Author's response to reviews

Title: Species diversity of non-tuberculous mycobacteria isolated from humans, livestock and wildlife in the Serengeti ecosystem, Tanzania

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Author's response to reviews: see over
The Editor,
BMC Infectious Diseases

Dear Editor,

RE: RESUBMISSION OF A MANUSCRIPT TITLED SPECIES DIVERSITY OF NON-TUBERCULOUS MYCOBACTERIA ISOLATED FROM HUMAN, LIVESTOCK AND WILDLIFE IN THE SERENGETI ECOSYSTEM, TANZANIA

Refer to the heading above.
I hereby submit a revised manuscript titled species diversity of non-tuberculous mycobacterium isolated from human, livestock and wildlife in the Serengeti ecosystem, Tanzania. This study was part of the study titled genetic and ecological drivers of tuberculosis at human/livestock/wildlife interface of the Serengeti ecosystem, Tanzania. I hereby submit a report on response to reviewer’s comments elaborating the issues and correction thought to be necessary.

Thank you for your cooperation.

I remain sincerely,

Bugwesa Z Katale

Responses to Reviewers report

Reviewer 1: Emily Henkle

1. I would suggest some additional comment on the use of solid media to culture NTM. You mention that 92% of samples in the Discussion paragraph 1 did not
grow mycobacteria and one reason might be failure to culture mycobacteria. Only 2.9% of sputum samples were NTM positive, which seems low. Here is a paper from Asia that found large differences depending on whether solid or liquid media was used.

Response: Additional comments on use of solid and comparison of solid media with liquid media has been added (lines 294-299). The paper by McCarthy et al. (2012) was cited to address the differences in recovery of NTM in solid or liquid media (lines 296-298).

2. Do you know the HIV status of the 472 human TB suspects? And how many were TB+? Please include if available.

Response: Based on study objectives, data on HIV status of human TB suspects were not collected. It has been addressed (lines 298-299).

Minor Essential Revision

3. Correct to Mycobacterium genavense (not genavanse)

Response: Corrected accordingly (line 272, 276, 305, 312, 330)

4. Read the discussion carefully

Response: The discussion was thoroughly reviewed for correction

Reviewer 2: Jakko van Ingen

#1 This study seems to lack a clear rationale / research question. If possible transmission of NTM from wildlife to cattle or from cattle to humans was to be evaluated, a molecular typing method would be expected. If the diversity of NTM in wildlife and cattle specimens suspect for mycobacterial infection would be investigated, more sensitive culture methods would be expected, including broth
based methods and incubation at 30 and 37 degrees Celsius. The overwhelming majority of tissue samples from lesions suggestive of mycobacterial disease did not yield positive cultures, as the authors report in the Discussion. That questions the validity of the microbiological methods.

Response: This study was part of a larger study that explored possibility of cross-transmission of tuberculosis at human-animal interface and to our knowledge a solid media was considered sufficient to provide intended results. We however, agree with the reviewer’s opinion that in future all considerations will be taken on board. We also consider this as a limitation of our study. Moreover, an additional comment on the use of solid media to culture NTM has been added in paragraph one of the discussions (lines 294-299).

#2 Given the availability of molecular methods, why weren't these directly applied to the tissue and sputum specimens?

Response: Dear reviewer, this study was conducted in Africa where resources are limited. We however agree that if appropriate molecular tools for direct detection of NTM from tissues were available we could definitely deploy in our study. The lab we used had protocol that allows only conventional followed by further molecular evaluation. Our efforts reached to characterization by sequencing which we think is sufficient to provide the necessary information.

#3 Given the nature of sample acquisition for the cattle and wildlife specimens, a discussion on the possibility of environmental contamination of the samples seems justified.

Response: It has been addressed (lines 308-312).

#4 in the discussion, comparisons are made with a wide array of studies identifying NTM in human and animal specimens. For such a comparison to be meaningful, several issues have to be tackled. 1) are these data from clinical or environmental samples? 2) are these clinical specimens from patients with suspected mycobacterial disease or not? 3) If from suspected cases, did patients meet disease criteria or not (Griffith DE et al., Am J Respir Crit Care Med 2007; ATS diagnostic criteria). 2) Were the samples acquired in prospective studies or retrospective analysis of odd strains observed in culture laboratories?

Response: The comparison made in this manuscript was not targeted to studies with the same methodology/diagnostic criteria to our study, but it intends to have an overview if NTM species isolated in this study has also recovered from other study irrespective of source of NTM species. For example no any sample was taken from the environment BUT we highlighted the NTM species in this study which have been isolated from the environment in other studies. In the present study, the samples were collected from field conditions where sophisticated diagnostic tools are not available. In most cases diagnosis of mycobacterium depends on clinical signs and microscopy (ZN stain) as stipulated by Griffith DE et al. (2007) on ATS diagnostic criteria (Am J Respir Crit Care Med 2007). These samples were collected from both hospitalized and outpatients with symptoms suggestive of TB symptoms in the study area (lines 191 -192). The samples were prospectively collected from clinical samples in human (sputum from suspect TB patients) and animals (tuberculous tissues).

#5 The animal samples, mainly for the wildlife samples, were not collected systematically. As a result, the data from these samples are not very meaningful, other than that they show which NTM are present in the environment. Why not focus on the human TB suspects and prospectively or retrospectively, but
systematically, identify all positive cultures by molecular methods and then go back to the patient to assess whether there could be true NTM disease (again using the ATS diagnostic criteria, Griffith DE et al 2007)? That would bring meaning to the identification data. Many of the very rare species isolated in this study are likely to be single positive cultures with limited clinical relevance. But to evaluate that, multiple specimens/cultures from each patient are needed, as well as clinical and radiological data.

Response: Samples from wildlife were opportunistic samples which are obtained during game hunting, natural deaths and road kill. Collection of wildlife samples is very limited based on policies and wildlife regulations in Tanzania. Under such circumstances, the only way to obtain samples is that what we used and we are hopeful that the information we provide is not useless but provides clues of what might be happening in this ecosystem. We plan, that in future more integrated methods (depending on availability) will be deployed for more elaborative findings.

#7 The suggestion of a one health approach to tackle NTM transmission is rather grandiose, as there is no evidence for animal-to-human transmission and environmental transmission is virtually impossible to prevent. I would use a one health approach to tackle issues of more importance to human and animal health than NTM (like tuberculosis, brucellosis, trypanosomiasis, etc.).

Response: We also agree with reviewer’s comments on NTM against zoonotic microbes in terms of priority for control. However, bearing in mind that this study was carried in the Serengeti ecosystem where human, livestock and wildlife are uniquely in intense contact with prevailing HIV infection in humans we are obliged to treat both tuberculous and non tuberculous mycobacteria equally due to the impact NTM may have on HIV infection in humans.

Minor essential revisions:

Please check the spelling of NTM species names throughout. For example: it is M. genavense not genavanse, etc.

Response: The spelling of NTM species was checked accordingly (line 272, 276, 305, 312, 330)

#1 The abstract says that gyrB gene sequencing was applied to identify NTM. There is no sign of that in the rest of the manuscript, particularly not in the Methods.

Response: This has been deleted out (line 71)

#2 If 16S rDNA gene sequencing was the only identification method applied, identification as M. chelonae should read M. chelonae-abscessus group, as 16S does not allow distinction between the species in this group.

Response: It has been addressed (lines 75, 272, 303, 330)

#3 line 303-305. The Gcebe study. The suggestion that NTM species diversity is higher in Africa than elsewhere has no scientific basis. The low number of species isolated in the current study actually does not support the notion by Gcebe et al.

Response: The statement from Gcebe et al. 2013 has been deleted.