Reviewer’s report

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

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Reviewer: Jakko van Ingen

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Major compulsory revisions:

#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.

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

#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.

#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?

#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.
#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.).

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

#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.

#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.

#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.

Level of interest: An article of limited interest

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:
I declare that I have no competing interests