Author’s response to reviews

Title: Genetic diversity of Leishmania donovani that causes cutaneous leishmaniasis in Sri Lanka: a cross sectional study with regional comparisons

Authors:

Udeshika Kariyawasam (ukariyawasam86@gmail.com)
Angamuthu Selvapandiyan (selvapandiyan@hotmail.com)
Keshav Rai (raikeshav@hotmail.com)
Tasaduq Wani (thwbi09@gmail.com)
Kavita Ahuja (kavita17111989@gmail.com)
Mizra Beg (m.adilbeg@gmail.com)
Hasitha Premathilake (hpremathilake@yahoo.co.uk)
Narayan Bhattarai (bhattarai03@yahoo.com)
Yamuna Siriwardena (yayureka@hotmail.com)
Daibin Zhong (dzhong@uci.edu)
Guofa Zhou (zhoug@uci.edu)
Suman Rijal (srijal@dndi.org)
Hira Nakhasi (Hira.Nakhasi@fda.hhs.gov)
Nadira D. Karunaweera (nadira@parasit.cmb.ac.lk)

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Author’s response to reviews:

Editor Comments:

Q1. Please clarify the roles of MAB and YS who are listed as authors but not mentioned in the author contributions section.

R1. The author contribution section has been changed accordingly on page 17 lines 379-382 as given below.
Conceived and designed the experiments-UK, NDK, HN, AS, SR, NRB, YS. Performed the experiments-UK, KA, THW, KR, MAB. Analyzed the data-UK, AS, HUP, DZ, GZ. Wrote the paper- UK, NDK. Read and approved the manuscript- all authors

Q2. Please ensure that authors’ names provided in the submission system are IDENTICAL to those given in the title page. Please ensure that authors’ names provided in the title page are given in full.

R2. Authors’ names provided in the submission system and in the title page are identical.

Q3. Please remove the duplicated figures and tables.

R3. The duplicated figures and tables have been removed.

Q4. Please provide figure titles/legends under a separate heading of 'Figure Legends' after the References. Figure files should contain only the image/graphic, as well as any associated keys/annotations. If titles/legends are present within the figure files, please remove them.

R4. The relevant changes have been introduced to page 23, lines 658-707.

Q5. Please ensure that an "Additional files" section is added (after the References/Figure legends) where you list the following information for each additional/supplementary file in the file inventory:

- File name (e.g. Additional file 1)
- Title of data
- Description of data

R5. The relevant changes have been introduced to page 25, lines 708-725.

Q6. Please have the text edited by a professional language editing service or a native English speaking colleague. There are several issues with grammar, wording, spelling, and/or punctuation that need to be addressed before acceptance for publication.
The manuscript was reviewed by a native English speaker Dr. John Hobson at Food and Drug Administration, USA is also acknowledged for language editing.

Q7. Please change the heading ‘Materials and Methods’ to ‘Methods’.

R7. The relevant changes have been introduced to page 04, line 80.

Q8. Please ensure that all figures/tables and supplementary files are cited within the text. Any items which are not cited may be deleted by our production department upon publication.

R8. All the figures/tables and supplementary files are cited within the text.

Q9. Please provide a list of all the abbreviations used in the manuscript. This list should be placed just before the Declarations section. All abbreviations should still be defined in the text at first use.

R9. All the abbreviations used in the manuscript are now listed in the manuscript on page 15, lines 325-351.

10. At this stage, please upload your manuscript as a single, final, clean version that does not contain any tracked changes, comments, highlights, strikethroughs or text in different colours. All relevant tables/figures/additional files should also be clean versions. Figures (and additional files) should remain uploaded as separate files.

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Reviewer reports:

Sanjay Mehta (Reviewer 3):

Overall the manuscript is much improved and is doing a better job at highlighting the unique findings of this work.
Minor comments

Abstract

Q1- Background S3 change "widely known" to "widely shown"

R1. The relevant section has been now changed in page 01 lines 06-08, as given below.

To investigate the different species or strain-specific differences of L. donovani in Sri Lanka we evaluated sequence variation of the kinetoplastid DNA (kDNA).

Q2- "sub clusters" should be written as sub-clusters

R2. The relevant change has been introduced to page 01 line 18.

Q3- Conclusion - revise as follows to clarify

This study reveals high levels of haplotype diversity of L. donovani in Sri Lanka with a distinct genetic association with clinically relevant phenotypic characteristics. The use of genetic tools to identify clinically relevant features of parasites has important therapeutic implications for leishmaniasis.

R3. The relevant change has been introduced to page 01 lines 22-25 as given below.

This study reveals high levels of haplotype diversity of L. donovani in Sri Lanka with a distinct genetic association with clinically relevant phenotypic characteristics. The use of genetic tools to identify clinically relevant features of Leishmania parasites has important therapeutic implications for leishmaniasis.

Background

Q4-line 47 change to "to the standard antimonial drugs routinely used in clinical practice."

R4- The relevant change has been introduced to page 03 lines 43-45.
Q5-Change line 54 to "A2 protein production [18, 20, 21], lipophosphoglycan (LPG) activity [22-24], gP63 gene expression [25, 26], acid phosphatase activity [18, 27] and variation in the mini-exon genes of chromosome 36 [17] are known factors that influence the virulence of…"

R5. The relevant changes have been introduced to page 03 lines 52-55.

Q6-line 59 - change to "of differences in virulence and pathogenicity."

R6. The relevant changes have been introduced to page 04 lines 56-57 as given below.

Further analysis of Sri Lankan L. donovani isolates is thus warranted in order to gain a better understanding of differences in virulence and pathogenicity.

Q7-line 61 change "usage" to analysis.

R7. The relevant change has been introduced to page 04 lines 59-61.

Q8-line 67 modify to containing a conserved region nearly 200 bp in length and a ~600 bp variable region."

R8. The relevant change has been introduced to page 04 lines 63-64, as given below.

The Leishmania parasite minicircle kDNA is approximately 1 kb in size, containing a conserved region that is ~200 bp in length and a variable region of ~600 bp.

Q9-line 68 clarify by removing extra information to "Minicircle sequence differences allow for accurate discrimination between species [32-34]."

R9. The relevant change has been introduced to page 04 lines 65-67 as given below.

Leishmania minicircle kDNA provides an ideal target for the genotyping of Leishmania parasite as the sequence differences allow for accurate discrimination between species [32–34].

Q10-line 72 similarly clarify by modifying "Previous studies using minicircle kDNA footprint assay based on PCR have enabled rapid identification of previously known or unknown species with a high level of sensitivity[35]."
R10. The relevant change has been introduced to page 04 lines 69-70.

Q11- line 75 - change "depends" to "depend"

R11. The relevant change has been made on page 04 line 72.

Q12- line 78 - to clarify "The aim of this study was to use the kDNA footprint assay to study L. donovani strain specific sequence diversity and determine the associations between sequence variations and distinct clinical characteristics in individuals with leishmaniasis in Sri Lanka."

R12. The relevant change has been introduced to page 04 lines 76-78, as given below.

The aim of this study was to use the kDNA footprint assay to study L. donovani strain specific sequence diversity and determine the association between sequence variations and distinct clinical characteristics in individuals with leishmaniasis in Sri Lanka.

Materials and Methods

Q13- Clarification line 91… change to "A patient was considered a 'poor responder', if the lesion size (ulceration area in case of ulcers or the induration area in case of non-ulcerative lesions) did not decrease at least by 50% from the pre-treatment size, as judged by the collaborating dermatologists, following a minimum of 10 IL-SSG injections given at weekly intervals, similar to previously used criteria."

R13. The relevant change has been introduced to page 05 lines 88-93, as given below.

A patient was considered a 'poor responder', if the lesion size (ulceration area in the cases of ulcerative lesions or the induration area in the cases of non-ulcerative lesions) did not decreased by a minimum of 50% of the pre-treatment size, as judged by the collaborating dermatologists, following a minimum of 10 IL-SSG injections given at weekly intervals, similar to previously described criteria [38–40].

Q14- Please clarify S1 under Laboratory and confirmation of Leishmaniasis

R14. The relevant change has been introduced to page 05 and 06 lines 103-110.
Q15- Line 128 - recombinants may be the wrong term here… don't you mean transformed colonies?

R15. The relevant change has been introduced to page 06 lines 126-127, as given below.

The resulting white colonies indicated transformed cells and blue colonies represented non-transformed ones.

Q16- Line 138 - Simplify the first sentence of the paragraph… break it up, as it is, it is too hard to follow.

R16. The relevant change has been introduced to page 06 lines 135-140, as given below.

A total of 301 L. donovani sequences of parasite isolates from Sri Lanka (n=38), India (n=27) and Nepal (n=26) were analyzed, along with minicircle sequences of 6 Leishmania reference species from different countries [34]. Multiple sequence alignment (MSA) was done, via CLUSTAL-X MSA Program, version 2.0, with a gap opening penalty of 10.00; gap extension penalty of 0.05; DNA transition weight of 0.50 [44, 45].

Q17- Line 173 add space after"in"

R17. The relevant change has been introduced.

Q18- Line 174 Reference the Maximum composite likelihood method

R18. The relevant change has been introduced to page 08 lines 170-172.

Q20. Please clarify what sequences were used for the multiple sequence alignment. The sequence shown in the supplementary material is only about 25 nucleotides long… as opposed to the 120bp listed in the methods

R20. All the sequences that were used in multiple sequence alignment were included in the additional file 1 in their full length (~120bp).