We have also improved the quality of this figure overall and hope that it will now be suitable.

Reviewer: Wanpen Chaicumpa

Thanks so much for your comments. We have answered the questions individually below.

Major Compulsory Revisions

1. Attempt should be made to identify the deduced SsTPx amino acid sequence
shared with the *P. cuniculi* which is absent in other organisms that did not give cross-reactivity with the SsTPx, at least by multiple alignments.

Response: Actually, we amplify the TPx gene sequence of *P. cuniculi* during the assay period. We found that *P. cuniculi* TPx shared 100% identity with that of *P. ovis*. Hence, we have re-amplified and sequencing this gene since the original paper was submitted. We have also submitted this sequence to GenBank, with the GenBank accession number of KF241278. We have added details of the homology alignment in the Results section and Fig. 1.

2. More details on evaluation of the stability of the antigen dotted strips; how were they evaluated; how were the strips kept; at what temperature?

Response: We have added the details about the evaluation of the dotted strips that referred to a previous study (Varghese A, Raina O, Nagar G, Garg R, Banerjee P, Maharana B, Kollannur JD: Development of cathepsin-L cysteine proteinase based Dot-enzyme-linked immunosorbent assay for the diagnosis of Fasciola gigantica infection in buffaloes. Veterinary parasitology 2012, 183(3-4):382.). The temperature at which the strips were kept has been added to the Methods section.

3. Discussion should include existing serological techniques used currently for diagnosis of *S. scabiei* infections in humans.

Response: Thank you for your advice. We only found a few papers regarding the detection of scabies using serological techniques in humans. The difficulties with
ELISA for the diagnosis of human scabies include the lack of an animal model for human scabies and recombinant purified mite antigens, host cross-reactivity to house dust mite allergens, and further technical problems. We have added more details of the serodiagnostic techniques for sarcoptes mange into the Discussion section of our revised manuscript.

4. The assumption that the recombinant SsTPx is an outer membrane protein is inappropriate.

Response: Thank you for your advice. We have deleted this sentence.

Minor Essential Revisions

1. Typing errors should be corrected, e.g., S. Scabiei should be S. scabiei, EcoRI and HindIII should be italicized correctly, OD600 should be OD600 nm.

Response: We have corrected all of the above-mentioned mistakes in revised manuscript.

2. References should be re-checked; scientific names should be italicized.

Response: All of the references have been re-checked carefully and scientific names have been italicized.

3. English should be revised.

Response: The revised manuscript has been modified by a professional editing
Discretionary Revisions

1. The SsTpx sequence should be deposited in the GenBank database.

Response: We have submitted the sequence of SsTPx to GenBank (GenBank accession number: KC693033) and added the SsTPx GenBank accession number to the Results section of the revised manuscript.

If you have any queries, please do not hesitate to contact me at the address below.

Thank you for your attention.

Yours sincerely,

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