RESEARCH ARTICLE

Who Has Mycobacterial Disease? A Cross Sectional Study in Agropastoral Communities in Tanzania

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Abstract

Objective
To determine and describe clinical symptoms, demographic characteristics and environmental exposures as determinants of pulmonary mycobacterial diseases among patients examined for tuberculosis in agropastoral communities in Northern Tanzania.

Methods
This was a cross sectional study. Sputum samples were collected from patients attending three hospitals in Tanzania, and were investigated for pulmonary tuberculosis by microscopy between November 2010 and June 2012. The patients were interviewed about background information, and potential exposure to mycobacteria.

Results
We examined 1,711 presumptive tuberculosis cases where 936 (54.2%) were males and 775 (45.3%) females. Of all the study participants, 277 (16%) were found to have sputum samples positive for mycobacteria; 228 (13%) were smear positive, 123 (7%) were culture positive and 74 (4%) were positive by both smear microscopy and culture. Of the 123 mycobacterial culture positive, 15 (12.2%) had non-tuberculous mycobacteria. Males were more likely than females to be positive for mycobacteria. Factors associated with mycobacterial disease were loss of appetite, age groups below 41 years, and being a male. Among HIV negative patients, loss of appetite, age below 20 years and being a male were associated with being mycobacterial positive. Among HIV positive patients, males and those patients with a persistently coughing family member were more likely to harbor mycobacteria.

Conclusion
The findings in this study show that both M. tuberculosis and non-tuberculous mycobacterial strains were prevalent in the study community. Some risk factors were identified.
Although the reported predictors may improve screening for mycobacterial diseases, their use requires some precaution.

Introduction

Mycobacteria are important acid-fast pathogens ranging from obligate intracellular parasites to environmental species [1]. Some mycobacteria are saprophytes and others are obligate parasites, most of them are found in soil and water in a free-living form or in diseased tissue of animals. Diseases caused by mycobacteria and the role of the environment as a reservoir of infections to human is well documented [2,3]. In communities where livestock, wildlife and humans share the same environment, there is opportunity for close interaction and increased potential risk of mycobacterial infection [4]. Mycobacterial diseases cause considerable morbidity and mortality in patients with human immunodeficiency virus (HIV) infection [5,6]. There is evidence that HIV is a major risk factor for clinical tuberculosis as well as for illnesses associated with certain opportunistic non-tuberculosis mycobacteria, such as Mycobacterium avium-intracellulare [7]. In addition to altering the risk of diseases caused by mycobacteria, the clinical characteristics of tuberculosis in HIV-infected individuals produce a more disseminated infection [8] and are more likely to be sputum-negative than persons without HIV [9].

Despite reports of existence of other mycobacterial infections in areas known to have high human-environment-livestock/wildlife interaction, available diagnosis is mainly for pulmonary tuberculosis. This is due to difficulties in diagnosing mycobacterial diseases as the clinical manifestation of most of mycobacterial lung diseases are often similar to those of many other diseases. Lack of a reliable, rapid, and inexpensive diagnostic tests to distinguish the pulmonary mycobacterial diseases remains a major obstacle to effective control of tuberculosis in sub-Saharan Africa where tuberculosis and HIV co-infection is common [10]. Sputum smear microscopy, the standard diagnostic test for pulmonary tuberculosis in low-income countries, fails to diagnose a large proportion of the patients [10,11]. Some earlier studies reported on how well clinical signs and symptoms can predict pulmonary mycobacterial diseases [12–14]. In HIV-infected adults with unexplained cough and negative sputum smears, the World Health Organization guidelines recommend clinical judgment and chest radiography for diagnosing tuberculosis.

To our knowledge, there is sufficient documentation of studies that have attempted to assess the diagnostic performance of clinical signs and symptoms [10,15]. Specifically, we aimed to describe the following among patients examined for pulmonary tuberculosis in pastoral communities in Northern Tanzania: 1) the demographic characteristics, 2) the associations between determinants and mycobacterial disease, and 3) the association between determinants and mycobacterial disease by HIV status.

Methods

Study design

This was a cross-sectional hospital-based study to assess risk factors for mycobacterial disease among hospital patients.

Study area and population

We enrolled presumptive tuberculosis patients who attended the Haydom Lutheran Hospital in Mbulu district of Manyara region, the Enduleni Catholic Hospital in Ngorongoro district of
Arusha region and the Mount Meru regional Hospital located in Arusha Municipal in northern Tanzania. We selected a study area known for its pastoral communities, and we selected three hospitals where we had previous experience in studying mycobacterial diseases and with a substantial number of patients examined for tuberculosis. We selected both government and private-not-for-profit. The study participants presented at the tuberculosis clinic for investigation. The participants had a reason or symptom that caused the clinician to refer them for investigation for tuberculosis, such as persistent cough for two weeks or more, loss of appetite, weight loss, evening fever, and hemoptysis.

For the purpose of this study, pastoralism refers to communities with farmers who grow crops and or keep livestock searching pastures and water. A presumptive tuberculosis patient (formerly "suspect") refers to an individual presenting to the health facility and being investigated for tuberculosis. In our study a tuberculosis case is an individual bacteriologically confirmed by smear microscopy or culture of a sputum sample. Literacy is the ability to read and write and speak Swahili (the national language). Education level is the highest grade of education that an individual has completed. Semi-urban means settings with business and employment as well as farming activities.

Data collection

We collected two sputum samples (spot and morning) from all consenting study participants. A specimen taken on the spot was used for routine examination at the hospital for immediate follow-up treatment, and the rest of the samples along with the morning sputum sample were transported to the Central Tuberculosis Reference Laboratory (CTRL) in Dar es Salaam. Sputum samples collected at Enduleni Catholic and Haydom Lutheran Hospitals were packed and transported to Mt. Meru Regional Hospital in Arusha on the same day of collection. Together with the samples collected at Mt. Meru Regional Hospital, the samples from Enduleni Catholic and Haydom Lutheran Hospitals were transported to the CTRL in Dar es Salaam on the second day from the day of collection. Cool boxes packed with ice cubes were used to maintain the temperature of the samples during transportation. Transport of the samples was done using public buses. In Dar es Salaam, the samples were send to the CTRL on the same day of arrival. Collection of sputum samples and processing for culture was done according to the national tuberculosis guidelines [16].

All study participants were interviewed about their demographic background and symptoms related to their illness, and about risk factors for mycobacteria especially tuberculosis. Interviews were conducted using a structured questionnaire. The interview was in Swahili, and the research assistants filled the questionnaire in English. Data collection was conducted from November 2010 to June 2013. Presence of mycobacteria was bacteriologically confirmed either by microscopy or culture.

Laboratory procedures

The sputum smears were stained using the Ziehl-Neelsen technique. Only the early morning specimen was used for culture because it was the most likely to grow mycobacteria, and it was least likely to be contaminated with other bacteria [17]. After decontamination and digestion of sputum samples with 4% sodium hydroxide (NaOH), a sterile phosphate buffer pH 6.8 was added to neutralize the effect of NaOH. The samples were concentrated by centrifugation at 3000g for 15 minutes. Supernatant was discarded and sediment was re-suspended in small amount (1–2 ml) of phosphate buffer and inoculated on the slants of solid Lowenstein Jensen (LJ) medium. Culture was considered positive if it grew any visible colonies. Samples that failed to show any growth after eight weeks of LJ incubation were classified as negative. Oxygen Preference Test and Twin 80 Test was further carried out. Species identification was done using
polymerase chain reaction (PCR). Growth on LJ media containing para-nitrobenzoic acid (PNB 500μg/ml) was considered as non-tuberculous mycobacteria. Participant HIV status was obtained from clinical records of the three hospitals.

Ethical considerations
This study was approved by the National Health Research Ethics Review Committee (NatREC) of the Medical Research Coordination Committee (MRCC) at the National Institute for Medical Research (NIMR) in Tanzania prior to its implementation (Approval Reference number: NIMR/HQ/R.8a/Vol. IX/1009). Furthermore, permission was sought and granted by regional, district and health facility authorities as required. Patients obtained information about the purpose, risks, benefits and comfort of the study participants either by reading or having the consent form read to them by the research assistants. All consenting patients signed the consent form prior to interviews and collection of sputum samples. The participants were free to decline interview at any point in time. Research assistants were trained on all important issues before commencement of data collection.

Data management
Data were double entered, validated and cleaned using EpiData version 3.1 (Epidata Association, Odense, Denmark) and STATA version 11 (STATA Corp Inc., TX, USA) for cleaning and analysis. The Pearson Chi square test was used to compare proportions between the groups. We considered $p<0.05$ as statistically significant. Multiple logistic regressions were used for assessing determinants of mycobacterial disease. Crude and adjusted odds ratios (OR) with 95% confidence intervals (CI) were reported. Variables giving $p\leq0.2$ in the univariate analysis were included as adjustment factors in the final multivariable regression model, and included sex, age, education, residence, agro-pastoralist, coughing family member, and smoking. Missing values were excluded from the regression models; the highest number of missing values was for the variable ‘age group’, where 6 values out of 159 (4%) were missing among HIV positives and 30 values missing among 505 (6%) HIV negatives.

Results
Demographic characteristics
A total of 1711 individuals were examined for tuberculosis and the socio-demographic characteristics of the study population are summarized and presented in Table 1. Of all the participants, 729 (42.6%) were from semi-urban and 979 (57.2%) were from rural areas. The mean age in years and the standard deviation (SD) of the study participants was 46 (20) for males and 44(20) for females. HIV test results were present in 664 participants of whom 159 (24%) were HIV positive (Fig 1).

Of the sputum samples from 1711 study participants who were identified through symptoms and signs of pulmonary tuberculosis, 277 (16%) were confirmed to have mycobacteria by smear microscopy and culture. Of the 1711 samples 228 (13%) were positive by smear microscopy, 123 (7%) by culture and 74 (4%) by both smear microscopy and culture. Among the 123 culture positive, 15 (12.2%) had non-tuberculous mycobacteria. Males were more likely than females to be positive for mycobacteria.

Association between determinants and mycobacterial disease
In Table 2, we show the association between the assessed potential determinants and mycobacterial disease among the study participants. We found higher risk of mycobacterial disease among men, and higher risk among those 40 years or younger compared to those over 50. Loss
of appetite was the only symptom significantly associated with being mycobacterial positive among study participants. Of all the study participants, 935 (55%) presented with a persistent cough lasting for two or more weeks (Fig 2A), 508 (30%) with loss of weight, 468 (27%) evening fever and 17 (6%) hemoptysis. In Fig 2B, we show a comparison of the proportion of reported symptoms by their HIV status.

Association between determinants of and mycobacterial disease among study participants with known HIV status

In Table 3, we present an assessment of the association between mycobacterial diseases and its determinants among the HIV positive participants: men were more likely than women to be

| Demographic characteristic | Tuberculosis suspects examined |
|----------------------------|-------------------------------|
|                            | n   | %   |
| Total                      | 1711| 100.0|
| Sex                        |     |     |
| Male                       | 927 | 54.2|
| Female                     | 775 | 45.3|
| Missing                    | 9   | 0.5 |
| Age group                  |     |     |
| ≤20                        | 148 | 8.6 |
| 21–30                      | 276 | 16.1|
| 31–40                      | 365 | 21.3|
| 41–50                      | 261 | 15.3|
| >50                        | 587 | 34.3|
| Missing                    | 74  | 4.3 |
| Education level            |     |     |
| No formal education        | 614 | 35.9|
| Primary school             | 728 | 42.6|
| Secondary school           | 290 | 17.0|
| Higher education           | 49  | 2.9 |
| Missing                    | 30  | 1.8 |
| Residence                  |     |     |
| Rural                      | 979 | 57.2|
| Semi-urban                 | 729 | 42.6|
| Missing                    | 3   | 0.2 |
| Literacy                   |     |     |
| Literate                   | 1067| 62.4|
| Illiterate                 | 622 | 36.4|
| Missing                    | 22  | 1.3 |
| Agropastoral involvement   |     |     |
| Primarily pastoralists     | 625 | 36.5|
| Primarily peasants         | 1053| 61.5|
| Missing                    | 33  | 1.9 |
| HIV status                 |     |     |
| Positive                   | 159 | 9.3 |
| Negative                   | 505 | 29.5|
| Test not done              | 1047| 61.2|

doi:10.1371/journal.pone.0153711.t001
positive for mycobacteria, and the presence of a family member with a persistent cough also predicted being positive for mycobacteria. In Table 4 we also show that among the HIV negative participants we found a higher risk of tuberculosis among men than women, among young (≤20 years) patients compared with adults over 50 years, and among those who presented with loss of appetite as a symptom for their illness.

Discussion

The current study shows that pulmonary mycobacterial diseases were common among the investigated patients. Men had higher risk of mycobacterial diseases both among HIV positives and HIV negatives, as well as the HIV positive patients who had a family member with persistent cough. Young adults and patients presenting with loss of appetite also were at increased risk of mycobacterial disease.

We demonstrate that non-tuberculous mycobacteria were prevalent among the participants examined in the agropastoral communities in northern Tanzania. Since patients with non-tuberculous mycobacteria present with acute or chronic illness that is clinically and radiologically indistinguishable from \textit{M. tuberculosis}, misdiagnosis of non-tuberculous mycobacteria infection could therefore lead to inappropriate anti-tuberculosis treatment. In Tanzania, the major diagnostic method for tuberculosis is sputum smear microscopy with culture only done at the CTRL and some few zonal laboratories. For that case non-tuberculous mycobacteria cases with positive smears will continue to be misclassified as \textit{M. tuberculosis} and subsequently treated with conventional anti-tuberculosis drugs to which some of them may be resistant and a large majority of non-tuberculous mycobacterial infections will remain undetected.

Occurrence of mycobacterial diseases in the study area

Information on the prevalence of diseases such as tuberculosis is vital for planning, implementation and evaluation of control strategies at local, national and global levels. In the current study, we report that the majority of the patients found to have mycobacteria had a positive sputum smear. We think part of the reason that many acid fast bacilli positives were negative on culture may have been due to long transport time. Still the proportion of "presumptive
Table 2. Determinants of mycobacterial diseases among 1711 patients examined for tuberculosis in three hospitals of Northern Tanzania, 2010–12.

| Determinant                          | Total Suspects | Mycobacteria (+) | OR (95%CI) | AOR* (95%CI) |
|--------------------------------------|----------------|------------------|------------|--------------|
|                                      | n (n (%))      |                  |            |              |
| Total                                | 1711 (277 (16.2)) |                  |            |              |

### Demographic characteristics

#### Sex
- Male: 927 (171 (18.4))
- Female: 776 (104 (13.4))
- Missing: 8 (2 (25.0))

#### Age group
- ≤20: 148 (29 (19.6))
- 21–30: 276 (55 (19.9))
- 31–40: 365 (73 (20.0))
- 41–50: 261 (36 (13.8))
- >50: 587 (75 (12.8))

#### Level of education
- No formal education: 728 (123 (16.9))
- Primary School: 290 (61 (21.0))
- Secondary School: 49 (6 (12.2))
- Higher Education: 615 (84 (13.7))
- Missing: 29 (3 (10.3))

#### Residence
- Semi-urban: 978 (159 (16.3))
- Rural: 730 (118 (16.2))
- Missing: 3 (0 (0.0))

#### Education status
- Literate: 1067 (190 (17.8))
- Illiterate: 623 (84 (13.5))
- Missing: 21 (3 (14.3))

#### Agropastoral involvement
- Primarily peasants: 1053 (186 (17.7))
- Primarily pastoralists: 626 (87 (13.9))
- Missing: 32 (4 (12.5))

### Environmental factors

#### Family size
- 6 or less: 562 (101 (18.0))
- More than 6: 1149 (177 (15.4))

#### Contact with person with tuberculosis
- Yes: 235 (37 (15.7))
- No: 1413 (230 (16.3))
- Missing: 64 (10 (15.6))

#### Shared a room with domestic animals
- Yes: 564 (76 (13.5))
- No: 1136 (198 (17.4))
- Missing: 11 (3 (27.3))

### Shared water source with animals

(Continued)
| Determinant                              | Total Suspects | Mycobacteria (+) | OR (95%CI) | AOR* (95%CI) |
|-----------------------------------------|----------------|------------------|------------|--------------|
|                                         | **n**          | **n (%)**        |            |              |
| Total                                   | 1711           | 277 (16.2)       |            |              |
| Yes                                     | 589            | 80 (13.6)        | 0.7 (0.6–1.0) | 0.7 (0.3–1.5) |
| No                                      | 1114           | 195 (17.5)       | REF        |              |
| Missing                                 | 8              | 2 (25.0)         |            |              |
| Presence of family member with cough    |                |                  |            |              |
| Yes                                     | 353            | 53 (15.0)        | 0.9 (0.7–1.3) |              |
| No                                      | 1328           | 218 (16.4)       | REF        |              |
| Missing                                 | 30             | 6 (20.0)         |            |              |
| Smoking                                 |                |                  |            |              |
| Yes                                     | 433            | 66 (15.2)        | 0.9 (0.7–1.3) |              |
| No                                      | 1257           | 205 (16.3)       | REF        |              |
| Missing                                 | 21             | 6 (28.6)         |            |              |
| Keeping animals                         |                |                  |            |              |
| Yes                                     | 625            | 86 (13.8)        | 0.8 (0.6–1.0) | 0.8 (0.4–1.7) |
| No                                      | 1053           | 186 (17.7)       | REF        |              |
| Missing                                 | 33             | 5 (15.2)         |            |              |
| Previously treated for tuberculosis     |                |                  |            |              |
| Yes                                     | 92             | 10 (10.9)        | 0.7 (0.4–1.3) |              |
| No                                      | 1605           | 263 (16.4)       | REF        |              |
| Missing                                 | 15             | 4 (26.7)         |            |              |
| Symptoms                                |                |                  |            |              |
| Cough                                   |                |                  |            |              |
| Yes                                     | 935            | 147 (15.8)       | 0.9 (0.7–1.2) |              |
| No                                      | 771            | 128 (16.6)       | REF        |              |
| Missing                                 | 5              | 2 (40.0)         |            |              |
| Hemoptysis                              |                |                  |            |              |
| Yes                                     | 107            | 17 (16.0)        | 1.0 (0.6–1.7) | 0.9 (0.5–1.9) |
| No                                      | 1598           | 257 (16.1)       | REF        |              |
| Missing                                 | 6              | 3 (33.3)         |            |              |
| Evening fever                           |                |                  |            |              |
| Yes                                     | 468            | 75 (16.1)        | 1.0 (0.8–1.3) | 1.0 (0.7–1.6) |
| No                                      | 1230           | 199 (16.2)       | REF        |              |
| Missing                                 | 13             | 3 (18.8)         |            |              |
| Loss of weight                          |                |                  |            |              |
| Yes                                     | 508            | 80 (15.8)        | 1.0 (0.7–1.3) |              |
| No                                      | 1191           | 193 (16.2)       | REF        |              |
| Missing                                 | 12             | 4 (26.7)         |            |              |
| Loss of appetite                        |                |                  |            |              |
| Yes                                     | 285            | 62 (21.8)        | 1.6 (1.2–2.2) | 2.1 (1.4–3.2) |
| No                                      | 1414           | 211 (15.0)       | REF        |              |
| Missing                                 | 12             | 4 (26.7)         |            |              |

*Adjustment factors included: Sex, Age, Education, Residence, Agropastoralist, Coughing family member, and Smoking.*

doi:10.1371/journal.pone.0153711.t002
tuberculosis patients who were finally reported to be mycobacterial positive (the yield) was higher than the national and regional smear positive tuberculosis rates [18]. Reports from other African countries have also documented varying prevalence of mycobacteria, indicating their public health importance in agropastoral communities [19,20]. Factors such as HIV, patient’s understanding about the disease, and an increased role of environmental sources and livestock/wildlife reservoirs have been reported to play role in the existence of mycobacterial diseases in humans [2,21,22].

Association between demographic determinants and mycobacterial diseases

Diagnosis of pulmonary tuberculosis based on a combination of clinical symptoms, sputum microscopy for acid-fast bacilli and chest radiography have been reported to be fairly sensitive, but nonspecific [23]. A study conducted earlier reported that age and symptoms were useful in predicting and screening for smear-negative pulmonary tuberculosis suspects and cases [24]. Predictors of mycobacterial diseases reported in this study were in line with those reported in a study conducted to evaluate the clinical, diagnostic and epidemiological characteristics of patients suspected to have pulmonary tuberculosis in Ethiopia [23]. This observation indicates that if used as a tool to support the diagnosis of mycobacterial diseases, clinical symptoms are useful, although their use may require some caution. The general rule in diagnosing mycobacterial diseases, including pulmonary tuberculosis involves examination of a patient with a cough or expectoration for two or more weeks by smear microscopy or chest radiograph [25]. In addition, in order to find patients with mycobacterial diseases, clinicians inquire about symptoms, risky exposures and habits that may suggest the need for further investigation [24]. In the current study, we found that sex, age and loss of appetite were associated with being
| Determinant                              | Total Suspects | Mycobacteria (+) | OR (95%CI) | AOR* (95%CI) |
|-----------------------------------------|----------------|------------------|------------|--------------|
| **Total**                               | 159            | 21 (13.2)        |            |              |
| **Demographic characteristics**         |                |                  |            |              |
| **Sex**                                 |                |                  |            |              |
| Male                                    | 78             | 15 (19.2)        | 3.0 (1.1–8.1) | 2.8 (1.0–8.0) |
| Female                                  | 81             | 6 (7.4)          | REF        | REF          |
| **Age group**                           |                |                  |            |              |
| ≤20                                     | 19             | -                | **         |              |
| 21–30                                   | 27             | 3 (11.1)         | 1.1 (0.2–7.5) |              |
| 31–40                                   | 54             | 12 (22.2)        | 2.6 (0.5–12.7) |              |
| 41–50                                   | 33             | 4 (12.1)         | 1.2 (0.2–7.5) |              |
| >50                                     | 20             | 2 (10.0)         | REF        |              |
| Missing                                 | 6              | -                |            |              |
| **Level of education**                  |                |                  |            |              |
| No formal education                     | 104            | 13 (12.5)        | **         |              |
| Primary School                          | 26             | 5 (19.2)         | **         |              |
| Secondary School                        | 3              | 0 (0.0)          | **         |              |
| Higher Education                        | 25             | 3 (12.0)         | REF        |              |
| **Residence**                           |                |                  |            |              |
| Semi-urban                              | 119            | 14 (11.8)        | REF        |              |
| Rural                                   | 40             | 7 (17.5)         | 1.6 (0.6–4.3) |              |
| **Education status**                    |                |                  |            |              |
| Literate                                | 133            | 18 (13.5)        | REF        |              |
| Illiterate                              | 26             | 3 (11.5)         | 0.8 (0.2–3.1) |              |
| **Agropastoral involvement**            |                |                  |            |              |
| Primarily peasants                      | 135            | 17 (12.6)        | REF        |              |
| Primarily pastoralists                  | 22             | 4 (18.2)         | 1.5 (0.5–5.1) |              |
| Missing                                 | 2              | -                |            |              |
| **Environmental factors**               |                |                  |            |              |
| **Family size**                         |                |                  |            |              |
| 6 or less                               | 63             | 8 (12.7)         | REF        |              |
| More than 6                             | 96             | 57 (15.4)        | 0.9 (0.3–3.0) |              |
| **Contact with person with tuberculosis**|                |                  |            |              |
| Yes                                     | 2              | 0 (0.0)          | **         |              |
| No                                      | 151            | 19 (12.6)        | REF        |              |
| Missing                                 | 6              | 2 (33.3)         |            |              |
| **Shared a room with domestic animals**  |                |                  |            |              |
| Yes                                     | 23             | 3 (13.0)         | 1.0 (0.3–3.7) |              |
| No                                      | 136            | 18 (13.2)        | REF        |              |
| **Shared water source with animals**    |                |                  |            |              |
| Yes                                     | 19             | 3 (15.8)         | 1.0 (0.3–3.9) |              |
| No                                      | 119            | 18 (15.1)        | REF        |              |
| **Presence of family member with cough**|                |                  |            |              |
| Yes                                     | 3              | 2 (66.7)         | 15.0 (1.3–173.9) | 11.05 (1.1–175.3) |
| No                                      | 153            | 18 (11.8)        | REF        |              |

(Continued)
mycobacteria positive, regardless of the HIV sero-status of the individual. Our findings align well with studies conducted in other developing countries [26–29].

Association between potential determinants and mycobacterial diseases by HIV status

Among the mycobacterial diseases associated with HIV infection, tuberculosis is of particular importance [30]. People infected with HIV have ten times higher risk of developing

Table 3. (Continued)

| Determinant                         | Total Suspects | Mycobacteria (+) | OR (95%CI) | AOR* (95%CI) |
|-------------------------------------|----------------|------------------|------------|-------------|
|                                     | n  | n (%)           |            |             |
| Total                               | 159 | 21 (13.2)       |            |             |
| Smoking                             |     |                 |            |             |
| Yes                                 | 14  | 3 (21.4)        | 2.0 (0.5–8.0) |             |
| No                                  | 143 | 17 (11.9)       | REF        |             |
| Missing                             | 2   | 1 (50.0)        |            |             |
| Keeping animals                     |     |                 |            |             |
| Yes                                 | 22  | 4 (18.2)        | 1.5 (0.5–5.1) |             |
| No                                  | 135 | 17 (12.6)       | REF        |             |
| Missing                             | 2   | 0 (0.0)         |            |             |
| Previously treated for tuberculosis |     |                 |            | **          |
| Yes                                 | 9   | -               | **         |             |
| No                                  | 149 | 21 (14.1)       | REF        |             |
| Missing                             | 1   | 1 (100)         |            |             |
| Symptoms                            |     |                 |            |             |
| Cough                               |     |                 |            |             |
| Yes                                 | 65  | 10 (15.4)       | 1.4 (0.6–3.5) |             |
| No                                  | 94  | 11 (11.7)       |            |             |
| Missing                             | -   | -               |            |             |
| Hemoptysis                          |     |                 |            |             |
| Yes                                 | 13  | 3 (23.1)        | 2.1 (0.5–8.5) |             |
| No                                  | 146 | 18 (12.3)       |            |             |
| Missing                             | -   | -               |            |             |
| Evening fever                       |     |                 |            |             |
| Yes                                 | 31  | 6 (19.4)        | 1.8 (0.6–5.1) |             |
| No                                  | 128 | 15 (11.7)       |            |             |
| Missing                             | -   | -               |            |             |
| Loss of weight                      |     |                 |            |             |
| Yes                                 | 34  | 6 (17.6)        | 1.6 (0.6–4.4) |             |
| No                                  | 125 | 15 (12.0)       | REF        |             |
| Missing                             | -   | -               |            |             |
| Loss of appetite                    |     |                 |            |             |
| Yes                                 | 27  | 6 (22.2)        | 2.2 (0.8–6.4) | 1.33 (0.3–5.2) |
| No                                  | 132 | 15 (11.4)       | REF        |             |

*Adjustment factors included: Sex, Age, Education, Residence, Agropastoralist, Coughing family member, and Smoking.

**Some cells had expected values < 5 making the analysis invalid.
Table 4. Determinants of mycobacterial disease among 505 HIV negative patients examined for tuberculosis in Northern Tanzania, 2010–12.

| Determinant                        | Total Suspects | Mycobacteria (+) | OR (95%CI) | AOR* (95%CI) |
|------------------------------------|----------------|------------------|------------|--------------|
|                                    | n   | n (%)           |            |              |
| Total                              | 505 | 82 (16.2)       |            |              |

Demographic characteristics

| Sex                  | n   | n (%) | OR (95%CI) | AOR* (95%CI) |
|----------------------|-----|-------|------------|--------------|
| Male                 | 264 | 49 (18.6) | 1.4 (0.9–2.3) | 2.2 (1.3–3.8) |
| Female               | 239 | 33 (13.8) | REF       |              |
| Missing              | 2   | -      |            |              |

Age group

| Age group | n   | n (%) | OR (95%CI) | AOR* (95%CI) |
|-----------|-----|-------|------------|--------------|
| <20       | 41  | 11 (26.8) | 2.5 (1.1–5.5) | 2.5 (1.0–6.3) |
| 21–30     | 78  | 12 (15.4) | 1.2 (0.6–2.6) | 1.2 (0.6–2.8) |
| 31–40     | 79  | 18 (22.8) | 2.0 (1.0–3.9) | 1.8 (0.9–3.7) |
| 41–50     | 59  | 10 (16.9) | 1.4 (0.6–3.0) | 1.8 (0.6–3.2) |
| >50       | 218 | 28 (12.8) | REF       |              |
| Missing   | 30  | 3 (10.0) |            |              |

Level of education

| Level of education | n   | n (%) | OR (95%CI) | AOR* (95%CI) |
|--------------------|-----|-------|------------|--------------|
| No formal education | 155 | 32 (20.6) | 1.6 (1.0–2.7) |              |
| Primary            | 58  | 10 (17.2) | 1.3 (0.6–2.7) |              |
| Secondary          | 7   | 1 (14.3) | 1.0 (0.1–8.7) |              |
| Higher             | 277 | 39 (14.1) | REF       |              |
| Missing            | 8   | -      |            |              |

Residence

| Residence | n   | n (%) | OR (95%CI) | AOR* (95%CI) |
|-----------|-----|-------|------------|--------------|
| Semi-urban | 173 | 32 (18.5) | REF       |              |
| Rural     | 332 | 50 (15.1) | 0.8 (0.5–1.3) |              |

Education status

| Education status | n   | n (%) | OR (95%CI) | AOR* (95%CI) |
|------------------|-----|-------|------------|--------------|
| Literate         | 220 | 43 (19.5) | REF       |              |
| Illiterate       | 279 | 39 (14.0) | 0.7 (0.4–1.1) |              |
| Missing          | 6   | -      |            |              |

Agropastoral involvement

| Agropastoral involvement | n   | n (%) | OR (95%CI) | AOR* (95%CI) |
|--------------------------|-----|-------|------------|--------------|
| Primarily peasants       | 166 | 36 (21.7) | REF       |              |
| Primarily pastoralists   | 338 | 46 (13.6) | 0.6 (0.4–0.9) |              |
| Missing                  | 1   | -      |            |              |

Environmental factors

| Environmental factors   | n   | n (%) | OR (95%CI) | AOR* (95%CI) |
|-------------------------|-----|-------|------------|--------------|
| Family size             |     |       |            |              |
| 6 or less               | 135 | 25 (18.5) | REF       |              |
| More than 6             | 370 | 57 (15.4) | 0.8 (0.4–1.5) |              |
| Contact with person with tuberculosis |     |       |            |              |
| Yes                     | 130 | 22 (16.9) | 1.1 (0.6–1.8) | 2.1 (1.0–4.5) |
| No                      | 365 | 59 (16.2) | REF       |              |
| Missing                 | 10  | 1 (10.0) |            |              |
| Shared a room with domestic animals |     |       |            |              |
| Yes                     | 313 | 43 (13.7) | 0.6 (0.4–0.1) | 1.4 (0.4–5.0) |
| No                      | 191 | 39 (20.4) | REF       |              |
| Missing                 | 1   | -      |            |              |
| Shared water source with animals |     |       |            |              |
| Yes                     | 306 | 41 (13.4) | 0.6 (0.4–1.0) | 0.6 (0.2–2.0) |
| No                      | 199 | 41 (20.6) | REF       |              |

(Continued)
Co-infection with HIV has a major effect on the natural history of many infectious diseases, particularly mycobacterial diseases [32]. HIV infection has been reported to affect the diagnosis of pulmonary tuberculosis in HIV positive patients [33]. In our study, we found that tuberculosis than healthy people, and pulmonary tuberculosis is still the most common form [31].

### Table 4. (Continued)

| Determinant | Total Suspects | Mycobacteria (+) | OR (95%CI) | AOR * (95%CI) |
|-------------|---------------|-----------------|------------|---------------|
|             | n             | n (%)           |            |               |
| **Total**   | 505           | 82 (16.2)       |            |               |
| Presence of family member with cough |               |                 |            |               |
| Yes         | 204           | 28 (13.7)       | 0.7 (0.4–1.2) | 0.7 (0.3–1.7) |
| No          | 296           | 54 (18.2)       |            | REF           |
| Missing     | 5             | -               |            |               |
| Smoking     |               |                 |            |               |
| Yes         | 224           | 30 (13.4)       | 0.7 (0.4–112) | 0.9 (0.5–1.8) |
| No          | 277           | 51 (18.4)       |            | REF           |
| Missing     | 4             | 1 (25.0)        |            |               |
| Keeping animals |         |                 |            |               |
| Yes         | 338           | 46 (13.6)       | 0.6 (0.4–0.9) | 0.5 (0.2–1.6) |
| No          | 166           | 36 (21.7)       |            | REF           |
| Missing     | 1             | -               |            |               |
| Previously treated for tuberculosis |     |                 |            |               |
| Yes         | 28            | 3 (10.7)        | 0.6 (0.2–2.0) |               |
| No          | 474           | 79 (16.7)       |            | REF           |
| Missing     | 2             | -               |            |               |
| **Symptoms** |               |                 |            |               |
| Cough       |               |                 |            |               |
| Yes         | 395           | 59 (14.9)       | 0.7 (0.4–1.1) |               |
| No          | 110           | 23 (20.9)       |            | REF           |
| Missing     | -             | -               |            |               |
| Hemoptysis  |               |                 |            |               |
| Yes         | 45            | 6 (13.3)        | 0.8 (0.3–1.9) |               |
| No          | 459           | 76 (16.6)       |            | REF           |
| Missing     | 1             | -               |            |               |
| Evening fever |            |                 |            |               |
| Yes         | 201           | 27 (13.4)       | 0.7 (0.4–1.2) | 0.7 (0.4–1.3) |
| No          | 304           | 55 (18.1)       |            | REF           |
| Missing     | -             | -               |            |               |
| Loss of weight |           |                 |            |               |
| Yes         | 245           | 38 (15.5)       | 0.9 (0.6–1.4) |               |
| No          | 258           | 44 (17.1)       |            | REF           |
| Missing     | 2             | -               |            |               |
| Loss of appetite |       |                 |            |               |
| Yes         | 131           | 29 (22.1)       | 1.7 (1.0–2.8) | 2.8 (1.5–5.2) |
| No          | 373           | 53 (14.2)       |            | REF           |
| Missing     | 1             | -               |            |               |

* Adjustment factors included in the final model were: Sex, Age, Education, Residence, Agropastoralist, Coughing family member, and Smoking.

doi:10.1371/journal.pone.0153711.t004
among HIV positive individuals, having a family member with a persistent cough was associated with being mycobacteria positive. It is well known that persons in the household of a tuberculosis patient are exposed to the bacteria and may develop disease, but for clinical practice, this is not among the “classic” risk factors for identifying patients to be examined for tuberculosis. In our study, this was not a significant risk factor among HIV negative patients, but for HIV positive patients, it was a very strong risk factor. Although there is no conclusive evidence that HIV sero-positive persons are more likely to acquire tuberculosis infection than HIV sero-negative individuals given the same degree of exposure [34], the risk of rapid progression is much greater among persons with HIV infection, as HIV impairs the host’s ability to contain new tuberculosis infection. HIV co-infection also increases the risk of progression of recently acquired infection to active disease [34,35]. The impact of HIV on the epidemiology, natural history, and clinical presentation of mycobacterial diseases, especially tuberculosis, has been well documented in previous studies, and it may explain the reported findings [36,37].

The reported and observed limited clinical symptoms show that when diagnosing mycobacterial diseases in persons with known or possible HIV infection, one has to consider using an appropriate diagnostic and screening approach. Although screening for mycobacterial diseases using symptoms does not require expensive equipment or specialized health personnel, the sensitivity and specificity of symptoms as a tool for diagnosis of tuberculosis has been reported to be lower in immune suppressed HIV individuals [33].

This study shows that for HIV negative individuals who had a family member who had been coughing for two or more weeks, being a male and aged 20 years or younger were significantly associated with being mycobacteria positive. Other studies show that screening by cough alone in HIV positive patients has low sensitivity [38–42], with up to 86% of tuberculosis cases being missed. In a study conducted in Cambodia, it was reported that the sensitivity of using symptoms rose when fever and weight loss were included as symptoms of mycobacterial disease [40]. This study has some limitations. Assessment of the HIV status of the suspected tuberculosis patients involved in this study relied on patient records available at the health facilities. As part of the national policy, all suspected tuberculosis patients are tested for HIV. However, due to poor record keeping and logistical issues, we found that more than half of the tuberculosis patients lacked HIV test results in the health facility register. This resulted to a lower strength of our associations, and care must be taken in interpretation when no associations between determinant and mycobacterial disease are found. Also, in the selection of all participants by symptoms, even the comparison group represents “suspects”, not healthy individuals. That means we already selected those with symptoms, so we cannot really say how well symptoms predict mycobacterial disease such as tuberculosis in the population, only how well it predicts disease among suspects. This will confound and weaken the associations between symptoms and disease, and in our study many symptoms will not predict disease in the normal population. Furthermore, many variables (e.g. symptoms) depend on participants’ recall and understanding about the disease and thus do not always represent the objective reality. However, this parallels the situation for clinicians, who are often more dependent on recall than objective reality.

**Conclusion**

The findings in this study show that both *M. tuberculosis* and non-tuberculous mycobacterial strains were prevalent in the study community. The high proportion of nontuberculous mycobacteria among the participants indicates clinical and environmental occurrence and possible human-environment-livestock risks of cross transmission. Some risk factors were identified which may improve screening for mycobacterial diseases, but their use requires some precaution.
Acknowledgments

We are grateful to the study participants, regional and district health authorities in Arusha and Manyara regions as well as local community leaders. The Wellcome Trust through the Afrique One Consortium financially supported the study. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Author Contributions

Conceived and designed the experiments: AMK EN BJN SGM. Performed the experiments: AMK YLL BJN JM. Analyzed the data: AMK EN GBK YLL BJN SGM SGH. Contributed reagents/materials/analysis tools: AMK YLL GBK BJN SGM. Wrote the paper: AMK EN JM YLL GBK BJN SGM SGH. Review and approval of the final draft of the manuscript: AMK EN JM YLL GBK BJN SGM SGH.

References

1. Brown-Elliott BA, Wallace RJ Jr. Clinical and taxonomic status of pathogenic non-pigmented or late-pigmenting rapidly growing mycobacteria. Clin Microbiol Rev. 2002; 15:716–746. PMID: 12364376
2. van Ingen J, Boeree MJ, Dekhuijzen PNR and van Soolingen D. Environmental sources of rapid growing non-tuberculous mycobacteria causing disease in humans. Clin Microbiol Infect. 2009; 15:888–893. doi:10.1111/j.1469-0691.2009.03013.x PMID: 19845700
3. Ameni G, Vordermeier M, Firdessa R, Aseffa A, Hewinson G, Gordon SV, et al. Mycobacterium tuberculosis infection in grazing cattle in central Ethiopia. Vet J. 2011; 188:359–361. doi: 10.1016/j.tvjl.2010.05.005 PMID: 20965132
4. Oloya J, Opuda-Asibo J, Kazwala R, Demelash AB, Skjerve E, Lund A, et al. Mycobacteria causing human cervical lymphadenitis in pastoral communities in the Karamoja region of Uganda. Epidemiol Infect. 2008; 136:636–643. PMID: 17599779
5. Washington L and Miller WT. Mycobacterial infection in immunocompromised patients. J Thorac Imaging. 1998; 13(4):271–81 PMID: 9799135
6. Beck K. Mycobacterial disease associated with HIV infection. J Gen Intern Med. 1991; 6(1):S19–23. PMID: 2005473
7. Horsburgh CR Jr. Epidemiology of disease caused by nontuberculous mycobacteria. Semin Respir Infect. 1996; 11(4):244–51. PMID: 8976578
8. Porter DHJ. Mycobacteriosis and HIV infection: the new public health challenge. Journal of Antimicrobial Chemotherapy. 1996; 37:113–120. PMID: 8918834
9. Elliott AM, Hayes RJ, Halwiindi B, Luo N, Tembo G, Pobee JD, et al. The impact of HIV on infectiousness of pulmonary tuberculosis: a community study. AIDS. 1993; 7:981–7. PMID: 8357557
10. Davis JL, Worodria W, Kisembo H, Metcalfe JZ, Cattamanchi A, Kawooya M, et al. Clinical and radiographic factors do not accurately diagnose smear-negative tuberculosis in HIV-infected inpatients in Uganda: a cross-sectional study. PLoS One. 2010; 5(3):e9859.
11. Steingart KR, Ng V, Henry M, Hopewell PC, Ramsay A, Cunningham J, et al. Sputum processing methods to improve the sensitivity of smear microscopy for tuberculosis: a systematic review. Lancet Infect Dis. 2006; 6:664–674. PMID: 17008175
12. Wilson D, Nachega J, Morroni C, Chaisson R, Maartens G. Diagnosing smear-negative tuberculosis using case definitions and treatment response in HIV-infected adults. Int J Tuberc Lung Dis. 2006; 10:31–38. PMID: 16466034
13. Were W, Moore D, Ekwaru P, Mwima G, Bunnell R, Kaharuza F, et al. A simple screening tool for active tuberculosis in HIV-infected adults receiving antiretroviral treatment in Uganda. Int J Tuberc Lung Dis. 2009; 13:47–53. PMID: 19105878
14. Palmer DL, SooHoo GH, Sopher RL. Clinical determinants of tuberculosis screening. South Med J. 1981; 74(2):170–4. PMID: 6781072
15. World Health Organization: Improving the diagnosis and treatment of smear negative pulmonary and extra pulmonary tuberculosis among adults and adolescents, recommendations for HIV-prevalent and resource-constrained settings. Geneva: WHO, Stop TB and HIV Departments; 2007. WHO/HTM/HIV/2007.01
16. National Tuberculosis and Leprosy Programme. Manual for the Management of Tuberculosis and Leprosy. Ministry of Health and Social Welfare. 8th Edition 2013. ISBN 978 9987 9708.
17. Ssengooba S, Kateete DP, Waija A, Bugumirwa E, Mboowa G, Namaganda C, et al. An early morning sputum sample is necessary for the diagnosis of pulmonary tuberculosis, even with more sensitive techniques: A prospective cohort study among adolescent TB-suspects in Uganda. *Tuberculosis Research and Treatment*. 2012 (2012: ), Article ID 970203.

18. National Tuberculosis and Leprosy Programme, Ministry of Health and Social Welfare. 2013 Annual Report.

19. Berg S, Firdessa R, Habtamu M, Gadisa E, Mengistu A, Yamuah L, et al. The Burden of Mycobacterial Disease in Ethiopian Cattle: Implications for Public Health. *PLoS ONE*. 2009; 4(4):e5068. doi:10.1371/journal.pone.0005068 PMID: 19352493

20. Mawak J, Gomwalk N, Bello C, Kandakai-Olukemi Y. Human pulmonary infections with bovine and environment (atypical) mycobacteria in Jos, Nigeria. *Ghana Med J*. 2006; 40(4):132–136. PMID: 17496986

21. Prim TP, Lucero CA, Falkinham JO. Health impacts of environmental mycobacteria. *Clin Microbiol Rev*. 2004; 17(1):98–106. PMID: 14726457

22. Chilima BZ, Clark IM, Floyd S, Fine PEM and Hirsch PR. Distribution of Environmental Mycobacteria in Karonga District, Northern Malawi. *Appl. Environ. Microbiol.* 2006; 72(4):2343–2350. PMID: 16597928

23. Bruchfeld J, Aderaye G, Palme IB, Bjorvatn B, Britton S, Feleke Y, et al. Evaluation of outpatients with suspected pulmonary tuberculosis in a high HIV prevalence setting in Ethiopia: clinical, diagnostic and epidemiological characteristics. *Scand J Infect Dis*. 2002; 34(5):331–7. PMID: 12069014

24. Tamhane A, Chheng P, Dobbs T, Mak S, Sar B, Kimerling ME. Predictors of smear-negative pulmonary tuberculosis in HIV-infected patients, Battambang, Cambodia. *Int J Tuberc Lung Dis*. 2009; 13(3):347–54. PMID: 19275795

25. Smith I. *Mycobacterium tuberculosis* pathogenesis and molecular determinants of virulence. *Clinical Microbiology Reviews*. 2003; 16(3):463–496. PMID: 12857778

26. Chilima C, Fielding K, Sillah JS, Bah B, Gustafson P, Wendorff D, et al. Investigation of the risk factors for tuberculosis: a case-control study in three countries in West Africa. *International Journal of Epidemiology*. 2005; 34(4):914–923. PMID: 15914505

27. Lienhardt C, Fielding K, Chihota VN, Hanifa Y, Grant AD, et al. Symptom and chest radiographic screening for infectious tuberculosis prior to starting isoniazid preventive therapy: yield and proportion missed at screening. *AIDS*. 2010; 24:S19–27. 49. doi:10.1097/01.aids.0000391018.72542.46 PMID: 21079424

28. Lawn SD, Brooks SV, Kranzer K, Nicol MP, Whitelaw A, Vogt M, et al. Screening for HIV-associated tuberculosis and rifampicin resistance before antiretroviral therapy using the Xpert MTB/RIF assay: a prospective study. *PLoS Med*. 2011; 8:e1001067. doi:10.1371/journal.pmed.1001067 PMID: 21818180

29. Corbett EL, Watt CJ, Walker N, Maher D, Williams BG, Raviglione MC, et al. The growing burden of tuberculosis: global trends and interactions with the HIV epidemic. *Arch Intern Med*. 2003; 163:1009–1021. PMID: 12742798

30. World Health Organization. TB/HIV: A Clinical Manual, Second Edition. *World Health Organization*, 2004.

31. Lockwood DN and Lambert SM. Human immunodeficiency virus and leprosy: an update. *Dermatologic Clinics*. 2011, 29(1):125–8. doi:10.1016/j.det.2010.08.016 PMID: 21095536

32. Rewata L, Rutherford M, Apriani L, Janssen W, Rahmadi A, Parwati I, et al. Improving diagnosis of pulmonary tuberculosis among HIV/AIDS patients: literature review and experience in a teaching hospital in Indonesia. *Acta Med Indones*. 2009; 41(1):57–64.

33. Whalen CC, Zalwango S, Chiunda A, Malone L, Eisenach K, Joloba M, et al. Secondary attack rate of tuberculosis in urban households in Kampala, Uganda. *PLoS One*. 2011; 6(2):e16137.

34. Sitaram HM and Bala K. Tuberculosis and HIV Double trouble. *World Journal of Pharmacy and Pharmaceutical Sciences*. 2015; 4(5):338–345.

35. Thomas DJ. Mycobacterial Diseases in HIV-Positive Patients. *Journal of Pharmacy Practice*. 2006; 19;1:10–16.

36. Tortoli E. Clinical manifestations of nontuberculous mycobacteria infections. *Clin Microbiol Infect*. 2009; 15:906–910. doi: 10.1111/j.1469-0691.2009.03014.x PMID: 19845702
38. Mohammed A, Ehrlich R, Wood R, Cillier F & Maartens G. Screening for tuberculosis in adults with advanced HIV infection prior to preventive therapy. Int J Tuberc Lung Dis. 2004; 8:792–795. PMID: 15182152

39. Shah SM, DeMissie L, Lambert J, Ahmed S, Leulseged T, Kebede T, et al., Intensified tuberculosis case finding among HIV-Infected persons from a voluntary counseling and testing center in Addis Ababa, Ethiopia. J Acquir Immune Defic Syndr. 2009; 50(5):537–45. doi: 10.1097/QAI.0b013e318196761c PMID: 19223783

40. Chheng P, Tamhane A, Natpratan C, Tan V, Lay V, Sar B, et al., Pulmonary tuberculosis among patients visiting a voluntary confidential counseling and testing center, Cambodia. Int J Tuberc Lung Dis. 2008; 12(3)(1):54–62. PMID: 18302824

41. Kimerling ME, Schuchter J, Chanthol E, Kunthy T, Stuer F, Glaziou P, et al. Prevalence of pulmonary tuberculosis among HIV-infected persons in a home care program in Phnom Penh, Cambodia. Int J Tuberc Lung Dis. 2002; 6:988–94. PMID: 12475145

42. Wood R, Middelkoop K, Myer L, Grant AD, Whitelaw A, Lawn SD, et al. Undiagnosed tuberculosis in a community with high HIV prevalence: implications for tuberculosis control. Am J Respir Crit Care Med. 2007; 175:87–93. PMID: 16973982