Author's response to reviews

Title: Isolation of non-tuberculous mycobacteria (NTM) from pastoral ecosystems of Uganda: Public Health significance

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Author's response to reviews: see over
Date: 20\textsuperscript{th} April 2011

To: The Editor, BioMed Central (BMC) Public health

From: Clovice Kankya (clokankya@yahoo.com)

Subject:

Submission of a revised version: MS: 1353869690486136

Research article: Isolation of non-tuberculous mycobacteria (NTM) from pastoral ecosystems of Uganda: Public Health significance

The Editor,

Dear Sir/Madam,

On behalf of fellow co-authors, I would like to thank you and your co-editors on the great work you are doing.

We have fully responded to the reviewers comments in the above manuscript. We are presenting point by point response to the reviewers concerns for the improvement of the Manuscript.

See attached response note below.

Yours faithfully,

Clovice Kankya (Corresponding author)
Authors response to reviewer’s comments

Title: Isolation of non-tuberculous mycobacteria (NTM) from pastoral ecosystems of Uganda: Public Health significance

Reviewer's report response:

The paper has been revised according to reviewers’ comments and giving a point-by-point response to the concerns. Details are given below.

Minor Changes

Line 41: should read “detected; 25.3%...”
It now reads as suggested

Line 50: “..humans to come in contact..
”Now reads as suggested

Conclusions: suggest be reworded to
“Drinking untreated water and living in close contact with cattle or other domestic animals may be risk factors associated with the possibility of humans and animals acquiring NTM infection from these ecosystems”
Conclusion rewording suggested has been effected.

Delete the last sentence.
Last sentence is deleted

Background
Line 68: would replace M. scrofulaceum with M. abscessus as most important pathogenic mycobacteria.
M. scrofulaceum has been replaced with M. abscessus as the most important pathogenic mycobacteria.

Line 78: replace ‘commonly’ with ‘traditionally’
Commonly has been replaced with traditionally

Line 88: replace ‘plays’ with ‘play’
Plays replaced with play

Paragraph starting line 99 doesn’t read well. Suggest rearrange from line 102, delete the sentence starting ‘Therefore, the increasing...” and moved to mid line 10. 7 Eg”.......the prevalence of HIV/AIDS is found to be as high as 7%. In addition a “One health, One ecosystem” study was carried out in Uganda, detecting NTM from humans with cervical lymphadenitis and cattle with lesions compatible with bovine tuberculosis. This demonstrates an increasing role for NTM as opportunistic pathogens in both immune-competent and immunocompromised individuals. As the source of these infections is not known, there is a clear need for linking data...”
Methods.

Table 1 – the numbers don’t add up. You have a subtotal of 196 for the water samples but the numbers in the table only add up to 186, which then gives you a total of 310 samples not 320. Subsequently the numbers in the text may be wrong. Please check and adjust.

Checked thoroughly and adjustments have been made. Numbers in table 1 and in the text now add up and conform to the changes made.

Not clear why the number of samples per household was so low – One of the major reasons for low yield of mycobacteria from environmental samples, is insufficient samples collected - cited by previous authors. You have 310/320 samples for 231 households, which is less than 2 samples per house.

In this study other samples were collected from the environment around household, and we tried to take a representative collection. However, we know this is a problem in such studies, and we believe we have covered this in the discussion.

Line 190 – should read “Thirty millilitres of drinking water was collected ..”

Need to explain why water wasn’t collected from every house – you state that it was only collected if there was biofilm or sediment in the sample - why??

Households that were drinking harvested rainwater from ironsheet houseroofs were not sampled or included in this study. We wanted to optimize the probability of finding mycobacteria and thus aimed at samples with visible sediment or biofilm.

How many dams were sampled every fortnight?

Nine valley dams were sampled

Line 230: delete “proportion of “ ; household should be plural

Results.

Second and third sentences don’t read well. Please state the proportion of isolates overgrown, and proportion with no growth.

The sentences are changed, and the number of samples with no growth and overgrowth by bacteria or fungi have been included.

Titles for figures and tables need to be more specific

Titles for figures and tables have been changed

I don’t know that you need to list all the species identified in the text, when they are in the table. Defer to the editors.

The mentioning of mycobacterial species has been considerably shortened down.

Line 280: ‘presences’ should read ‘presence’

It now reads presence

Line 283: change ‘getting exposed’ to ‘exposure’

It now reads exposure
Discussion.

I don’t think you need to list species again in the first sentence.
Trimmed, no more listing done

Line 302 – you could comment on the % reduction in yield of mycobacteria with decontamination that has been documented by previous authors using spiked samples.
It is indeed possible that some of the mycobacterium was killed during the disinfection process, hence contributing to the % reduction e.g Malachite green which inhibits the growth of contaminating organisms is also capable of contributing to % reduction of mycobacteria.

In addition, the sample transportation process from Uganda to Norway could have resulted in change in temperature hence leading to reduction in mycobacteria yield.

Paragraph 3 – would move the last sentence to start in middle of line 306, after “..Uganda. Other studies have detected..”
Edited according to suggestions

Line 309: …humans stand at risk…
Reworded to read humans stand at risk

Line 312: delete from “…and represents…mycobacteria” as this is already stated.
Deleted as suggested

Line 319; delete “The reason could be”
“The reason could be” has been deleted

Paragraph starting line 323 needs to be rewritten as the English is poor.Eg “ Humans can be infected/colonized with NTM without having disease and exposure to NTM in the environment is common. One of the major limitations of this study is the lack of accurate case diagnoses with disease in the communities sampled. As resources are limited patients and animals presenting with granulomatous lesions in Uganda usually do not receive culture confirmation of species involved. Therefore the species of NTM isolated in the environment can not be reliably linked to those causing disease in the patients exposed to them. However, the species isolated from the Ugandan ecosystems have been found to cause disease in patients in other parts of the world. …
Cite examples of this, then go onto talk about HIV in
Revised as suggested.

Most of page 17 is too wordy and needs to be condensed.
Condensation effected.

I would deleted from line 348 “It is also speculated.” to line 353
This section has been deleted.

Line 354: reverse ‘potential the’
‘Potential the” has been reversed to read “the potential”

Line 373: “ The present study suggests drinking…”
Stated as suggested

Line 375: ‘to be exposed’ to ‘exposure’
Replaced to be exposed with exposure

Line 380: delete sentence starting “Therefore, the present…” – too repetitive
The sentence starting with “Therefore, the present…” has been deleted

**Conclusion:** Suggest reword “This study detected a wide range of potentially pathogenic NTM in the environment around the pastoral communities in Uganda. Humans drinking untreated water and living in close contact with cattle or other domestic animals may be at risk of NTM infection. Further studies are required to assess to what extent humans and animals are infected by these environmental species.
Suggested re-wording in the conclusion has now been effected.

**Table 1.**
Numbers don’t add up – see text
Numbers now add up both in the table in the text.

**Table 2**
Instead of putting a reference to which are rapid and slow growers, why don’t you split the column M. Species table into two sections – rapid and slow
Now split into two sections –Rapid and slow growing mycobacteria.

**Table 3**
Do same as for Table 2 – split into rapid and slow growers Need to include denominators for the numbers in each column. – ie how many samples were collected in each month – so then what proportion of samples positive is more meaningful
Now split into two sections –Rapid and slow growing mycobacteria and the percentage of samples sampled each month is included in the legend.

**Table 4**
The wording of the title needs to be improved. Suggest Multivariate logistic regression analysis examining factors associated with the likelihood of isolating nontuberculous mycobacteria from household environments in the districts of Nakasongola and Mubende, Uganda.
The suggested rewording of the title is already effected.

**Figure 1**
Reword title. Frequence of isolation of NTM (number of isolates or proportion?) relative to monthly rainfall (milliliters).
Title reworded

**Figure 2.**
Title: A spider web presentation of the potential routes of exposure to Environmental mycobacteria in the pastoral communities of Nakasongola and Mubende, Uganda.
Title has been reworded as suggested

*Put these in the legend under the table. RGM _ rapid growing mycobacteria SGM – slow growing mycobacteria*
These legends have been included on the tables 2 and 3