Mycobacteria in northern Tanzania:

Exposure and risk of disease among agropastoralists and programmatic challenges in investigation of re-treatment cases

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Title: **Mycobacteria in northern Tanzania: Exposure and risk of disease among agropastoralists and programmatic challenges in investigation of re-treatment cases**

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Dedication

To the memory of my beloved Father, the late **Martin Meza** and my Mother, T**wingilage Mwandawila Sanga**

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I thank His Almighty God for blessing me with this opportunity and keeping me strong throughout the period of my studies.

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List of acronyms

AIDS	Acquired immunodeficiency syndrome
AOR	Adjusted Odds Ratio
CI	Confidence Intervals
COR	Crude Odds Ratio
CTRL	Central Reference Laboratory
DST	Drug Susceptibility Testing
E	Ethambutol
EAG	Union Ethics Advisory Group
EPTB	Extra-pulmonary Tuberculosis
H	Isoniazid
HIV	Human Immunodeficiency Virus
MAC	*Mycobacterium avium*-complex
MDR-TB	Multidrug Resistant Tuberculosis
MOTT	Mycobacteria Other Than Tuberculosis
MTB	*Mycobacterium tuberculosis*
NatREC	National Health Research Ethics Review Committee
NIMR	National Institute for Medical Research
NTLP	National Tuberculosis and Leprosy Programme
NTM	Non-tuberculous Mycobacteria
OR	Odds Ratios
PTB	Pulmonary tuberculosis
R	Rifampicin
S	Streptomycin
SPSS	Statistical Package for the Social Sciences
STROBE	Strengthening the Reporting of Observational Studies in Epidemiology
TB	Tuberculosis
WHO	World Health Organization
Z	Pyrazinamide

Abstract

Background: The genus mycobacterium includes several species that can cause disease. *Mycobacterium tuberculosis*-complex is transmitted from person to person by air and usually affects the lungs. Non-tuberculous mycobacteria (NTM) are transmitted from natural sources in the environment and are often considered not a public health hazard. A number of mycobacterial diseases occur due to close contacts between humans, domestic animals and the environment.

Objective: The objective of this study was to examine patients suspected of having mycobacterial diseases and describe: 1) the association between mycobacterial diseases and patient characteristics, 2) experienced risk factors, 3) knowledge and perceptions about TB in agropastoral communities, and 4) at national level the investigation practices of retreatment cases of mycobacterial disease in Tanzania.

Methods: Three studies were conducted to address the stated objectives. In order to identify risk factors for mycobacteria, a cross-sectional study was conducted on 1711 patients examined for tuberculosis (TB) (TB suspects). The suspects were enrolled from Mt. Meru Hospital (the Arusha Regional Hospital), Enduleni Lutheran Hospital in Ngorongoro district, and Haydom Lutheran Hospital in Mbulu district. The study areas were purposively selected because of their strong human and animal habitat overlap. The participants were examined for mycobacteria by sputum microscopy and culture at Central Tuberculosis Reference Laboratory in Dar es Salaam.

In a cross-sectional study, we selected 41 patients among the 277 confirmed mycobacteria positive from the study mentioned above. Interviews were conducted to compare the patients with 68 relatives and 55 neighbors about their perceived risk of exposure to mycobacteria and knowledge about mycobacterial diseases. In a study on the national TB and laboratory registers, we assessed the investigation practices for mycobacteria by reviewing of records of all TB patients notified as re-treatment cases, and the number submitting their sputum samples for culture and DST at the reference and three zonal laboratories between 2002 and 2010.

Results: We examined 1711 patients suspected of having TB of whom 927 (54.2%) were males. Out of all the study participants, 277 (16%) were found to have sputum samples positive for mycobacteria, 228 (13.3%) were smear positive and 123 (7.2%) were culture positive. Of the 123 mycobacterial culture positive, 15 (12.2%) had NTM. Of the 1711 study participants, 664 had

known HIV sero-status, of whom 159 (23.9%) were HIV positive. Males were more likely than females to have sputum samples positive for mycobacteria.

Comparing 41 patients with tuberculosis with 68 relatives and 55 neighbors, we found that participants aged over 50, those aged ≤20 years, 21-30; 31-40 were more likely to be positive for mycobacteria. Being mycobacterial positive was also associated with loss of appetite, living in a household with a family size of more than six individuals, being in contact with a person who had TB, sharing a room with domestic animals, sharing water sources with domestic animals, or a family member with persistent cough significantly.

Knowledge about risk factors for mycobacterial diseases was generally low both among the 41 TB patients and among their 68 relatives and 55 neighbors. There was an association between believing to be at risk of mycobacterial disease and eleven assessed practices with some inherent risk of exposure to mycobacteria; there was higher risk among respondents who do not boil, filter or treat their drinking water, and among the respondents who had shared dwelling with a known TB patient, and livestock keepers. Of all the 164 respondents, 9 (5.5%) reported to be aware of traditional medicines or procedures in their community that a person with symptoms of TB may use and get relief. Drinking untreated water, consumption of raw animal products such as milk, meat and blood, smoking and drinking alcohol were among the habits reported as routes for mycobacterial diseases.

We reviewed 40940 retreatment TB patients notified by the NTLP, and found that 3871 (10%) had their sputum samples received at the reference and zonal laboratories, for culture and DST, 3761 (9%) were processed for culture and 1589 (3.9%) were found to be culture-positive; 1415 (3.5%) had DST performed.

Conclusions:

Among patients suspected and examined for TB, many had mycobacterial disease and over 10% had non-tuberculous mycobacteria (NTM). Predictors of having mycobacteria were loss of appetite, presence of a coughing family member, being an exclusive animal keeper, age below 40 years, and being a male. Knowledge of risk factors for mycobacterial diseases was generally low. Although awareness about mycobacterial diseases among the study community was high, specific knowledge on causes, prevention, and treatment of the disease was poor, and some misconceptions existed on modes of transmission and symptoms. Only 10% of notified

retreatment cases had their sputum samples submitted for DST, implying that there are problems with the logistics of getting sputum samples to a central reference laboratory.

List of publications

Paper I: Who has mycobacterial disease? A cross sectional study in agropastoral communities in Tanzania

Submitted and accepted for publication in Plos One: PONE-D-15-36107.

Paper II: Experienced and perceived risks of mycobacterial diseases: A cross sectional study among agropastoral communities in northern Tanzania. PLoS ONE 10(6):e0130180. DOI:10.1371/journal.pone.0130180

Paper III: Knowledge and perceptions about tuberculosis in agropastoral communities in northern Tanzania: A cross-sectional study. BJMMR. 2015;10(3):1-9.
DOI: 10.9734/BJMMR/2015/18973.

Paper IV: Are sputum samples of re-treatment tuberculosis reaching the reference laboratories? A 9-year audit in Tanzania. PHA 2013; 3(2):156-159.
http://dx.doi.org/10.5588/pha.12.0103

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1.0. INTRODUCTION

1.1. Mycobacteria and mycobacterial diseases

Mycobacterial diseases refer to a range of disorders caused by bacteria which are members of Actinobacteria family. The genus Mycobacteria comprises more than 120 different species. The species are divided into three main groups; *Mycobacterium tuberculosis*-complex, non-tuberculous mycobacteria (NTM) and *Mycobacterium leprae*. Under appropriate conditions, mycobacteria cause different forms of mycobacterial disease,[1-5] and most of them have the ability to go into a metabolic inactive state, called latency. The most important species of this genus are *Mycobacterium tuberculosis (M. tuberculosis),* the causative agent of tuberculosis (TB), and *Mycobacterium leprae,* which causes leprosy, a skin disease*;* the latter will not be further mentioned in this thesis. **Box 1** shows a list of the most common mycobacteria.[6]

Classical mycobacteria are transmitted by cough from a sick person to another person in the near surroundings, and usually affect the lungs; NTM are transmitted from natural sources in the environment and are considered as not a public health hazard.[7] Other NTM are commonly found in soil and water, while some species have been detected in dust, municipal water, hot tub water and dairy products.[8] NTM are considered as opportunistic pathogens, several species are capable of causing serious illnesses resembling TB,[9] and can cause opportunistic infections and hypersensitivity reactions. Some of the NTM have been reported to cause pulmonary disease, skin and soft tissue infections and disseminated infections in both HIV negative and immunocompromised individuals.[10-12] Earlier studies conducted in Tanzania have indicated existence, risk factors and clinical role of mycobacterial diseases in different communities.[13-16]

Some symptoms in diseases caused by mycobacteria are specific for each species; pulmonary infections share some common symptoms such as cough, fever, fatigue, night sweats, and loss of appetite. Many mycobacterial diseases can therefore be suspected clinically. However, some individuals may be infected and may present without any symptoms.[17] In case of exposure, infection and development to disease, identification of mycobacteria is accomplished either by confirming the presence of the pathogen or examination of the host immune response to the pathogen.[18,19]

Box 1: Major Groupings of Organisms Belonging to the Genus *Mycobacterium*

***Mycobacterium tuberculosis* Complex**		
M. tuberculosi,		
M. bovis		
M. bovis BCG		
M. africanum		
M. caprae		
M. canettii		
M. microti		
M. pinnipedii		
Nontuberculous mycobacteria		
Slow-growing nonphotochromogens	**Rapid-growing, potentially pathogenic**	**Others**
M. avium complex	*M. fortuitum*	**Photochromogens**
M. avium	*M. chelonae*	*M. kansasii*
subsp. *avium*	*M. abscessus subsp. abscessus*	*M. asiaticum*
subsp. *silvaticum*	*M. abscessus subsp. bolletii*	*M. marinum*
subsp. *paratuberculosis*	*M. smegmatis*	
M. intracellulare	*M. peregrinum*	**Scotochromogens**
M. celatum	*M. immunogenum*	*M. szulgai*
M. ulcerans	*M. mucogenicum*	*M. scrofulaceum*
M. gastri	*M. neworleansense*	*M. interjectum*
M. genavense	*M. brisbanense*	*M. gordonae*
M. haemophilum	*M. senegalense*	*M. cookii*
M. malmoense	*M. porcinum*	*M. hiberniae*
M. shimoidei	*M. houstonense*	*M. lentiflavum*
M. xenopi	*M. boenickei*	*M. conspicuum*
M. heidelbergense	*M. wolinskyi*	*M. heckeshornense*
M. branderi	*M. goodii*	*M. tusciae*
M. simiae	*M. septicum*	*M. kubicae*
M. triplex	*M. mageritense*	*M. ulcerans*
M. conspicuum	*M. canariasense*	*M. bohemicum*
	M. alvei	
	M. novocastrense	**Noncultivatable**
	M. cosmeticum	*M. leprae*
	M. boenickei	
	M. canariasense	**Rarely pathogenic or not yet associated with infection**
	M. setense	*M. agri, M. aichiense, M. austroafricanum, M. aurum, M. brumae, M. chitae, M. chubuense, M. diernhoferi, M. duvalii, M. fallax, M. flavescens, M. gadium, M. gilvum, M. hassiacum, M. komossense, M. moriokaense, M. murale, M. neoaurum, M. obuense, M. parafortuitum, M. phlei, M. pulveris, M. rhodesiae, M. senegalense, M. sphagni, M. thermoresistibile, M. tokaiense, M. vaccae M. elephantis, M. lacticola, M. mageritense, M. phocaicum*

Sources: *Scotts Diagnostic, 2015.* 6

Studies have shown that there is a complex disease transmission pattern between livestock, pastoral communities and their environment.[20-23] In humans, reduced immune-competence as a

result of HIV, malnutrition, pre-existing lung disease, excessive alcoholism, and smoking are among the documented risk factors and the root causes for NTM.[24-26] Some individuals are more susceptible to mycobacterial diseases than others.[27] Although it is largely *M. tuberculosis* that is found in active TB in humans and *Mycobacterium bovis* (*M. bovis)* in cattle, mycobacterium species can cross the species barrier.[28,29] Thus, cases of *M. bovis* in humans are well known. Cases of *M. tuberculosis* have been reported in animals such as cats, dogs and elephants,[30-32] where the sources of these infections have commonly been traced to humans. However, there are many cases of *M. tuberculosis* in animal collections, where it is arguably an important cause of morbidity and mortality from infectious disease in captive wildlife.[33]

In the agropastoral ecosystems, studies have identified mixed infections caused by mycobacteria in humans, animals and their environment.[21,34] The risk of occurrence of mycobacterial infections at the human-environment-livestock/wildlife pastoral interface is influenced by the socio-demographic, environmental and household related factors as well as knowledge. However, in most of the agropastoral communities mycobacterial infections have been given little attention.[20]

1.2. Agropastoral communities and health

For the purpose of this thesis agropastoralism refers to small-scale farming combined with keeping livestock.[35] Agropastoralism accounts for the livelihoods of 50-100 million people in developing countries, while about 60% of these populations live in more than 21 African countries confined to the most arid regions of the continent.[36,37] With a population of about 45 million,[38] 82% of the Tanzanians derive their main livelihood from agriculture (including the livestock sector).[39] A decade ago, about 2.2 million practiced pastoralist or agro-pastoralist production.[40]

1.3. Epidemiology of mycobacterial diseases

General overview

After infection with mycobacteria the progression to disease often occurs with a period of latency.[41] An infectious case exposes the environment by coughing expectorate with bacteria up from the lungs, and if exposed only some of them are infected. Most of those who are infected will control the bacteria, and may either eradicate them or make them go into latent disease, but a few infected persons may develop disease; in the natural course of the disease the patient may be spontaneously cured, or develop chronic disease, or may die. Pulmonary mycobacterial disease refers to TB, a disease caused by *M. tuberculosis* for which humans are the main reservoir.

Similar disease occasionally results from the closely related mycobacteria, *M. bovis*, *M. africanum*, and *M. microti* together known as the *M. tuberculosis* complex.[42] In our setting the most common species is *M. tuberculosis* followed by *M. bovis*.

TB is transmitted through the air when someone who is sick with TB disease of the lungs or throat coughs, speaks, laughs, sings, or sneezes. Anyone near the sick person with TB disease can breathe the tubercle bacilli into their lungs. The TB bacilli can stay in the body without making you sick. This is called "latent TB infection". This means the TB bacilli in the body are inactive. The inactive bacilli cannot be transmitted to another person. Several factors determine the probability of transmission, including the infectiousness of the source patient and the nature of the environment where exposure occurs.[32] The diagnosis of latent TB infection is done with either the tuberculin skin test or an interferon-gamma release assay. Up to 5% of immunocompetent persons will later progress to TB disease, even decades after infection, if they are not treated for latent TB infection. If the bacilli become active, they will multiply and the person will get sick with TB disease. When the mycobacteria are active and are multiplying in the body, this is called "tuberculosis disease". The bacilli usually settle in the lungs, and from there can be transported to the lymphatic system and further into the blood, and then to any part of the body and settle there, causing "extra-pulmonary tuberculosis". Pulmonary TB disease is diagnosed using a combination of chest radiography and microscopic examination, culture and nucleic acid amplification testing of sputum. Serology tests measures how a person's immune system reacts to the bacilli that causes TB.

M. bovis and closely associated acid-fast bacilli also cause disease in humans.[43] The infection occurs through ingestion or inhalation, and extra-pulmonary lesions of the lungs may occur in association with the consumption of infected animal products such as milk and meat. Moreover, human-to-human transmission is rare[44] and is probably responsible for only a very small percentage of the cases. *M. bovis* is most often not identified, as it cannot be distinguished by smear microscopy alone but needs Lowenstein Jensen culture media without pyruvate. The real incidence of *M. bovis* in humans in developing countries continues to be under-estimated due to the scarcity of appropriate laboratory facilities to isolate and to differentiate *M. bovis* strains.[45,46] Reports indicate that *M. bovis*, including multidrug-resistant *M. bovis*, has been isolated from some HIV infected patients, but the scope of the problem has not been investigated in countries where HIV infection is widespread. However, the occurrence of *M. bovis* disease in humans, domesticated and wild animals confirms the relevance of this zoonosis.[45]

1.4. The epidemiology of mycobacterial diseases

A clear regional variation in the occurrence of mycobacterial species has been documented.[5,47] This has also been observed for the prevalence of mycobacterial diseases such as TB and bovine TB.[14,48,49] The epidemiology of mycobacteria is particularly interesting due to their ability to exist for a long time in latent phase in the immune system of the host. The interaction between the bacterium and the host can be presented in a simplified fashion. The host can be *exposed* to bacteria, usually followed by their disappearance, but it can also lead to colonization or infection of the host. This is most often taken care of by the host immune system, but can also lead to "disease" with clinical signs and symptoms; in the natural course of the disease the outcomes of the disease may be spontaneous cure, or chronic disease, or death. The most fatal species is *M. tuberculosis*.

While there is a reasonable body of literature regarding the distribution of the *M. tuberculosis* complex, information on the epidemiology and public health implications of NTM is limited.[47] The importance of the later is due to their presence in the environment where human activities have had direct impacts on their ecology and hence their epidemiology.[21]

TB is a major public health problem throughout the world. About a third of the world's population is estimated to be infected (colonized) with the tubercle bacilli and are at risk of developing active disease. In 2013, an estimated 9.0 million people developed TB and 1.5 million died from the disease, 360 000 of whom were HIV-positive. TB is slowly declining each year and it is estimated that 37 million lives were saved between 2000 and 2013 through effective diagnosis and treatment.[50] Pulmonary diseases caused by NTM are being diagnosed with increasing frequency worldwide.[51]

The WHO identified 22 high TB burden countries which account for more than 80% of the global TB burden. Nine of these countries were in Africa. Tanzania ranks 15th on the list of high-burden TB countries in the world with 37% TB patients also infected with HIV.[50] **Box 2** shows the reported cases of TB in Tanzania for 2012. A total of 65,732 cases of all forms were notified in 2013, which shows an increase of 1,840 cases or 2.9% compared to the year 2012. The rapid increase of TB has mainly been attributed to the HIV/AIDS epidemic, but factors such as population growth and urban overcrowding have also contributed. In 2014 deaths due to TB in Tanzania were reported to be 6,044, representing 1.7% of total deaths.[51]

Box 2: Reported cases of tuberculosis in Tanzania, 2012

Indicators	Notified cases, n (%)
All cases of tuberculosis	**63,892**
New cases	
- Pulmonary smear positive	25,138 (39.3)
- Pulmonary smear negative	21,393 (33.5)
- Extra-pulmonary	14,595 (22.8)
Total new cases	61,126 (95.7)
Retreatment cases:	
- Relapse	1,052 (1.6)
- Failure	154 (0.2)
- Return to control	201 (0.3)
- Others	1,359 (2.1)
Total retreatment cases	**2,766 (4.3)**
Children (<15 y)	5,283 (8.6)
Notification rates	142 cases / (100000 pop. *x* yr)
Notification rates	56 cases / (100000 pop. *x* yr)

Source: *NTLP Annual Report, 2013* [52]

The presence of mycobacterial infections in livestock represents another health risk to the community.[53-55] In Tanzania subsistence farmers live in close contact with their livestock such as pigs, cattle, goats and chicken. The close intimacy between human and livestock where both share a common ecosystem pose a health risk to the people in terms of zoonotic transmission of infections. In Tanzania for example, *M. bovis* has been isolated from human lymph biopsies[14,56] and cow's milk and tissue samples from slaughter houses.[56] Generally bovine TB in Tanzania is endemic[16,57,58] indicating high possibility of cross transmission of between humans, livestock, and wildlife. The phenomenon is of particular importance in rural communities where people share habitats with livestock and wildlife (particularly in areas near national parks and game reserves).

For humans the most common sources of zoonotic mycobacterial infections are pigs and cattle.[54,59] A study conducted in pastoral communities in Uganda documented a high prevalence of mycobacterial infections in slaughter pigs in Mubende district.[60] Previous findings show that atypical mycobacteria were prevalent.[57,61] Mdegella et al[58] and Durnez et al[61] found a prevalence of 14% and 19% of atypical mycobacteria in milk samples that could expose milk consumers to milk-borne zoonotic infections. Consumption of undercooked meat and un-pasteurised milk is a common practice in most pastoral communities in Tanzania.[48,62] The reported prevalence of atypical mycobacteria alerts for possibility of immune compromised individuals such as

HIV/AIDS patients and puts them into a risk of being infected with opportunistic infections. Reports show that atypical mycobacteria that are shed in cattle milk include *M. gordonae, M. smegmatis, M. fortuitum, M. phlei, M. flavescenes* and *M. avium intracellular*.[58]

1.5. Treatment of mycobacterial diseases

Mycobacterial diseases are curable. Treatment depends on the particular mycobacteria and the illness that it causes, the severity of the illness, the age of the patient and any other health problems that the patient might have (such as HIV). Treatment of mycobacterial diseases is given by one or more of the following: 1) antituberculous drugs: usually combination therapy and for a prolonged period of time; 2) other antibiotics for atypical mycobacterial infections: usually combination therapy and for a prolonged period of time; 3) surgical debridement of ulcers; 4) drainage of mycobacterial abscesses; and 5) excision of infected lymph nodes. For this purpose, we briefly discuss the treatment of diseases due to *M. tuberculosis and Mycobacterium avium*-complex (MAC).

In Tanzania, TB treatment is initiated when the diagnosis has been confirmed. Latent TB infection can be treated with isoniazid (H) for 9 months to prevent progression to TB disease. Treatment of drug-susceptible TB varies from six to eight months depending on whether they have been treated before (the category of the disease).[63] Four drugs are necessary in the initial phase for the 6-month regimen to be maximally effective. The treatment regimen for all adults with previously untreated TB consists of a 2-month initial phase of H, rifampicin (R), pyrazinamide (Z), and ethambutol (E). According to the NTLP guidelines,[63] the recommended regimens are shown in **Box 3**.

Box 3: NTLP recommended TB treatment regimens in Tanzania

2RHZE/4RH*	For new adult TB patients, never before treated for more than a month.
2SRHZE/1HRZE/5RHE*	This is given to patients treated for more than a month: For patients who are rifampicin resistant - Smear positive patients who failed first treatment - Smear positive patients returning after being lost to follow-up - Smear positive patients with relapse - Other previously treated patients, e.g. smear negative and EPTB

*S=Streptomycin, R=Rifampicin, H=Isoniazid, Z=Pyrazinamide, E=Ethambutol; (Source: *NTLP Manual, 2013* [63])

All patients are monitored to assess their response to TB treatment. A smear-positive result at the end of the second, fifth, and seventh months may indicate; i) inadequate supervision and patient adherence in the initial and/or continuation phase; ii) doses of anti-TB drugs are below the recommended range; iii) resolution is slow because the patient had extensive cavitations and a heavy initial bacillary load; iv) there are co-morbid conditions that interfere either with adherence or with response; v) the patient may have drug-resistant *M. tuberculosis* that is not responding to first-line treatment; vi) non-viable bacteria remain visible by microscopy; or vii) presence of mycobacteria other than TB. Therefore, causes of drug-resistant TB are many, but drug-resistant TB is essentially a man-made public health problem. Failure from treatment at the end of the second, third, fifth, and seventh months may indicate drug resistance or NTM. According to the WHO and national guidelines, it is recommended that culture and DST is done before initiation of the retreatment regimen. Thus, all proven treatment failure patients have to submit their sputum specimen for culture and DST.

1.6. Rationale of the study

The health status of agropastoral communities across the globe is often poor, and infectious diseases occurring among them include mycobacterial diseases such as TB.[64] Tanzanian agropastoralists are at a health disadvantage because they are often left behind the development processes and excluded from many health services as a result of their unique cultural, political and geographic situation.[65] There are disparities in health between rural pastoralists and their urban and agropastoral counterparts. Links between human and livestock health in Tanzania have been reported.[14] A number of mycobacterial diseases occur with increased frequency due to increased close contacts between humans and their domestic animals. Focusing on health issues related to agropastoralists lifestyles is important as these groups often face unique social and environmental challenges leaving them vulnerable from both resource and political perspectives which have important implications for health.

Although the initial diagnosis of mycobacterial disease often is based on the initial clinical presentation of the patient, definitive diagnosis usually involves the isolation and identification of the infecting organism in the laboratory.7 Because of the slow growth rate of the mycobacteria, isolation, identification, and drug susceptibility testing can take 4 to 8 weeks or longer. Groups of related mycobacteria cause similar disorders; this clinical similarity also make it impossible to differentiate diseases caused by the different species.

In settings where the routine diagnosis does not discriminate between *Mycobacterium tuberculosis* and other NTM, mycobacterial disease is often TB and hence first line treatment is for TB.[66,67] However, *M. tuberculosis* and NTM mixed infections have also been reported.[68,69] It is important that all samples from re-treatment patients undergo culture and test for possible drug resistance, either in naturally resistant NTM or in MTB with acquired resistance.

Most re-treatment cases are cured but some fail and some recur later, and these need retreatment.[70] When compared to new cases, re-treatment cases have more treatment failure or loss to follow up.[71-75] The Global Plan to STOP TB (2006–2015) proposed that by 2015, all previously treated patients should have access to culture and drug susceptibility testing at the beginning of treatment to identify MDR-TB as early as possible. This would allow confirmation of their disease and eventually allow more effective treatment regimens.[76] Also, some NTM do not respond to TB treatment and need another treatment. Therefore, a case of mycobacterial disease formerly treated should have investigation of the bacteria, and this is reflected in the NTLP guidelines. The implementation of this at programmatic level seemed suboptimal but had not been closer investigated in Tanzania.

We think mycobacterial diseases remains significantly underrepresented as causal agents of extra pulmonary and pulmonary TB especially in rural areas. In the human-animal ecosystems, humans and animals (both livestock and wildlife) share the same micro-environments and water sources particularly during the dry season, thereby increasing the risk of transmission of mycobacterial diseases between infected and susceptible hosts. Consumption of un-pasteurized milk and meat products from infected animals as an important cultural practice poses a risk of transmitting mycobacteria to people living at the human-animal interface.[77] Lack of knowledge about the cause, mode of transmission, and symptoms, as well as appropriate treatment not only affects the health-seeking behavior of patients, but also could affect control strategies, thereby sustaining the transmission of the diseases in the community.[78]

The current study was conducted among patients examined for mycobacteria/TB in agropastoral communities in Northern Tanzania 1) to describe patient characteristics, and to assess the association between mycobacterial disease and its determinants by HIV status; 2) to assess experienced risk factors of mycobacterial diseases; 3) to determine knowledge and perceptions

about TB in agropastoral communities in Northern Tanzania; and 4) We also studied at national level investigation practices of re-treatment cases of mycobacterial disease in Tanzania.

2.0. OBJECTIVES

1. In agropastoral communities in Northern Tanzania we assessed:
   i) The demographic determinants of mycobacterial diseases
   ii) Experienced and perceived risks of mycobacterial diseases
   iii) Knowledge and perceptions about TB

2. To outline and evaluate the number of annually notified patients with mycobacterial disease receiving re-treatment regimen as compared to the number of sputum samples received by the reference laboratories for culture and drug susceptibility testing.

3.0. MATERIALS AND METHODS

The data for the papers in this thesis are basically from two different sources. **Paper I** used data from patients examined for TB. **Paper II** and **III** are based on material from selected TB cases for the study in Paper I and controls (relatives and neighbors). **Paper IV** uses national programmatic and laboratory registers for 2002 – 2010 to identify weaknesses in investigation practices for mycobacteria.

Definition of terms

Mycobacteria: Organisms belonging to the genus Mycobacterium. It includes some species which are pathogenic for humans and animals such as *M. tuberculosis*.

Atypical tuberculosis: Is used to signify a group of closely related diseases caused by bacterial organisms belonging to non-tuberculous mycobacteria (NTM) or Mycobacterium other than TB (MOTT).

Mycobacterium tuberculosis: The bacteria that is the most common cause of TB in humans. Primarily causes pulmonary disease. Persons are infected when exposed to the organism.

Mycobacterium tuberculosis complex: Refers to a genetically related group of *Mycobacterium* species that can cause TB in humans or other organisms. Members of the group includes: *Mycobacterium tuberculosis, Mycobacterium africanum, Mycobacterium bovis and the Bacillus Calmette–Guérin strain, Mycobacterium microti*, *Mycobacterium canetti*, *Mycobacterium caprae*, *Mycobacterium pinnipedii*, *Mycobacterium suricattae, Mycobacterium mungi.*

Environmental mycobacteria: Mycobacteria which do not cause TB or leprosy. They are also called non-tuberculous mycobacteria (NTM), MOTT).

A tuberculosis suspect: Refers to an individual presenting to the health facility and being investigated for TB, often because of the following symptoms or signs of TB: a productive cough for more than two weeks, shortness of breath, chest pain, blood-stained cough, weight loss, fever, night sweats, and fatigue.

A tuberculosis case: An individual who is bacteriologically confirmed by smear microscopy or culture of sputum sample.

New patients: Those who had no history of prior TB treatment or who had received less than one month of anti-TB drugs regardless of their smear or culture results. New patients may have positive or negative bacteriology and may have disease at any anatomical site. This includes all EPTB patients.

Retreatment TB patients comprised the following: *treatment failures:* patients with a positive sputum smear at five months of treatment or later; *relapses:* patients successfully completed treatment and who come back with smear positive pulmonary TB; *return after being lost to follow up:* patients who return to the programme sputum smear positive after being lost-to-follow-up (>1 months); *retreatment other:* any patient not fitting the above three definitions such as those previously treated but with unknown outcome of that previous treatment, and/or who have returned to treatment with smear negative PTB or bacteriologically negative EPTB.

3.1. Material and methods for Paper I

Data for this study was collected as part of a large study on epidemiology and diagnosis of mycobacterial diseases in Tanzania. The main study was a Post Doctoral Fellowship Program Project conducted by the National Institute for Medical Research, Muhimbili Research Centre in collaboration with Sokoine University of Agriculture and University of Bergen. The program aimed to a) improve the diagnosis of both *MTB*-complex and environmental mycobacterial diseases by introduction and validation of new molecular techniques; b) determine the prevalence of mycobacterial diseases in urban and rural agropastoral populations and assess associated factors (this current study); c) carry out drug sensitivity studies for NTM to help rationalize regimens for NTM infections; d) confirm the observation of zoonotic transmission of TB and establish its extent and dynamics in both rural agropastoral and urban populations.[79] It involved collection of sputum samples from TB presumptive (suspects) in rural and urban agropastoral communities Arusha and Manyara regions. Wellcome Trust through the Afrique One Consortium funded the study. The different objectives in the program were implemented separately involving other researchers. Several publications of findings resulting from this study have been made.

Study area and population

The study was conducted in the catchment areas of 3 hospitals in Northern Tanzania: Mount Meru Hospital in Arusha municipal, Enduleni Catholic Hospital in Ngorongoro district and in Haydom Lutheran Hospital in Mbulu district. According to the 2012 Census the selected districts had a population of 650,370. [80] Prominent local tribes include the Maasai in Ngorongoro, the Arusha and the Meru in Arusha, and the Datoga and Iraqw in Mbulu district, all with many agropastoralists.

Study design

This was a cross sectional hospital based study to describe the characteristics, and to assess the association between mycobacterial disease and its determinants.

Sampling and sample size estimation

This study involved multistage sampling in which three districts were purposefully selected from Arusha and Manyara regions, one Urban district and two rural districts. The regions were purposely selected because of their clear human and animal habitat overlap. They have both well developed urban district and rural agropastoral communities that border with national parks and therefore share habitats with wild animals. Sample size calculation was based on the previous study that shows prevalence of MTB among outpatient attendees with prolonged cough was 12.8%.[81] Using Epi info version 6 Statcalc, the selected minimum sample size was 1600. Taking into consideration the 10% drop-out, the minimum total sample size was 1760. The sample size was divided between the three health facilities, Mt. Meru Regional Hospital, Enduleni Catholic and Haydom Lutheran Hospitals according to the number of patients accommodated in each these hospitals.

Selection of study subjects

Targeting presumptive TB patients attending the selected health facilities, eligible patients were referred to the TB clinic for investigation. Some common reasons or symptoms that made the clinician to refer them for investigation for mycobacterial diseases included persistent cough for two weeks or more, loss of appetite, weight loss, evening fever, and hemoptysis.

Data collection

We collected two sputum samples (spot and morning) from all consenting study participants. The participants were interviewed about their demographic background and symptoms related to their

illness. The specimen taken on the spot was used for routine examination at the hospital for immediate follow-up treatment (this information was not used in this study), and the rest of the sample along with the morning sputum sample were transported to the CTRL in Dar es Salaam. At each participating hospital, collection of sputum samples was done under supervision of the laboratory technician who was responsible for the study.

Transport of sputum samples

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.

Laboratory procedures

At the CTRL, the samples were processed on the third day from the day of collection. This was the case even when the samples arrived during the weekend. Investigations done included smear microscopy and culture for mycobacteria. The sputum smears were stained using Ziehl-Nielsen technique as described by Bancroft and Gamble.[82] For culture only the early morning specimen collected at home was used, as it was the most likely to grow tubercle bacilli, and the least likely to be contaminated with other bacteria. Sputum samples were processed for culture according to the standard CTRL culture protocol. All culture bottles were incubated at 37^{o}C and monitored daily. Samples that failed to show any growth after 8 weeks of Lowenstein Jensen incubation were classified as negative.

HIV results

Participant HIV status was obtained from clinic records.

Data management and analysis

Study personnel, according to established ethical standards handled patient records and specimen results in strict confidence. A copy of the results obtained was immediately sent to the attending clinician for further management of the patient. The data were double entered, validated and cleaned using EpiData version 3.1. Data was then exported to SPSS version 20 for Windows

(SPSS Inc, Chicago, IL, USA) and STATA version 11 (STATA Corp Inc., TX, USA) for cleaning and analysis. Pearson Chi square statistics test was used to compare group differences for categorical variables. Associations and relationships were considered statistically significant if $p<0.05$. Logistic regression was used for modeling multiple determinants predicting mycobacterial disease. Crude and adjusted odds ratios (OR) with 95% confidence intervals (CI) were reported. Variables that were considered significant at $p\leq0.2$ in the univariate analysis were included in the multivariate analysis.

3.2. Material and methods for Papers II and III

Study design

This was a cross sectional hospital and community based study to describe experienced and perceived risks of mycobacterial diseases, knowledge and perceptions about TB in agropastoral communities in Northern Tanzania

Selection of patients, relatives and neighbors

Out of the 1711 sputum samples from the presumptive TB patients, 277 (16.2%) were proven to be mycobacterial positive either by being smear and or culture positive. A selected number of patients with confirmed diagnosis of TB by sputum smear microscopy and/or culture at central laboratory were followed up and their relatives and neighbors were also included for interviews to allow assessment of their knowledge, perception and practice towards mycobacterial diseases for **Papers II** and **III**. For this purpose, patients included fulfilled the following criteria: 1) proven TB patients reporting to the outpatient departments of the selected health facilities for the first time; 2) residents of the districts served by the selected health facilities; 3) consenting to participate; 4) participants reachable by mobile phone and address. For each selected patient, at least one relative and one neighbor were also included into the study to allow comparison of knowledge and perceptions about TB and risks from mycobacterial diseases between them.

Data collection

Using patient addresses recorded at the health facility during the initial visit, a list of patients for follow up and interviews to assess their knowledge on TB, experiences and perception on risks of mycobacterial diseases was prepared. Only TB patients whose sputum samples were found to have smear positive and/or culture positive at the CTRL were eligible. Prior to visits, patients were reached by phone to confirm their availability and readiness to be visited and interviewed in their homes. For each consenting patient, relatives who were present and neighbors living in the

vicinity were also interviewed. Data was collected using two structured questionnaires: one for patients and one for relatives and neighbors. The questionnaires were translated into Swahili (the national language) and pre-tested for clarity and cultural acceptability. The collected information included; baseline information including sex, age, area of residence, marital status, occupation and level of education. Also perceptions on selected risk factors for mycobacterial infections, like number of people sharing a dwelling, history of sharing house with a person with TB, past history of TB infection, smoking and livestock keeping, consumption of raw animal products, household primary source of water, source of drinking water for animals/livestock, and preparation of drinking water for the family.

3.3. Materials and methods for Paper IV

Study area

The study used national data from the archive of the TB program in Tanzania.

Study design

This was a cross sectional analysis of routine NTLP data.

Study setting and population

The data were collected and analyzed between June and November 2012 in Tanzania. The study included a) records of all TB patients who were notified as re-treatment cases between 2002 and 2010, and b) culture and DST results at the reference and three zonal laboratories.

Data collection and analysis

Data were obtained from nine annual NTLP reports and 21 laboratory registers from the zonal and reference laboratories which covered the nine year period. Data were double entered by two independent encoders, and validated using EpiData Entry software version 3.1 (EpiData Association, Odense, Denmark). Discordances were resolved by crosschecking with the paper registers. Data were exported to SPSS version 17.1 for analysis.

Management of sputum samples from notified retreatment TB cases

Retreatment TB cases receive a retreatment drug regimen according to national and WHO guidelines. Tanzania has more than 500 public and private health facilities which carry out sputum smear microscopy for TB diagnosis. Each patient diagnosed as being a retreatment case is required to submit one sputum sample for culture to one of the three zonal reference

laboratories located in different regions of the country, or to the CTRL which is located in Dar es Salaam. Primary responsibility for sending sputum specimens of retreatment cases lies within the health facilities that diagnose the retreatment cases. If a specimen grows on culture at the zonal laboratory, the positive isolates are then sent to the CTRL for DST. Sputum samples and isolates are transported through the normal postal service.

Routinely used culture and DST methods

While anti-TB DST is only done at the CTRL, sputum culture is carried out at four sites, namely the zonal TB reference laboratories in Bugando Medical Centre, Kilimanjaro Christian Medical Centre, Mbeya Referral Hospital and CTRL. Culture is done on Lowenstein Jensen (LJ) solid media. Positive culture isolates are sent to the CTRL for DST. DST is done by resistance ratio method for the first-line anti-TB drugs: Rifampicin, Isoniazid, Ethambutol and Streptomycin.

Ethics approval

Ethical considerations

The study for **Papers I, II and III** was approved by the National Health Research Ethics Review Committee (NatREC) of Medical Research Coordination Committee (MRCC) at National Institute for Medical Research (NIMR) in Tanzania prior to its implementation (Approval Reference number: NIMR/HQ/R.8a/Vol. IX/1009) (**Appendix I**). Furthermore, permission was sought and granted by regional, district and health facility authorities as required. Information about the purpose, risks, benefits and comfort of the study participants was communicated to the patients either by allowing them to read the consent form or reading to them by the research assistants. All consenting patients signed the consent 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. The study for **Paper IV** received clearance from the National Health Research Ethics Committee (NatREC) of the Medical Research Coordinating Committee in Tanzania and also met the Union Ethics Advisory Group (EAG) approved criteria for analysis of routinely collected program data (Appendix II).

4.0. SUMMARY OF THE RESULTS

This section provides a summary of the main findings of the studies as presented in the four individual papers in Section 9 of this thesis, and a few contextual results as well. The results for **Paper I** are entirely based on the main cross sectional study carried out in a study population of 1711 patients who were investigated for mycobacteria (mostly "presumptive" TB). Study population for **Papers II** and **III** were the patients diagnosed with mycobacterial disease in **Paper I**, as well as their selected relatives and neighbors as shown in **Figure 1**. **Paper IV** looks into a special problem in treating mycobacterial diseases: some cases need full retreatment for the disease. This paper uses programmatic annual reports of TB retreatment patients to highlight the challenges in the process of laboratory verification of the diagnosis.

Of the 1711 presumptive TB patients who were identified through symptoms and signs of TB, 277 (16%) were confirmed to have mycobacteria by smear microscopy (136), culture (49) and both (92). Among the 92 confirmed positive cases by both smear microscopy and culture; 36 were identified as NTM whereas of the 49 cases diagnosed as AFB smear negative but culture positive 15 of them were identified as NTM.

Among the 1711 presumptive TB, HIV test results was available for 664 (39%) of whom 159 (24%) were positive. NTM mycobacteria were identified in 3 (2%) of the HIV positive, 13 (3%) in HIV negative patients.

4.1. Paper I: Who has got mycobacterial disease in agropastoral communities in Tanzania? A cross sectional study

We examined 1711 patients who attended a clinic for investigation for mycobacteria, seeking health care after experiencing one or more of the following symptoms: persistent cough for two weeks or more, shortness of breath, chest pain, cough with blood-stained sputum, weight loss, fever, night sweats and fatigue.

Of all the assessed demographic characteristics, males were more likely to be positive for mycobacteria than females, and compared to participants aged over 50 years, those aged less than 40 years were more likely to be positive for mycobacteria. Among HIV negative participants we found a higher likelihood of being mycobacterial positive in men than in women.

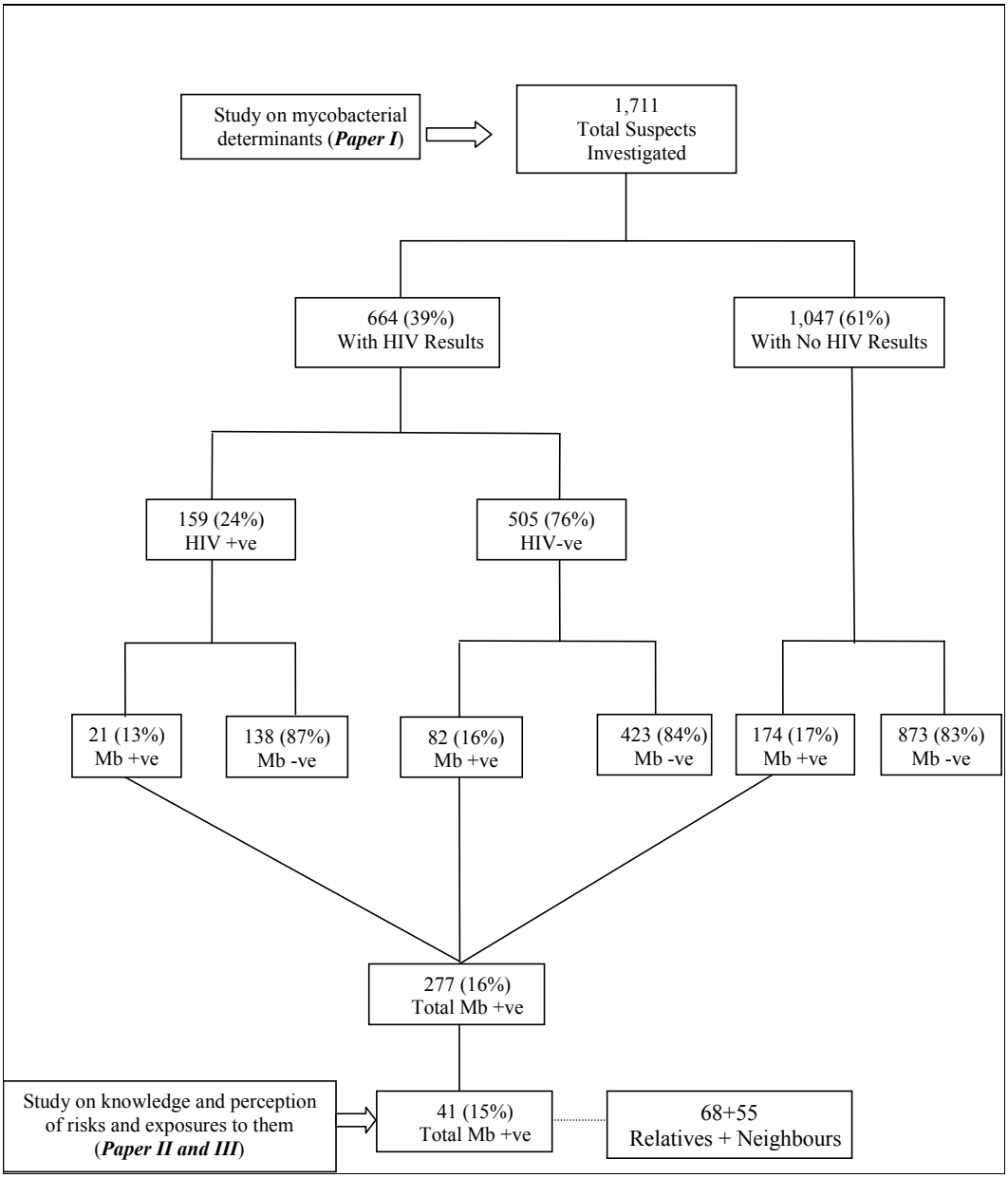

Figure 1: Flowchart of study participants in Papers I-III. Mb = mycobacteria; +ve = positive; –ve = negative.

We assessed five known symptoms and eight environmental factors (involving practices and exposures to mycobacteria) as determinants of mycobacterial diseases. Cough was present in 935 (55%) respondents of whom 147 (16%) had mycobacterial positive sputum samples in laboratory. Hemoptysis was reported in 17 (6%) of the 935 TB presumptive. Among the assessed symptoms, loss of appetite was the only symptom associated with mycobacterial disease. Symptoms reported by patients who had evidence of mycobacteria are shown on **Figure 2**. A higher proportion of HIV negative than HIV positive respondents presented with TB like symptoms. Among the 505 HIV negative patients, 82 (16%) had positive test for mycobacteria; among the 159 HIV positive patients 21 (16%) had a positive test for mycobacteria. Among HIV positive study participants none of the symptoms was associated with being mycobacterial positive. Among HIV negative patients, loss of appetite was associated with being mycobacterial positive.

Regarding environmental determinants, we found that most of the study participants belonged to households with a family size of more than six individuals (1149; 67%). A substantial proportion of the study participants reported to have been in contact with a person who had TB (235; 12%), shared a room with domestic animals (564; 33%), shared water sources with domestic animals (589; 34%), or had a family member with persistent cough (353; 21%). Overall, none of these environmental determinants was found to be associated with being mycobacterial positive. Presence of a family member with a persistent cough significantly predicted being positive for mycobacteria among HIV positive study participants. None of the assessed environmental determinants significantly determined mycobacterial disease among the HIV negative study participants.

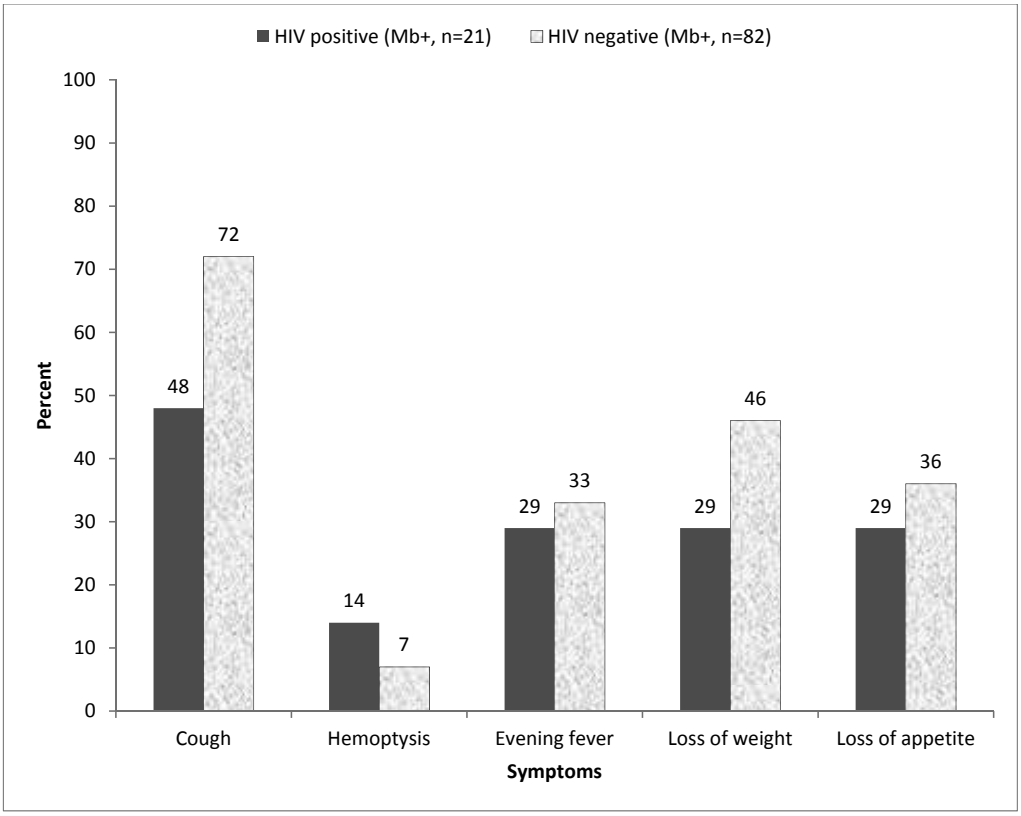

Figure 2: Proportion of patients with mycobacteria reporting selected symptoms in Tanzania, by HIV status.

4.2. Paper II: Experienced and perceived risks of mycobacterial diseases: A cross sectional study among agropastoral communities in northern Tanzania

Selected TB patients (41) from **Paper I** were compared with a selection of their relatives (68) and neighbors (55). The proportion of livestock keepers was higher among study participants in Mbulu (67; 41%) than in Ngorongoro (10; 6%) and Arusha districts (11; 7%), ($p<0.01$). A higher proportion of participants who had primary school education had shared dwelling with TB patients than those without any education ($p=0.01$).

Eleven known risk factors for mycobacterial diseases were assessed. Overall, 64/164 (39%) respondents were aware of a risk factor for mycobacterial diseases. Among those who reported to know a risk factor, 20 (12%) mentioned living with a person who had TB, 11 (7%) sharing eating

and drinking utensils and 11 (7%) mentioned being close to someone with infectious mycobacterial disease. Feeling at risk of mycobacterial disease was reported by 21 (45%) of the TB patients, 28 (41%) of the relatives and 15 (27%) of all the 55 neighbors. A smaller proportion of those aged 21-30 years felt at risk compared to those over 50 years.

We assessed 11 practices with some inherent risk of exposure to mycobacteria. Respondents who do not boil, filter or treat their drinking water considered themselves to be at risk of mycobacterial diseases ($p=0.05$); so did the respondents who had shared dwelling with a known TB patient ($p<0.01$) and livestock keepers ($p<0.01$).

4.3. Paper III: Knowledge and perceptions about TB in agropastoral communities in northern Tanzania: A cross-sectional study

This study used the same participants as in Paper II, with 41 TB cases selected from the study for Paper I, and in addition 68 of their relatives and 55 of their neighbors. Of all the 164 study participants, only 2 (1%) neighbors had never heard about TB. Sources of information about TB were health workers (99; 60%), family/friends or neighbors (27; 16%), teachers (1: 1%) and newspapers (2: 1%). There were 123 (75%) respondents who thought that TB was caused by microbes, 50 (31%) thought animals were responsible, and 111 (68%) suggested that transmission occurs during sexual intercourse. Overall, 65 (40%) of the respondents thought that TB can be prevented and 107 (65%) considered it to be treatable. Of the 164 respondents, 9 (6%) reported to be aware of traditional medicines or procedures in their community that a person with symptoms of TB may use and get relief. Respondents suggested that TB was more common in adulthood (69%), among alcohol abusers (40%), among smokers (27%), among people eating raw animal products such as meat, blood and milk (6%), and in childhood (23%). Some misconceptions existed on mode of transmission and symptoms of TB.

4.4. Paper IV: Are sputum samples of retreatment TB reaching the reference laboratories? A 9-year audit in Tanzania

Some TB patients who have taken the standard first-line regimen may be required to take a course of retreatment with a stronger regimen. These cases may have developed drug resistance. The National TB and Leprosy Programme (NTLP) requires that all notified cases of retreatment TB in Tanzania submit to TB reference laboratories sputum samples for culture and drug

susceptibility testing (DST). We therefore conducted a study to determine if the number of annually notified retreatment TB cases corresponded to the number of sputum samples received at the reference laboratories, and to assess the number of samples that were culture positive and had DST results. The data were extracted from NTLP annual reports and from the Central Reference Laboratory.

The 40940 notified retreatment cases included 900 (2%) treatment failure, 1890 (5%) return after being lost to follow-up, 15 283 (38%) relapses and 22 867 (60%) 'Retreatment Others'. Of the 40940 retreatment TB cases notified by the NTLP from 2002 to 2010, 3871 (10%) had their smear microscopy positive sputum samples received at the central reference and zonal laboratories for culture and at the central reference laboratory for DST. A total of 3761 (9%) sputum samples were processed for culture. Of these positive cases, only 1589 (42%) were found to be culture-positive, and 1415 (38%) had DST performed.

5.0. DISCUSSION

5.1. Discussion about the methods

Selection of the study communities

The choice of the agropastoral communities enabled us to gather evidence on prevalent determinants and risk factors for mycobacterial diseases due to the interaction between human-environment-livestock/wildlife. An overriding weakness of the study for **papers I-III** was that the study involved participants who were recruited for another study which had broader objectives other than the current study. This affected the sample size and the strengths of some inferences made. One of the important criteria for inclusion into the study was an individual has to present with selected symptoms for mycobacterial diseases. They included persistent cough for two weeks or more, loss of appetite, weight loss, evening fever, and hemoptysis. The study participants were very similar as there were no healthy controls to allow comparison. This amounted to a weak study population for generalization of some important conclusions. Furthermore, participants in **Papers II** and **III** were selected based on being reachable by telephone (mainly mobile phones). Available evidence shows that in 2012 about 80% of adults in rural Tanzania owned mobile phones, over two thirds of them young adults.[83] Therefore selection of participants reachable by mobile phone and address recorded during initial visit to the health facility may have introduced a selection bias. If patients excluded with no mobile phones were more poor and elderly, and the poor were likely to have less education; this may have biased our results particularly on knowledge (see later discussion about bias).

Study design and settings

The choice of the cross-sectional study design for the first objective to determine demographic determinants of mycobacterial diseases was adequate. Our second and third objectives also used cross sectional design to determine experienced and perceived risks for mycobacterial diseases, and knowledge and perceptions about TB. This design is not strong for showing any causal relationship in the associations analyzed. The comparison groups shared many common characteristics such as the environment, culture and lifestyles, which are some empirical risk factors for mycobacterial infections. These **Papers II** and **III** are not case-control studies where the cases and controls are selected based on the outcome measurement, case/not case, and results give risk of being case compared to non-case. The fourth objective uses records of sputum samples tested in the laboratory among national retreatment cases. A retrospective cohort study

design was much more appropriate for this purpose. However, the use of programmatic data as mainly a prevalence study, justifies the usefulness of our findings.

The location of the selected health facilities for the study lead to the transportation of the collected sputum samples to reach the CTRL in two days amounting to a delay in processing of the samples. This may have affected the growth of some of the mycobacteria leading to low number of culture positive.

Validity

The validity of a study refers to the degree to which a test is capable of measuring what it is intended to measure. A study is valid if its results correspond to the truth and accurately represents the features of a phenomenon under investigation.[84] The results can be valid for the study population (internal validity) and in addition be valid for other populations (externally validity).

Internal validity

The internal validity of a study can be compromised by bias, by confounding and by chance. Regarding bias, in this study there may be selection bias and information bias.

Selection bias

Although the study area fits well on the concept of the dynamics facing the human-environment-livestock/wildlife interface, other areas with similar interactions in the country could have been selected. The selection of health facilities took into consideration of public (Mt. Meru Regional Hospital) and faith-based (Haydom Lutheran Hospital and Enduleni Catholic Hospital) ownership in the catchment areas of the study sites. In all the selected health facilities TB services are provided free of charge, ensuring little bias between the rich and the poor who accessed the services. Although selection of rural, urban/semi-urban health facilities could result into good results for comparison, laboratory logistics were likely to be worse at Haydom Lutheran and Enduleni Catholic Hospitals than at Mt. Meru Regional Hospital. Due to transport problems of the patients, rural health facilities were likely to have fewer participants than the urban health facility.

A major bias for **Papers II** and **III** lies in the selection of presumptive TB patients as an entry point for interview. We enrolled only those with recorded mobile phone numbers and valid address. Despite the findings by Nyamba and Mlozi[83] that around 80% of rural population have mobiles it is likely that the poorest with no mobile phones were excluded. These could represent an important group with characteristics that could have made a difference in our findings. Although we assume that the study participants were fairly similar, neighbors involved in this study did not have TB, partly because they were economically stronger with improved life condition and education.

Information bias

The interviews were done in Swahili (the national language), which is not the mother tongue of most of the communities in the study area. There may be misunderstanding to the questions asked and their responses. We do not know about concrete examples, but possibility of bias is there. However, some steps were taken to minimize bias: ***First,*** quality of data collection and management was assured through training of data collectors and data entrants for double entry. ***Secondly,*** use of clear TB patient (in both, the TB presumptive and re-treatment studies) definitions. ***Thirdly,*** quality of data collection and management was assured through training of data collectors and data entrants for double entry. ***Fourthly***, the studies were done independently without any interference from the health authorities including the National TB and Leprosy Program (NTLP). ***Fifth,*** neither Welcome Trust nor Afrique One Consortium as funding institutions played role on data analysis and publication.

Confounding: Effects of confounders were reduced as adjustments were done in regression analysis in **Paper I** and **Paper II**.

Chance

The sample size of paper I was fairly large giving some precision in result estimates. However, the sample size of papers II and III is small, and this is partly because they used available study patients who were enrolled for a different study. Therefore, the results have to be interpreted carefully as it has low statistical power, particularly the negative findings.

External validity

External validity or generalizability is the extent to which the results of a study apply outside the study area we think the results may apply to all agropastoralists populations in Tanzania, and

perhaps the results can be generalized to other African countries with similar customs. The problem of collecting and testing retreatment patients is likely to be similar in many developing especially African countries. All African countries use the "DOTS strategy" (a global plan to stop TB) for programmatic management of TB, and use the same regimens for new and retreatment cases recommended by WHO. All countries have a proportion of cases of retreatment, and this proportion is much higher than in Tanzania.

5.2. Discussion of the main findings

Discussion of the general findings

According to the national policy, smear microscopy is the basis for the diagnosis of TB in the country.[52] However, among the disadvantages of smear microscopy for detection of TB cases is its inability to differentiate between *M. tuberculosis*-complex and NTM.[85] The findings in our study raise concern on the role of the NTM on diagnosis of mycobacterial diseases in the study community. This is in line with findings from a study which reported increased isolation of NTM from patients. This implies that more patients with acid-fast bacilli positive samples receive inappropriate or unnecessary empirical anti-TB treatment.[86] The lack of diagnostic facilities to distinguish between *M. tuberculosis*-complex and NTM remains to be a challenge.

Earlier studies report on various risk factors for infection with NTM. In their review, Marras and Daley[87] report on risk factors identified in several studies, among others they include HIV, advancing age, and male sex. The main difference between the risk factors reported in the review is the importance of history of previous TB which has not been pronounced in our findings. Although Aliyu et al.[69] reported risk factors for NTM similar to those reported in the current study, the difference lies in the target population where their focus was urban-based TB presumptive. In their study, they report occupational exposure and seasonal harmattan dusty winds being among the risk factors.

Discussion for Paper I: Who has mycobacterial disease? A cross sectional study in agropastoral communities in Tanzania

Symptom screening for mycobacterial diseases, especially TB, seems to be attractive because it is simple, does not need expensive equipment, and has been applied in several surveys.[88-90] Symptom screening is usually the first step for TB case finding, their sensitivity and positive predictive value are high.[91] According to the WHO,[92] national TB programs have traditionally defined a TB "suspect" as someone with cough lasting greater than 2 or 3 weeks. Poor

performance of symptom screening for detecting TB had also been found in several studies elsewhere.[93-97] We report that roughly half of the study participants presented with cough as the main symptom and of these, less than a quarter were found to have the disease (by being smear and or culture positive). While there has been ongoing debate about the importance of cough in screening for mycobacterial diseases especially for TB in people living with HIV, this study shows that more HIV negative study respondents presented with the studied symptoms than their HIV positive counterparts. Earlier studies have also shown that chronic cough is less sensitive for TB disease in people living with HIV than HIV negatives; hence, using symptom as a screening rule will miss some cases and contribute to diagnostic delays.[88,94]

Our study has similar socio-demographic, environmental, and household related risk factors as a study in Uganda in pastoral households.[20] We show that loss of appetite and presence of a family member with a persistent cough was associated with mycobacterial disease. These findings were also in line with other studies in Uganda.[20,58]

Discussion of Paper II: Experienced and perceived risks of mycobacterial diseases: A cross sectional study among agropastoral communities in northern Tanzania

Risk of exposure to mycobacteria reported in **Paper II** were similar to those reported in previous studies conducted in Uganda.[20,60] From the reported findings, it is evident that if the community is not made aware of the consequences and the relationship between their lifestyles and activities which pre-dispose them to infection, mycobacterial diseases in the study area are likely to go beyond public health control. Required interventions may include addressing the economic problems in order to improve the way they attain their livelihood, changing the attitude and related socio-cultural behavior of the community.

In our study, the community perceived that smoking was a risk factor to mycobacterial diseases. This is scientifically in line with findings by other scholars who have documented smoking as a risk factor to acquisition of mycobacterial infections.[96,98]

Although earlier findings show that agropastoral communities had basic awareness about mycobacterial diseases such as TB,[65] our study shows that two third of the respondents did not have knowledge of any risk factors for mycobacterial diseases. This is not in-line with reported findings from Uganda[25] where a large proportion of pastoral community members were found to have broad knowledge on mycobacterial infections especially TB.

Routes for mycobacterial infections suggested by participants were drinking untreated water, consumption of raw animal products such as milk, meat and blood, cigarette smoking and drinking alcohol. Interestingly, even the respondents themselves who reported consuming these things considered themselves to be at risk from mycobacterial diseases. Similar behaviors were perceived as exposure routes for acquiring mycobacterial infections in Uganda.[25] A study among cattle from two districts in central Tanzania found that 0.4% and 1.7% of milk samples contained *M.bovis*.[58] From this study, the authors concluded that despite of the relatively low proportion of samples showing bovine TB, the consumers were at risk and the authors suggested that farmers have to be educated about the health risks of consuming raw milk. Similarly, Mfinanga et al[59] reported that the consumption of raw milk was a risk factor for bovine TB in northern Tanzania, the same setting as our study area. In their study, they found that all ethnic groups had habits and beliefs that increased their risk of being infected with both bovine and human mycobacteria.

Mfinanga et al[14] found that several practices by pastoralists might increase the risk of zoonotic TB. Not surprisingly, we found that rural dwellers compared to urban were more often livestock keepers and more exposed to potential sources of infection, and shared dwelling with livestock. Participants who had shared dwelling with TB patients felt more at risk, and so did livestock keepers. Our findings show that the risk factors for mycobacteria in the different agropastoral communities were similar. This observation is well supported by findings from such studies as a that which was conducted in pastoral communities in the northern part of Tanzania, where the risk factors for bovine TB in humans were found to be traditional practices such as sleeping in the same house with animals, lack of knowledge regarding the disease and its risks, raw milk consumption and poor ventilation of houses.[16] In a study in Uganda, sharing of water and utensils with animals and poor hygiene of the utensils was identified as possible mycobacterial infection transmission routes in a study in agropastoralists in Uganda.[25] These findings shows that to reduce the risk of acquiring the diseases in both pastoral and agropastoral communities there is need to promote health education about transmission, prevention and risk factors for mycobacterial diseases.

Discussion for Paper 3: Knowledge and perceptions about TB in agropastoral communities in northern Tanzania: A cross-sectional study

This study demonstrates that almost all participants had heard about TB, but specific knowledge on causes, prevention and treatment of the disease was poor, and some misconceptions existed on

modes of transmission and symptoms. These findings were similar to the results from earlier studies conducted in Ethiopia.[36,99,100]

In a study conducted in the general community in a rural district of Malawi, Chizimba and others[101] reported that sources of information was important in determining perception of risk of TB. In their study community, individuals who were exposed to mass media and interpersonal communication had increased perception of risk of TB as compared with individuals exposed to only interpersonal communication. However, in the current study, we show that health workers and family/friends or neighbors were a common source of information about TB; teachers and newspapers were not common sources. Our findings are in line with those reported in earlier studies in pastoral communities, where friends or pulmonary TB patients were the main sources of information about TB.[59,36] However, Melaku et al[100] reported that radio, health service providers, and friends were common sources of information, noting that newspapers and televisions were not commonly used in the study area. We speculate that among others, this might be due to the fact that agropastoralists have less access to newspapers, TV, radio, health care and other social services.[102] This requires the health authorities to choose the most feasible channels of health education on TB that fits with the agropastoral mode of life. These include selecting and empowering individuals from agropastoralists, as well as training and recruitment as agropastoralists' community health workers. Also it is important that health authorities finds ways of improving agropastoralists' knowledge-gap on TB and design strategies to create positive attitude to avoid misconceptions. There is a need to launch and promote extensive health education to raise the awareness specifically about TB symptoms, means of transmission, prevention, and treatment in relation to the community misconceptions. Training should also be provided to community leaders regarding activities of the TB control program for them to make ordinary people seek appropriate help.

While three quarters of the respondents said that TB was caused by microbes, and one third thought animals were responsible, some misconceptions existed on mode of transmission of TB and treatment. Over two third of the respondents suggested that transmission occurs during sexual intercourse. Similar misconceptions were reported in previous studies in Ethiopia[100] and Zambia.[103]

In many countries in Africa people use traditional healers prior to attending formal health facilities for their ailments such as treatment of TB as reported in this study community. Similar

findings have also been reported by other studies in Tanzania,[104] Peru[105] and Republic of Vanuatu.[106] Findings from a study involving agropastoralists in Uganda shows that two third of the respondents visited traditional healers and used local herbs.[25] Although this can be associated with the overburdened public health care services that have not been able to effectively deal with the burden of TB, it has been reported to have some consequences on treatment delay for patients with TB.[104-,108] Considering the position of the traditional healers in pastoral communities some suggest that educating the traditional healers may lead to improvement in the process of diagnosis and referral. Another strategy involves penetrating cultural paradigms underlying some tribes in agropastoral communities. This is based on the fact that although some individuals can correctly be informed they may not act accordingly.[104] A way to come around this problem could be to implement changes assisted by chiefs (e.g. the Maasai) and traditional healers in leading positions. This shows that in order to transform traditional beliefs and perceptions about diseases to biomedical knowledge, it is important to have a clear understanding of traditional indigenous knowledge, attitudes, practices, myths, beliefs and perceptions.

Although two thirds of all the respondents said TB can be treated, they believed that it cannot be prevented. This implies that not knowing that they can prevent themselves from being infected; they are at higher risk of getting the disease. Legesse et al,[36] in a study involving pastoralists concluded that lack of knowledge about TB could affect the health-seeking behavior of patients thus, sustain the transmission of the disease in the community.

Discussion for Paper IV: Are sputum samples of retreatment TB reaching the reference laboratories? A 9-year audit in Tanzania

The primary aim of the TB control programmes across the world is to reduce mortality and morbidity due to TB by interrupting the chain of TB transmission.[109] Incorrectly treated TB sometimes relapses and will then be given stronger regimen. The retreatment regimen is also given to patients who fail treatment, or who interrupted treatment and come back.[110] Vijay et al,[111] in a study conducted in India associated the high number of retreatment patients with poor quality of TB services with unsupervised short course regimen. Similarly, Nabukenya-Mudiope et al[112] associated this with the presence of high TB-HIV co-infection rates in their study area. Similar observations were reported in previous studies elsewhere.[110,113]

In order to offer the optimum treatment regimen to TB retreatment patients, national guidelines requires that their sputum samples undergo culture and DST for *M. tuberculosis.*[114] Monitoring this process inform the NTLP about the implementation. In this study we show that only 10% of the retreatment cases notified during the audit period had their sputum samples submitted to the laboratories for culture and DST. The logistics of getting sputum samples to a central reference laboratory is not easy in Tanzania, and has proved difficult in other countries. Despite national guidelines in many countries recommending culture and DST for all retreatment patients, this does not always happen. A country-wide survey in Malawi showed that in retreatment patients only 40% of specimens arrived at the reference laboratory.[115] A study in China showed that less than one third of patients who should have undergone culture and DST actually did so.[116] In the mid-west region of Nepal, only 15% of retreatment TB patients submitted sputum specimens, 15% of sputum samples arrived at the reference laboratory, and less than 10% of patients had a culture and DST result.[117] This therefore shows that even in good TB programmes there will always be some who fail to comply with the guidelines.

6.0. CONCLUSION AND RECOMMENDATIONS

Both *M. tuberculosis*-complex and NTM strains were prevalent in the study community. The high proportion of NTM among those diagnosed to have TB indicates clinical and environmental occurrence and possible human-environment-livestock risks of cross transmission.

Our findings shows that the following were determinants of the likelihood of being positive for mycobacteria: i) *Demographic characteristics*: being a male, and age less than 40 years, ii) *Symptoms*; loss of appetite, iii) *Environmental factors* (exposures and practices); living in a households with a family size of more than six individuals, being in contact with a person who had TB, sharing a room with domestic animals, sharing water sources with domestic animals, or a family member with persistent cough.

Knowledge of risk factors for mycobacterial diseases was generally low. Mycobacterial disease exposures and practices reported in our study include; being in contact with a person who had TB, sharing a room with domestic animals, sharing water sources with animals, having a family member who had a persistent cough, being a smoker, keeping animals, and having been previously treated from TB.

Although awareness about mycobacterial diseases among the study community was high, specific knowledge on causes, prevention, and treatment of the disease was poor, and some misconceptions existed on modes of transmission and symptoms. There are some misconceptions such as transmission of TB to occur during sexual intercourse; there seem to be some involvement of traditional healers in the treatment of TB. The reported low knowledge implies that the community members cannot prevent themselves from being infected; they are therefore at risk from getting the disease. Drinking untreated water, consumption of raw animal products such as milk, meat and blood, smoking and drinking alcohol were among the behaviors reported as routes for mycobacterial diseases. These findings can help designing appropriate control measures against mycobacterial diseases.

The findings show that there are problems with the logistics of getting sputum samples of retreatment cases to a central reference laboratory. This affects the implementation of the national guidelines on submission and having culture and DST for sputum samples from all retreatment patients in the country.

RECOMMENDATIONS

The high prevalence of NTM in the study community, their implication on diagnosis of mycobacterial diseases and epidemiology of TB in the study community requires some attention. We therefore recommend for further research to elucidate their role in causing disease in the study community.

The study community is not aware of the relationship between their lifestyles and usual activities that predispose them to mycobacterial diseases. Mycobacterial diseases in the study community are therefore likely to go beyond public health control. There is a need to implement interventions addressing changing perceptions and altitude towards risks for mycobacterial diseases.

There is a need for health education to transform the traditional beliefs and perceptions about mycobacterial diseases to biomedical knowledge. Also to promote health education about transmission, prevention and risk factors for mycobacterial diseases. This can be achieved by strengthening school health programmes. There is a need to find out better ways and to strengthen community health education to raise awareness on TB symptoms, means of transmission, prevention, and treatment against the community misconceptions.

Involving traditional healers and community leaders may contribute to the improvement of diagnosis and referral of patients. This can solve the prevailing misconceptions in the study community by penetrating cultural paradigms underlying some tribes.

The TB programme has to regularly review and improve the logistics for culture and DST for TB retreatment cases.

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8.0. ORIGINAL PAPERS

Paper I: Who has mycobacterial disease? A cross sectional study in agropastoral communities in Tanzania

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I

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 1711 presumptive tuberculosis cases where 936 (54.2%) were males and 775 (45.3%) females. Out of all the study participants, 277 (16%) were found to have sputum samples positive for mycobacteria; 228 (13.3%) were smear positive and 123 (7.2%) were culture positive..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*-complex and non-tuberculous mycobacterial strains were prevalent in the study community. Loss of appetite, presence of a coughing family member, being an exclusive animal keeper, age below 40 years, and being a male were predictors of having mycobacteria. Although the reported predictors may improve screening for mycobacterial diseases, their use requires some precaution.

Key words: Mycobacterial disease; HIV; Agropastoralist; Tuberculosis; Tanzania.

Running title: Mycobacterial disease in Tanzania

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 ddifficulties in diagnosing mycobacterial diseases as the clinical manifestations 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 mycobacterial diseases 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 Endulen 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 presumptive 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 like 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, often because of the following symptoms or signs of tuberculosis. In our study a tuberculosis case is an individual bacteriologically confirmed by smear microscopy or by culture. Literacy is used as the ability to read and write and speak Swahili. Education level is the highest grade of education that an individual has completed. Semi-urban means in a town with business and employment as well as farming.

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. We investigated sputum samples using smear microscopy and culture. 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 (the national language) and the research assistants filled the questionnaire in English. Data collection was conducted from November 2010 to June 2013. A case of tuberculosis 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 4 Paranitrobenzoic 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, Chicago, IL, USA) and STATA version 11 (STATA Corp Inc., TX, USA) for cleaning and analysis. The Pearson Chi square statistics test was used to compare group differences for categorical variables. Associations and relationships were considered statistically significant if $p<0.05$. Multiple logistic regressions were used for assessing multiple determinants predicting mycobacterial disease. Crude and adjusted odds ratios (OR) with 95% confidence intervals (CI) were reported. Variables that were considered significant at $p\leq0.2$ in the univariate analysis were included in the regression model. In the final model we adjusted all the risk factors with the following variables: sex, age, education, residence, agropastoralist, coughing family member, and smoking. During multivariate analysis five potential symptoms, eight selected risky exposures/habits and six demographic characteristics giving a total of 19 known determinants of mycobacterial diseases were assessed. For the assessment of association between determinants of and mycobacterial disease among all study participants; out of 19; three symptoms, five risk exposure and four demographic giving a total of 12 determinants were significant during

univariate analysis. However, after running multivariate logistics analysis one symptom and two demographic amounting to three were significant determinants of mycobacterial disease (**Table 2**). For the HIV positive category of respondents; out of 19, three determinants were significant during univariate analysis. However, after multivariate logistic analysis; one risk exposure and demographic characteristic giving a total of two determinants were significant. The last category of study participants was the HIV negative individuals; two symptoms, eight risk exposure and two demographic giving a total of 12 determinants, were significant during univariate analysis. After multivariate analysis one symptom and risk exposure, two demographic characteristics giving a total of four were significant determinants of mycobacterial disease among HIV negative respondents (**Table 3**).

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;** 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, (**Figure 1**).

Of the sputum samples from 1711 presumptive tuberculosis patients 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, a total of 228 (2%) were positive by smear microscopy and 123 (3%) by 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 (**Figure 2a**), 508 (30%) with loss of weight, 468 (27%) evening fever and 17 (6%) hemoptysis. In **Figure 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 3a**, 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 positive for mycobacteria, and the presence of a family member with a persistent cough also predicted being positive for mycobacteria**.** In **Table 3b**, we 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 tuberculosis 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 presumptive pulmonary tuberculosis patients 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 *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 *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 diagnosed as mycobacteria positive had a positive sputum smear. We think part of the reason that many acid fast bacilli positives were negative on culture may have been long transport time. Still the proportion of "presumptive 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, knowledge, 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 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 the risk of developing tuberculosis compared with healthy people, and pulmonary tuberculosis is still the most common form (31). Co-infection with human immunodeficiency virus (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 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 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 TB 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 TB 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 results in the health facility register. This resulted in 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 knowledge 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 shows that both *M. tuberculosis*-complex and non-tuberculous mycobacterial strains were prevalent in the study community. The high proportion of nontuberculous mycobacteria among those diagnosed to have tuberculosis indicates clinical and environmental occurrence and possible human-environment-livestock risks of cross transmission. Loss of appetite, presence of a coughing family member, being an exclusive animal keeper, age below 40 years, and being a male were predictors of having mycobacteria among patients attending clinics for pulmonary mycobacterial examination. Although the reported predictors may improve screening for mycobacterial diseases, their use requires some precaution.

Competing interests

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Acknowledgments

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LIST OF TABLES AND FIGURES

Table 1: Demographic characteristics of 1711 patients examined for tuberculosis in three hospitals of Northern Tanzania, 2010-12.

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
Missing	1047	61.2

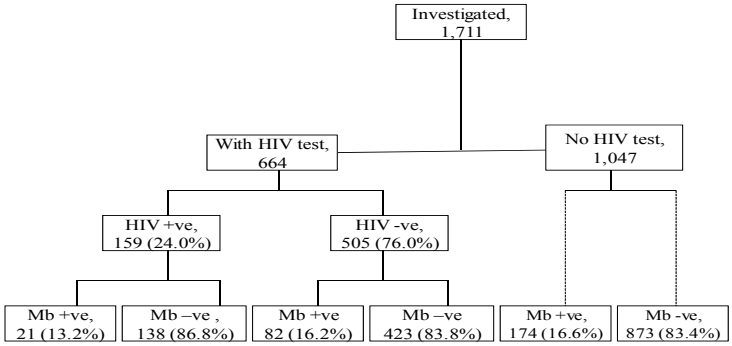

Figure 1: Flowchart of investigations for mycobacteria among study participants, by HIV status, in Northern Tanzania, 2010-12.

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)	**1.5 (1.1-1.9)**	**1.5 (1.1-2.0)**
	Female	776	104 (13.4)	REF	
	Missing	8	2 (25.0)		
Age group					
	≤20	148	29 (19.6)	**1.7 (1.0-2.7)**	**1.6 (1.0-2.9)**
	21-30	276	55 (19.9)	**1.7 (1.2-2.5)**	**1.9 (1.2-2.9)**
	31-40	365	73 (20.0)	**1.7 (1.2-2.4)**	**1.7 (1.2-2.6)**
	41-50	261	36 (13.8)	1.1 (0.7-1.7)	1.0 (0.6-1.6)
	>50	587	75 (12.8)	REF	REF
	Missing	74	9 (12.2)		
Level of education					
	No formal education	728	123 (16.9)	**1.3 (1.0-1.7)**	0.9 (0.6-1.4)
	Primary School	290	61 (21.0)	**1.7 (1.2-2.4)**	1.1 (0.7-1.9)
	Secondary School	49	6 (12.2)	0.9 (0.4-2.1)	0.5 (0.2-1.4)
	Higher Education	615	84 (13.7)	REF	
	Missing	29	3 (10.3)		
Residence					
	Semi-urban	978	159 (16.3)	REF	
	Rural	730	118 (16.2)	1.0 (0.8-1.3)	
	Missing	3	0 (0.0)		
Education status					
	Literate	1067	190 (17.8)	REF	
	Illiterate	623	84 (13.5)	0.7 (0.5-1.0)	
	Missing	21	3 (14.3)		
Agropastoral involvement					
	Primarily peasants	1053	186 (17.7)	REF	
	Primarily pastoralists	626	87 (13.9)	0.8 (0.6-1.0)	
	Missing	32	4 (12.5)		
ENVIRONMENTAL FACTORS					
Family size					
	6 or less	350	65 (18.6)	0.8 (0.6-1.1)	0.9 (0.6-1.2)
	More than 6	1149	177 (15.4)	REF	
	Missing	212	36 (17.0)		

Contact with person with tuberculosis					
	Yes	235	37 (15.7)	1.0 (0.7-1.4)	
	No	1413	230 (16.3)	REF	
	Missing	64	10 (15.6)		
Shared a room with domestic animals					
	Yes	564	76 (13.5)	0.8 (0.6-1.0)	1.1 (0.5-2.2)
	No	1136	198 (17.4)	REF	
	Missing	11	3 (27.3)		
Shared water source with animals					
	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 TB					
	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)		

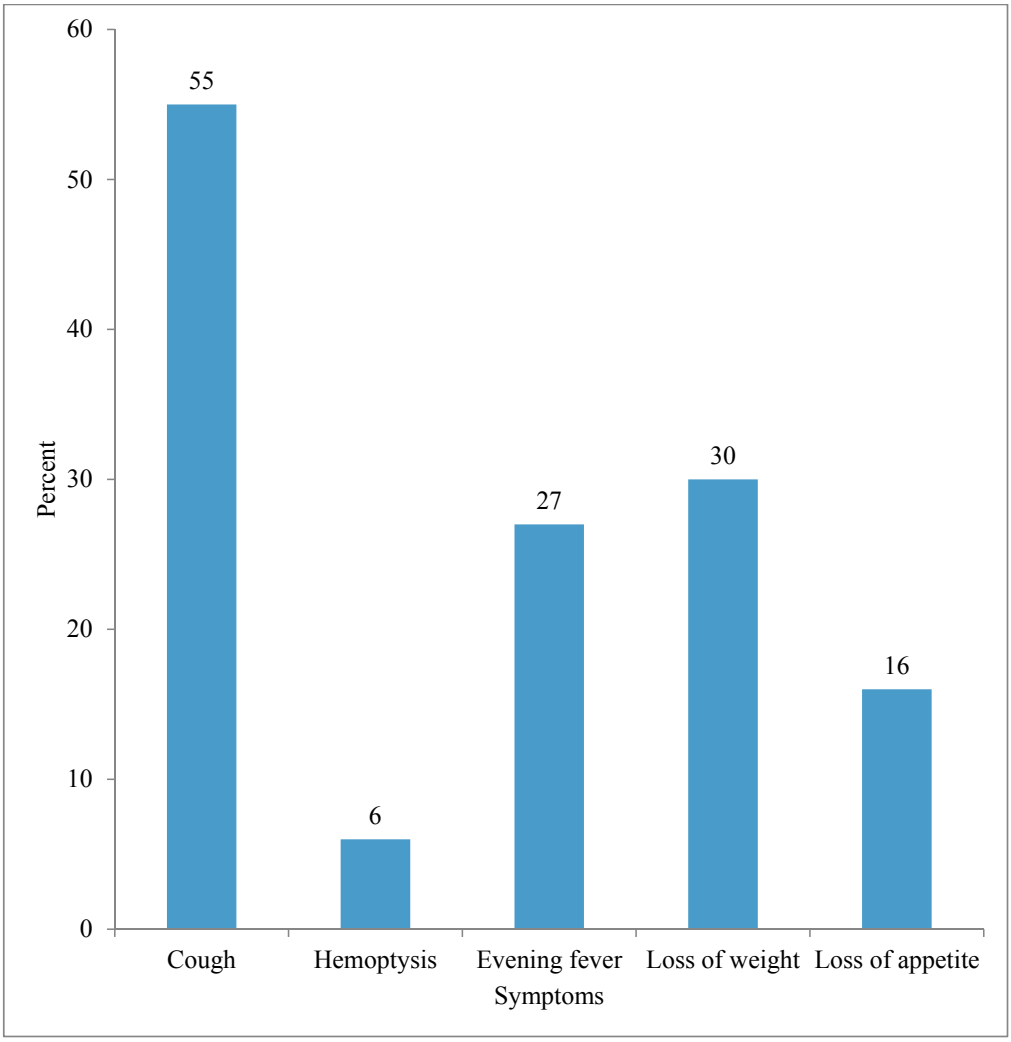

Figure 2a: Proportion of patients with symptoms for investigation of mycobacterial diseases among 1711 patients attending three tuberculosis clinics in Northern Tanzania, 2010-12.

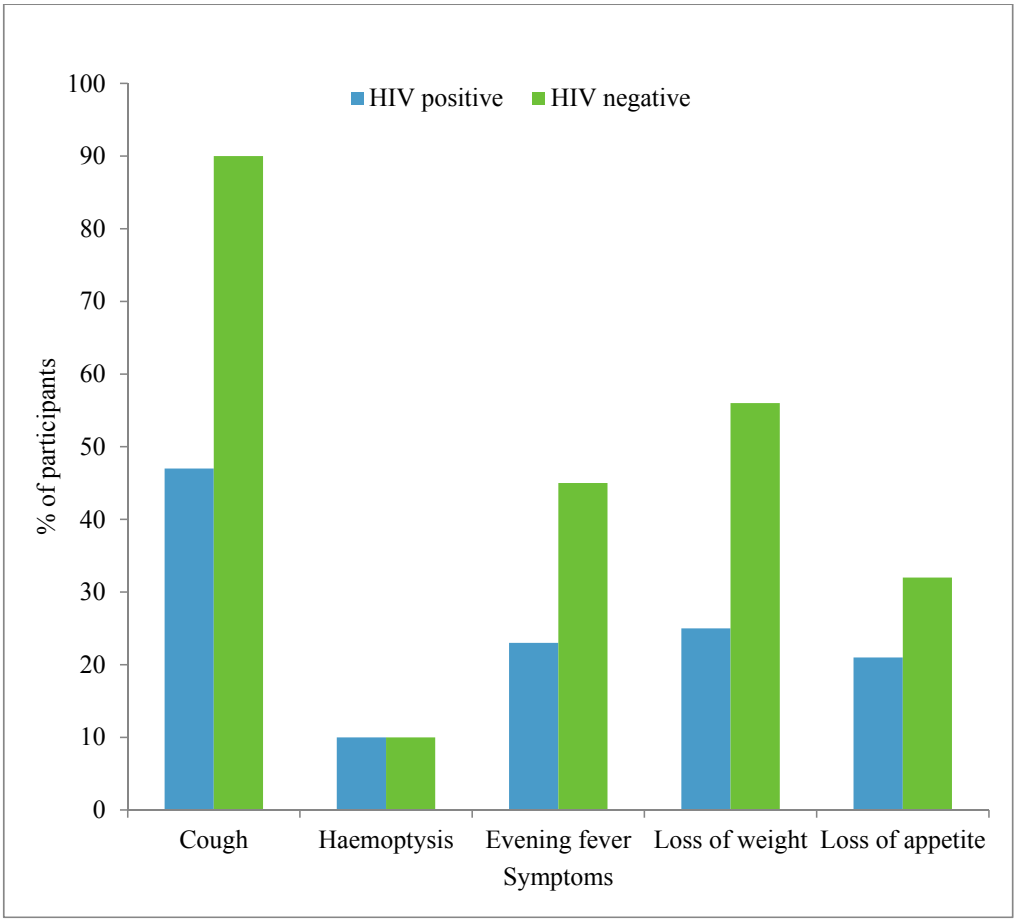

Figure 2b: Reported symptoms by 664 patients investigated for mycobacterial diseases by HIV status in Northern Tanzania, 2010-12.

Table 3 a): Determinants of mycobacterial disease among 159 HIV positive patients examined for tuberculosis in northern Tanzania, 2010-12.

Determinant	Total Suspects	Mycobacteria (+)	OR (95%CI)	AOR* (95%CI)
	n	n (%)		
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	27	4(14.8)	REF	
More than 6	96	13(13.5)	0.9 (0.3-3.0)	
Missing	36	4(11.1)		
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	
Missing	3	1(33.3)		
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 TB				
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 3 b): Determinants of mycobacterial disease among 505 HIV negative patients examined for tuberculosis in Northern Tanzania, 2010-12.

Determinant	Total Suspects n	Mycobacteria (+) n (%)	OR (95%CI)	AOR* (95%CI)
Total	**505**	**82 (16.2)**		
DEMOGRAPHIC CHARACTERISTICS				
Sex				
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				
≤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 (0.8-3.7)
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				
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				
Semi-urban	173	32(18.5)	REF	
Rural	332	50(15.1)	0.8 (0.5-1.3)	
Education status				
Literate	220	43(19.5)	REF	
Illiterate	279	39(14.0)	0.7 (0.4-1.1)	
Missing	6	-		
Agropastoral involvement				
Primarily peasants	166	36(21.7)	REF	
Primarily pastoralists	338	46(13.6)	0.6 (0.4-0.9)	
Missing	1	-		
ENVIRONMENTAL FACTORS				
Family size				
6 or less	73	14(19.2)	REF	
More than 6	370	57(15.4)	0.8 (0.4-1.5)	
Missing	62	11(17.7)		
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	
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 TB				
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.

Paper II: Experienced and perceived risks of mycobacterial diseases: A cross sectional study among agropastoral communities in northern Tanzania

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II

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RESEARCH ARTICLE

Experienced and Perceived Risks of Mycobacterial Diseases: A Cross Sectional Study among Agropastoral Communities in Northern Tanzania

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Abstract

Objective

The current study was conducted to assess experienced risk factors and perceptions of mycobacterial diseases in communities in northern Tanzania.

Methods

We conducted a cross-sectional study in Arusha and Manyara regions in Northern Tanzania. We enrolled tuberculosis (TB) patients attending Mount Meru Hospital, Enduleni Hospital and Haydom Lutheran Hospitals in Arusha municipality, Ngorongoro and Mbulu districts, respectively. Patient addresses were recorded during their first visit to the hospitals. Patients with confirmed diagnosis of TB by sputum smear microscopy and/or culture at central laboratory were followed up and interviewed using pre-tested questionnaires, and selected relatives and neighbors were also interviewed. The study was conducted between June 2011 and May 2013.

Results

The study involved 164 respondents: 41(25%) were TB patients, 68(41.5%) were their relatives and 55(33.5%) their neighbors. Sixty four (39%) knew a risk factor for mycobacterial disease. Overall, 64(39%) perceived to be at risk of mycobacterial diseases. Exposure to potential risks of mycobacterial diseases were: keeping livestock, not boiling drinking water, large family, smoking and sharing dwelling with TB patients. Rural dwellers were more often livestock keepers ($p<0.01$), more often shared dwelling with livestock ($p<0.01$) than urban dwellers. More primary school leavers reported sharing dwelling with TB patients than participants with secondary and higher education ($p = 0.01$).

Competing Interests: The authors have declared that no competing interests exist.

Conclusion

Livestock keeping, sharing dwelling with livestock, sharing household with a TB patient were perceived risk factors for mycobacterial diseases and the participants were exposed to some of these risk factors. Improving knowledge about the risk factors may protect them from these serious diseases.

Background

Mycobacterial infection is a global public health problem in humans, animal populations and ecosystems, particularly in developing countries [1,2]. *Mycobacterium tuberculosis (MTB)* is the principal cause of human tuberculosis worldwide [3], but environmental mycobacteria (non-tuberculous mycobacteria, NTM) are opportunistic pathogens whose role in human and animal disease is increasingly recognized [4]. *Mycobacterium bovis* and a wide range of species of NTM have been isolated and characterized from environment shared by humans and animals [3, 5, 6]. Many mycobacterial infections are preventable and the associated diseases are treatable with multiple special antibiotics for a long duration [7].

In the last few decades, the incidence of classical tuberculosis in humans has been on the increase alongside tuberculosis-like disease caused by environmental mycobacteria [8,9]. Tanzania ranks fourteenth among TB high burdened countries in the world [2]. A total of 65,732 cases of all forms were notified in 2013, which shows an increase of 1,840 cases or 2.9 percent compared to the year 2012. The notification rate of tuberculosis (all forms) in 2013 remained at 142 cases per 100,000 populations as for the year 2012. Notification rate of new smear positive tuberculosis cases decreased from 56 to 53 cases per 100,000. This however, shows that TB is still a major burden in the country [10]. In the HIV/AIDS era, mycobacterial infections have become a subject of interest because of their potential co-infection with HIV/AIDS [11]. It has been estimated that one-third of HIV/AIDS deaths are due to mycobacterial diseases, particularly tuberculosis [12].

The human-mycobacterial interaction with its impact on human health is complex and likely broader than currently recognized [1]. Human activities are likely to influence the distribution and prevalence of mycobacteria. The role of open water sources with its livestock/wildlife as reservoirs of infections to human are well documented [11,13]. In agropastoral ecosystems with close interaction between humans, livestock and wildlife affects the health of humans. These semi-arid areas in general suffer from poor quality pastures and seasonal water availability [4]. In such areas, large herds of cattle, domestic animals such as goats and sheep, wildlife, and humans all share the same environment, providing opportunities for close interaction and potential risk of mycobacterial infection to these communities [12].

Effective prevention and control measures of mycobacterial diseases require comprehensive initiatives that address the primary barriers for the prevention of mycobacterial infection. In this regard it is important to understand the perceptions that influence behavior and response to mycobacterial diseases. Knowledge about risk factors for TB has been studied in Tanzania, but information on potential risk factors of mycobacterial diseases in general for individuals, families and communities in the most at-risk areas of Tanzania is limited. This study aims to study selected socio-cultural risk factors and knowledge about them in this area. Our specific objectives were to assess the following among TB patients, their relatives and neighbors in three selected districts with many agropastoralists in Tanzania: 1) their knowledge about some known risk factors; 2) their exposure to these risk factors; 3) the association between exposure to risk and their risk perception.

Methods

Design

We conducted a cross-sectional study to assess knowledge and exposure to risks of mycobacterial infections among TB patients, their relatives and neighbors.

Setting

The study was conducted in the catchment areas of 3 hospitals in Northern Tanzania: Mount Meru Hospital in Arusha municipal, Enduleni Catholic Hospital in Ngorongoro district and in Haydom Lutheran Hospital in Mbulu district. The TB patients were enrolled from these hospitals. According to the 2012 Census the selected districts had a population of 650,370 [14]. The notification rate of all types of TB in Arusha and Manyara regions in 2011 were between 150–199 per 100 000 population [15]. Prominent local tribes include the Maasai in Ngorongoro, the Arusha and the Meru in Arusha, and the Datoga and the Iraqw in Mbulu district, and many homes in all these groups are agropastoralists.

Sampling of the study subjects

This study was part of a larger study where a total of 1,711 eligible TB suspects attending the three selected health facilities were enrolled for a study on diagnostics of mycobacterial infections. Sputum samples from the enrolled participants were processed and examined for smear microscopy at the local health facility to allow case management; this information was not used in this study. At the same time, another sample from the enrolled patients was collected and send to the Central Tuberculosis Reference Laboratory (CTRL) in Dar es Salaam for smear microscopy culture and drug sensitivity testing was also done for all positive isolates. From the 1,711 TB suspects, there were 277 proven TB patients from whom we selected participants. The inclusion criteria for TB patients in this study were: 1) smear or culture positive samples; 2) residents of the study area; 3) consenting to participate; 4) traceable by mobile phone and address recorded at the health facility. Using patient addresses recorded at the health facility during the initial visit, the patients were listed for follow up and interviews to assess their experiences and perception on mycobacterial diseases. Prior to visits for interview patients were called by phone and consent was requested. For each consenting TB patient, relatives who were present and neighbors living in the vicinity were also interviewed.

Data collection

Data was collected using two structured questionnaires: one for patients and one for relatives and neighbors. The questionnaires included standard questions adapted from previous studies [16] and some developed based on existing literature [17]. The questionnaires were translated into Swahili (the national language) and pre-tested for clarity and cultural acceptability, and the information collected was as follows: Baseline information including sex, age, area of residence, marital status, occupation and level of education; perceptions on selected risk factors for mycobacterial infections, like number of people sharing a dwelling, history of sharing house with a person with TB, past history of TB infection, smoking and livestock keeping, consumption of raw animal products, household primary source of water, source of drinking water for animals/livestock, and preparation of drinking water for the family. Some known risk situations assessed in this study are listed in Table 1 [12,18,19]. Eligible TB patients were enrolled between June 2011 and May 2012, relatives and neighbors between 2012 and 2013.

Table 1. Situations associated with risk of mycobacterial diseases (references in brackets).

Livestock keeping	[3,4]
Never boil, filter or treat drinking water	[4]
Large family ≥5 individuals	[4]
Smoker or ex-smoker	[4,18]
Sharing dwelling with a TB patient	[4]
Sharing water source with animals	[4]
Sharing dwelling with livestock	[4]
Sleeping 4≥ individuals in one room	[4]
Consume raw milk	[12,13]
Consume raw meat	[18,19]
Consume raw blood	[4]

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Ethical considerations

Ethics approval was given by the National Health Research Ethics Review Committee of the National Institute for Medical Research in Tanzania. Both regional and district administrative officials gave permission to conduct the study in their areas. All respondents were asked to sign a consent form prior to participation in the study.

Data management and analysis

All responses in the study questionnaires were coded and double entered in Epi-data version 3.1. Data was transferred to Statistical Package for Social Sciences version 18 for windows (SPSS Inc, Chicago, USA) for analysis. Pearson Chi square statistics test was used to compare categorical variables. Significance level was set at $p<0.05$.

Results

In Table 2 we present a summary of the socio-demographic characteristics of the study participants. The study involved 164 respondents of whom 41(25%) were confirmed TB patients and 68(41.5%) were their relatives and 55(33.5%) their neighbors. The mean age of the study participants was 39 (range 14–76) years.

Knowledge about some known risk factors

Table 3 shows the responses by the three groups of participants to questions about risk factors for mycobacterial diseases. Overall, 61% of the 164 respondents reported that they did not know any risk factor for mycobacterial diseases: 49% of TB patients, 59% of relatives and 73% of neighbors. Living with a person who had TB, sharing eating and drinking utensils, and having infectious diseases were risk factors known for the infections.

Perception of being at risk from mycobacterial diseases

Overall, 64(39%) of the 164 study participants perceived to be at risk from mycobacterial diseases; 51% of the TB patients, 41% of the relatives and 27% of the neighbors. In Table 4 we present the respondents' perceptions of being at risk from mycobacterial diseases by demographic characteristics. A smaller fraction of the neighbors than the patients felt at risk. Compared to the age group over 50 years, a smaller proportion of those aged 21–30 years felt at risk. Respondent's marital status, level of education, residence and occupation were not significantly associated with perception of being at risk from mycobacterial diseases.

Table 2. Socio-demographic characteristics of study participants by study group in northern Tanzania, 2011–12.

Characteristic	Total n (%)	TB patients n (%)	Relatives n (%)	Neighbors n (%)
Sex				
Male	84 (51)	24 (58)	28 (41)	32 (58)
Female	80 (49)	17 (42)	40 (59)	23 (42)
Age group				
≤20	14 (9)	4 (10)	7 (10)	3 (6)
21–30	39 (24)	6 (15)	17 (25)	16 (29)
31–40	46 (28)	12 (29)	19 (28)	15 (27)
41–50	26 (16)	3 (7)	14 (21)	9 (16)
>50	39 (24)	16 (39)	11 (16)	12 (22)
Marital status				
Married	119 (73)	26 (63)	52 (77)	41 (75)
Others (single, widow)	45 (27)	15 (37)	16 (23)	14 (25)
Region				
Arusha	59 (36)	19 (46)	25 (37)	15 (27)
Manyara	105 (64)	22 (54)	43 (63)	40 (73)
District				
Mbulu	106 (65)	22 (54)	43 (63)	41 (75)
Ngorongoro	18 (11)	2 (5)	10 (15)	6 (11)
Arusha	40 (24)	17 (41)	15 (22)	8 (14)
Level of education				
No formal education	38 (23)	13 (32)	19 (28)	6 (11)
Primary	99 (60)	19 (46)	44 (65)	36 (65)
Secondary and above	27 (17)	9 (22)	5 (7)	13 (24)
Residence				
Urban	47 (29)	20 (49)	17 (25)	10 (18)
Rural	117 (71)	21 (51)	51 (75)	45 (82)
Occupation				
Peasant	117 (71)	23 (56)	53 (78)	41 (75)
Employed	13 (8)	2 (5)	4 (6)	7 (13)
Business	23 (14)	10 (24)	8 (12)	5 (9)
None	11 (7)	6 (15)	3 (4)	2 (4)

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Association between perceived risk and exposure to potential risks of mycobacteria

We asked the participants about eleven practices with some inherent risk of exposure to mycobacteria, listed in Table 1. In Table 5 we display some of these common situations with risk of exposure to mycobacteria and the reported perceptions of risks for mycobacterial diseases by the study respondents. Respondents who do not boil, filter or treat their drinking water considered themselves to be at risk of mycobacterial diseases ($p = 0.05$); so also did the respondents who had shared dwelling with a known TB patient ($p<0.01$). Participants who had shared dwelling with TB patients felt more at risk ($p<0.01$) as did livestock keepers ($p<0.01$) and those who never boil or treat their drinking water ($p = 0.05$). Other associations were not statistically significant. Rural dwellers were more often livestock keepers and more exposed to this potential source of infection ($p<0.01$), and they shared dwelling with livestock more frequently than urban dwellers ($p<0.01$). Males and females had similar exposures to situations with

Table 3. Knowledge about some known risk factors for mycobacterial diseases in northern Tanzania, 2011–12.

Response	Total n (%)	Patients n (%)	Relatives n (%)	Neighbors n (%)
All respondents	164 (100)	41 (100)	68 (100)	55 (100)
Yes, I know a risk factor	64 (39)	21 (51)	28 (41)	15 (27)
No, I don't know risk factors	100 (61)	20 (49)	40 (59)	40 (73)
Selected known risk factors				
Living with a person with TB	20(12)	6(15)	9(13)	5(9)
Sharing eating and drinking utensils	11(7)	5(12)	6(9)	-
Infectious diseases can transmit	11(7)	4(10)	5(7)	2(4)
Having ever suffered from TB	7(4)	3(7)	3(4)	1(2)
Inhaling infected air from coughing person	7(4)	2(5)	3(4)	2(4)
Coughing or fevers	2(1)	-	1(1)	1(2)
Dirty environment	2(1)	-	-	2(4)
Attending public events	1(1)	-	1(1)	-
Witchcraft	1(1)	1(2)	-	-
Congestion	1(1)	-	-	1(2)

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potential exposure, although more males consumed raw meat (14: 23%) than females (4: 8%, $p = 0.05$). Patients and relatives and neighbors had similar exposure. There were more livestock keepers among participants in Mbulu (67; 41%) than in Ngorongoro (10; 6%) and Arusha districts (11; 7%), ($p<0.01$). A higher proportion of participants who had (at least started) primary school education had shared dwelling with TB patients than those without any schooling ($p = 0.01$).

Discussion

Studies focusing on individual experience on situations and perceived risks of mycobacterial diseases in rural and urban agropastoral communities in Tanzania are limited. The current study shows that livestock keeping, sharing dwelling with livestock, sharing household with a TB patient, living in congested households and consumption of raw meat were the main potential exposures for mycobacterial diseases in the study area. Similarly, rural residence, being a male, a peasant and holding a primary school education were significant socio-demographic characteristics associated with exposure to risky practices. The study shows that sixty one percent of the respondents did not perceive that they were at risk of being infected with mycobacteria.

Previous studies have shown that individuals who perceive to be at risk of contracting a disease are likely to take measures to protect themselves against the disease [20]. Sixty percent of our study participants consider themselves not at risk, and were therefore not likely to take any measures for protection from mycobacterial diseases. Overcrowding is a known risk factor for TB [21–23], and we found that almost half of the participants were living in houses with many persons; however, less than 15% shared sleeping room with four or more.

Despite evidence indicating that cultural and socio-economic factors, among others, increases the likelihood of mycobacterial transmission, consumption of raw animal products including raw milk, meat and drinking untreated water poses a risk of transmitting mycobacteria to humans [20]. Earlier reports associate the consumption of raw milk and meat with extrapulmonary TB [24–26], and Ameni and colleagues [27] reported no association between consumption of raw milk and occurrence of human TB. In agropastoral communities milk is often produced and consumed at household level as opposed to milk pooled for a wider commercial

purpose which is pasteurized. Therefore milk has minimal role in transmitting mycobacteria to people other than the household.

We showed that among TB patients the perception of risk and exposure to it was statistically associated for people sharing dwelling with TB patients and livestock keepers, whilst other risk factors were not. This underlines the fact that knowledge about risk does not in itself create a protected environment, as many other circumstances play a role. Furthermore, in our analysis the relation between the timing of the exposure and the perception is not known. Measures for protection from TB patients were still in place in hospital isolation wards during the initial weeks of treatment.

Although awareness does not necessarily translate into behavior, these findings have public health implications calling for appropriate community interventions to reduce the risk of

Table 4. Perceptions of being at risk of mycobacterial diseases, by demographic characteristics in northern Tanzania, 2011–12.

Characteristic		Consider being at risk?		
	Total	Yes, n (%)	No, n (%)	Risk Ratio and 95% CI
Sex				
Male	84	34(41)	50(60)	1.09; (95%CI 0.735–1.584)
Female	80	30(38)	50(63)	
Age group				
< = 20	14	5(36)	9(64)	0.66; (95%CI 0.310–1.419)
21–30	39	8(21)	31(80)	0.38; (95%CI 0.193–0.754)
31–40	46	16(35)	30(65)	0.65; (95%CI 0.395–1.055)
41–50	26	14(54)	12(46)	1.00; (95%CI 0.632–1.583)
>50	39	21(54)	18(46)	
Marital status				
Married	119	49(41)	70(59)	1.24; (95%CI 0.775–1.968)
Others (single, widow, widower)	45	15(33)	30(67)	
Region				
Arusha	59	21(51)	38(64)	0.87; (95%CI 0.575, 1.314)
Manyara	105	43(41)	62(59)	
District				
Mbulu	106	43(41)	63(59)	
Ngorongoro	18	3(17)	15(83)	0.41; (95%CI 0.143–1.184)
Arusha	40	18(45)	22(55)	1.11; (95%CI 0.734–1.676)
Type respondent				
Patients	41	21(51)	20(49)	
Relative	68	28(41)	40(59)	0.80; (95%CI 0.532–1.214)
Neighbor	55	15(27)	40(73)	0.53; (95%CI 0.315–0.900)
Level of education				
No formal education	38	17(45)	21(55)	1.34; (95%CI 0.708–2.545)
Primary	99	38(38)	61(62)	1.15; (95%CI 0.639–2.075)
Secondary and above	27	9(33)	18(67)	
Residence				
Urban	47	18(38)	29(62)	0.97; (95%CI 0.636–1.493)
Rural	117	46(39)	71(67)	
Occupation				
Peasant	117	47(40)	70(60)	
Employed	13	4(31)	9(69)	0.77; (95%CI 0.329–1.783)
Business	23	10(44)	13(57)	1.08; (95%CI 0.646–1.813)
None	11	3(27)	8(73)	0.68; (95%CI 0.252–1.827)

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Table 5. Exposure to situations with known potential risk of mycobacterial diseases in northern Tanzania, 2011–12.

Exposure	Responded	Consider being at risk? Yes, n (%)	No, n (%)
Livestock keeping	88	51 (58)	37 (42)
Never boil, filter or treat drinking water	77	47 (61)	30 (39)
Large family ≥5 individuals	75	45 (60)	30 (40)
Smoker or ex-smoker	71	40 (56)	31(44)
Sharing dwelling with a TB patient	67	46 (69)	21 (31)
Sharing water source with animals	49	26 (53)	23 (47)
Sharing dwelling with livestock	37	23 (62)	14 (38)
Sleeping 4≥ individuals in one room	21	14 (67)	7 (33)
Consume raw milk	26	14 (54)	12 (46)
Consume raw meat	18	10 (56)	8 (44)
Consume raw blood	16	7 (44)	9 (56)

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mycobacterial diseases in agropastoral communities in Tanzania and similar settings. Such interventions include health education to improve awareness, perceptions and change in practice. For the interventions to be effective, both the community and health system has to address the complex socio-cultural aspects surrounding the agropastoralists.

Our study has several limitations. Firstly; despite the reported findings, not all the 277 proven TB patients were enrolled into the study as many did not fulfill inclusion criteria. The small sample size limited the analysis we could carry out from our research questions. Secondly; the presence of mycobacterial pathogens in the environment was investigated in another study, but was not part of this study. Thirdly; as part of a larger study on mycobacteria, this resulted into low sample size for some of the outcomes.

Conclusion

Our study shows that two third of the participants did not know a risk factor for mycobacterial disease. Livestock keeping, sharing dwelling with livestock, sharing household with a TB patient, congestion and consumption of raw meat were perceived risk factors for mycobacterial diseases. The participants were exposed to some of these risk factors. Exposure to these common potential risks was substantial but perception about the risk was limited. Improving knowledge about the risk factors may protect them from these serious diseases.

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Author Contributions

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

AMK EN GBK YLL BJN SGM SGH. Review of the manuscript: AMK EN GBK YLL JM BJN SGM SGH.

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Paper III: Knowledge and perceptions about tuberculosis in agropastoral communities in northern Tanzania: A cross-sectional study

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Knowledge and Perceptions about Tuberculosis in Agropastoral Communities in Northern Tanzania: A Cross-Sectional Study

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Authors' contributions

This work was carried out in collaboration between all authors. Authors AMK, SGM, JM, EN and BJN designed the study and wrote the protocol. Authors AMK, BJN and YLL wrote the first draft of the manuscript and managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aim: To determine knowledge and perceptions about tuberculosis in agropastoral communities in Northern Tanzania.
Study Design: This was a cross sectional study on habits and attitudes to tuberculosis.
Methods: The study was conducted between June 2011 and May 2012.We enrolled tuberculosis patients registered at Mount Meru Hospital in Arusha municipal, Enduleni Hospital in Ngorongoro district, and Haydom Lutheran Hospital in Mbulu district. In addition we selected for comparison some of their household relatives and individuals from the neighborhood. Data was collected using a structured questionnaire. Knowledge about tuberculosis was assessed by questions concerning causes, symptoms, modes of transmission and prevention and treatment. Key variables for

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assessment of perception on tuberculosis included: individuals considered most at risk, and misconceptions.
Results: We recruited 164 respondents of whom 25% were confirmed tuberculosis patients, 41.5% relatives of the patients and 33.5% neighbors. Females constituted 48.8% of all respondents. Of all the participants, only two of the neighbors had never heard about tuberculosis in their life time. Even though 99% had heard about tuberculosis, specific knowledge on causes, prevention and treatment was poor. A total of 67.7% thought that transmission of tuberculosis occurs during sexual intercourse. Respondents thought that risk from tuberculosis was higher among adults (68.9%), alcohol users (39.6%), smoking (26.8%), consumption of raw animal products (6.1%) and childhood (23.2%).
Conclusion: Our study shows that study participants had heard about tuberculosis but specific knowledge was low. Misconceptions surrounding causes, transmission, prevention and treatment of the disease were common. Selection of appropriate channels for public health education and awareness programmes targeting knowledge about prevention and control of tuberculosis in agropastoral communities may improve this situation.

Keywords: Knowledge; perception; tuberculosis; agropastoralists; Tanzania.

1. INTRODUCTION

Tuberculosis constitutes a significant global public health concern, especially in developing countries [1,2]. A third of the world's population is infected with *Mycobacterium tuberculosis* [3]. In addition to the public health impact, the economic effects of tuberculosis are severe [3,5].

Lack of knowledge about tuberculosis may play an important role in delayed diagnosis and treatment [6]. Many patients only seek healthcare after a long duration of disease and transmission, perhaps a consequence of lack of knowledge [7]. Further evidence from a large survey shows that a person's response to tuberculosis disease is associated with his prior knowledge of it [8], and better knowledge of tuberculosis is shown to be associated with better health-seeking behavior [9]. Delayed diagnosis and treatment leads to increased risk of transmission. The impact of treatment of tuberculosis is partly determined by the patient's health-seeking behavior, which may in turn be influenced by the patients' demographic characteristics, knowledge and socio-cultural factors [10]. Cattle can also transmit mycobacteria to man which may cause disease, like adenitis [11,12].

Agropastoral communities in Tanzania live a nomadic life with cattle being their main source of livelihood and peasants in these areas equally keep livestock alongside farming. Studies document various limiting factors to access education and health services [13,14,15]. The socio-behavior and indigenous knowledge associated with tuberculosis in most African countries have been documented previously [16,1]. Effective community-based prevention and control measures of tuberculosis in agropastoral communities require comprehensive initiatives that address the primary barriers. However, their knowledge, awareness, perceptions, attitude and practices towards prevention and control of tuberculosis among agropastoral communities in Tanzania is largely unknown. The objectives of this study were to determine the knowledge and perceptions of agropastoral communities' on tuberculosis in the Northern part of Tanzania.

2. METHODS

2.1 Study Design and Population

We conducted a cross-sectional study in Northern part of Tanzania. The study population included tuberculosis patients at Mount Meru Hospital in Arusha urban, Enduleni Hospital in Ngorongoro district and Haydom Lutheran Hospital in Mbulu district. For each patient we also enrolled at least one relative and one neighbor to allow comparison between them.

2.2 Sampling of the Study Subjects

This study was part of a larger study which enrolled 1,711 tuberculosis suspects for the purpose of improving the diagnosis of mycobacterial infections. A tuberculosis suspect was defined as an individual presenting to the health facility with any of the following symptoms or signs of tuberculosis: a productive cough for more than two weeks, shortness of breath, chest pain, blood-stained cough, weight loss, fever,

night sweats, and fatigue. Sputum samples were collected, processed and examined for smear microscopy at the local health facility to allow diagnosis of tuberculosis and treatment initiation; this information was not used in this study. The suspects were asked to collect another sample which was sent to the Central Reference Laboratory (CTRL) in Dar es Salaam for further analysis. At the CTRL, the samples were processed and examined for smear microscopy and culture. Drug susceptibility testing was also done for all positive isolates. Out of the 1,711 sputum samples from the tuberculosis suspects, 277 (16.2%) were proven to have tuberculosis either by being smear positive and or culture positive and from them we selected patients for follow up and interviews. The inclusion criteria in the current study were: 1) proven tuberculosis patients reporting to the outpatient departments of the selected health facilities for the first time; 2) residents of the districts served by the selected health facilities; 3) consenting to participate; 4) participants reachable by mobile phone and address. Only tuberculosis patients whose sputum samples were found to have smear positive and/or culture positive at the CTRL were eligible. Prior to visits, patients were reached by phone to confirm their availability and readiness to be visited and interviewed in their homes.

2.3 Data Collection

Data collection was done between June 2011 and May 2012. Following enrolment of participants, data was collected using a structured questionnaire to assess participants' knowledge about tuberculosis concerning the following aspects: 1) causes; 2) symptoms; 3) modes of transmission; and 4) prevention and treatment. Aspects of perception of tuberculosis were also included those considered most at risk and misconceptions concerning TB. Sociodemographic characteristics were collected: age, sex, marital status, rural or urban residence, occupation and level of education.

2.4 Ethical Consideration

Ethics approval was granted by the National Health Research Ethics Review Committee in Tanzania. Both regional and district administrative officials gave permits to conduct the study in their areas. The study respondents consented by signing a consent form prior to participation into the study.

2.5 Data Management and Analysis

Data was double entered using Epi-data version 3.1 and transferred to Statistical Package for Social Sciences version 18 for windows (SPSS Inc, Chicago, USA) and Stata version 11 (STATA Corp Inc., TX, USA) for cross-checking, data cleaning and analysis. To assess knowledge about tuberculosis and other mycobacteria among the participants, we calculated the percentage of people who provided correct response to questions concerning the six symptoms related to tuberculosis, three ways on transmission, five items related to treatment and five core knowledge of tuberculosis. We used Pearson Chi square (χ^2) statistics to compare participants' responses by demographic characteristics and factors associated with core knowledge on tuberculosis among the respondents. We used a two-tailed probability level of $p<0.05$ as the level of statistical significance.

3. RESULTS

3.1 Demographic Characteristics of Participants

We recruited 164 respondents of whom 41 (25%) were confirmed tuberculosis patients, 68 (41.5%) relatives of the patients and 55 (33.5%) neighbors. The distribution of the socio-demographic characteristics of the study respondents is shown in Table 1.

3.2 Awareness and Knowledge about Tuberculosis

Of all the 164 study participants, only 2 (1%) neighbors had never heard about tuberculosis. Overall, health workers were a common source of information on tuberculosis (99; 60.4%) and family/friends or neighbors (27; 16.2%). Teachers (1: 0.6%) and newspapers (2: 1.2%) were not common sources of information on tuberculosis.

In Table 2 we show participants' knowledge about causes, modes of transmission, symptoms, prevention and treatment, and at risk groups from tuberculosis. There were 123 (75.0%) respondents who thought that tuberculosis was caused by microbes, 50 (30.5%) thought animals were responsible, and 111 (67.7%) suggested that transmission occurs during sexual intercourse.

Overall, 65 (39.6%) of the respondents thought that tuberculosis can be prevented and 107 (65.2%) considered it to be treatable. Of the 164 respondents, 9 (5.5%) reported to be aware of traditional medicines or procedures in their community that a person with symptoms of tuberculosis may use and get relief.

3.3 Perceptions on Selected Determinants of Tuberculosis

In Table 3 we present a list of selected perceptions on tuberculosis. Respondents considered that high risk from tuberculosis was associated with adulthood (68.9%), alcoholism (39.6%), smoking (26.8%), consumption of raw animal products such as meat, blood and milk (6.1%) and childhood (23.2%). Some misconceptions existed on mode of transmission and symptoms of tuberculosis.

4. DISCUSSION

This study shows that almost all participants had heard about tuberculosis, but specific knowledge on causes, prevention and treatment of the disease was poor, and some misconceptions existed on modes of transmission and symptoms. Adults, alcoholics, smokers and young children were thought to be at higher risk of tuberculosis.

Table 1. Socio-demographic characteristics of the study participants

Characteristics	All respondents	Patients	Relatives	Neighbors	*P*-value
	n (%)	n (%)	n (%)	n (%)	
Total	164 (100)	41 (100)	68 (100)	55 (100)	
Sex					
Males	84 (51.2)	24 (58.5)	28 (41.2)	32 (58.2)	0.01
Females	80 (48.8)	17 (41.5)	40 (58.8)	23 (41.8)	
Age group					
<20	12 (7.3)	4 (9.8)	5 (7.4)	3 (5.5)	0.41
20-29	35 (21.3)	6 (14.6	17 (25.0)	12 (21.8)	
30-39	44 (26.8)	10 (24.4)	19 (27.9)	15 (27.3)	
40-49	29 (17.7)	4 (9.8)	13 (19.1)	12 (21.8)	
50+	44 (26.8)	17 (41.5)	14 (20.6)	13 (23.6)	
District					
Mbulu	106 (64.6)	22 (53.7)	43 (63.2)	41 (74.5)	0.03
Ngorongoro	18 (11.0)	2 (4.9)	10 (14.7)	6 (10.9)	
Arusha	40 (24.4)	17 (41.5)	15 (22.1)	8 (14.5)	
Residence					
Urban	47 (28.7)	20 (48.8)	17 (25.0)	10 (18.2)	$P < 0.001$
Rural	117 (71.3)	21 (51.2)	51 (75.0)	45 (81.8)	
Marital status					
Married	119 (72.6)	26 (63.4)	52 (76.5)	41 (74.5)	0.31
Other[i]	45 (27.4)	15 (36.6)	16 (23.5)	14 (25.5)	
Occupation					
Peasants	117 (71.3)	23 (56.1)	53 (77.9)	41 (74.5)	0.03
Employed	13 (7.0)	2 (4.9)	4 (5.9)	7 (12.7)	
Business	23 (14.0)	10 (24.4)	8 (11.8)	5 (9.1)	
None	11 (6.7)	6 (14.6)	3 (4.4)	2 (3.6)	
Level of education					
No formal education	38 (23.2)	13 (31.7)	19 (27.9)	6 (10.9)	0.02
Started primary school	99 (60.4)	19 (46.3)	44 (64.7)	36 (65.5)	
Secondary and above	27 (16.5)	9 (22.0)	5 (7.4)	13 (23.6)	

Table 2. Knowledge about tuberculosis among tuberculosis patients and their relatives and neighbors in northern Tanzania, 2012

Variable	Respondents	Patients	Relatives	Neighbors	*P*-value
	n (%)	n (%)	n (%)	n (%)	
Total	164 (100)	41 (100)	68 (100)	55 (100)	
Suggested causes of tuberculosis					
Microbes	123 (75.0)	37 (90.2)	46 (67.7)	40 (72.7)	0.17
Animals	50 (30.5)	21 (51.2)	22 (32.4)	7 (12.7)	$P < 0.001$
Alcohol	24 (14.6)	5 (12.2)	8 (11.8)	10 (18.2)	0.55
Smoking	15 (9.1)	6 (14.6)	5 (7.4)	4 (7.3)	0.02
Dirty air	10 (6.1)	3 (7.3)	1 (1.5)	6 (10.9)	0.09
Others	7 (4.3)	3 (7.3)	2 (2.9)	2 (3.6)	0.24
Suggested modes of transmission of tuberculosis					
During sexual intercourse	111 (67.7)	32 (78.1)	46 (67.7)	33 (60.0)	0.17
Air e.g. when a person with tuberculosis coughs/sneezes	70 (42.7)	25 (61.0)	30 (44.1)	15 (27.3)	$P < 0.001$
Sharing eating/drinking utensils	48 (29.3)	10 (24.4)	24 (35.3)	14 (25.5)	0.36
Smoking	30 (18.3)	8 (19.5)	15 (22.1)	7 (12.7)	0.40
Consumption of raw animal products (e.g. milk, meat)	11 (6.7)	5 (12.2)	3 (4.4)	3 (5.6)	0.26
Others	20 (12.2)	7 (17.1)	9 (13.2)	4 (7.3)	
Suggested symptoms of tuberculosis					
Coughing/coughing up blood	111 (67.7)	32 (78.1)	46 (67.7)	33 (60.0)	0.17
Cough lasting for 2 weeks or more	99 (60.4)	25 (61.0)	29 (42.7)	20 (36.4)	0.05
Severe headache	74 (45.1)	24 (58.5)	20 (29.4)	30 (54.6)	$P < 0.001$
Ongoing fatigue	59 (36.0)	21 (51.2)	27 (39.7)	11 (20.0)	$P < 0.001$
Fever/fever without clear cause for 7 or more days	40 (24.0)	11 (26.8)	16 (23.5)	13 (23.6)	0.92
Chest pain	25 (15.2)	9 (22.0)	12 (17.7)	4 (7.3)	0.11
Shortness of breath	25 (15.2)	9 (22.0)	12 (17.7)	4 (7.3)	0.11
Nausea	13 (7.9)	7 (17.1)	5 (7.4)	1 (1.8)	0.02
Others	14 (8.5)	4 (9.8)	5 (7.4)	5 (9.1)	0.90
Suggestions on prevention and treatment of tuberculosis					
Tuberculosis can be prevented	65 (39.6)	19 (46.3)	33 (48.5)	13 (23.6)	0.01
Tuberculosis can be cured	107 (65.2)	31 (75.6)	46 (67.7)	30 (54.6)	0.09

***Some cells had expected values < 5; the analysis did not meet criteria.*

Table 3. Selected perceptions of tuberculosis among tuberculosis patients and their relatives and neighbors in northern Tanzania, 2012

Variable	All	Patients	Relatives	Neighbors	*P*-value
	n (%)	n (%)	n (%)	n (%)	
Total	**164 (100)**	**41 (100)**	**68 (100)**	**55 (100)**	
Perceived high risk groups from tuberculosis					
Adults	113 (68.9)	32 (78.1)	48 (70.6)	33 (60.0)	0.07
People who consume alcohol	65 (39.6)	18 (43.9)	30 (44.1)	17 (30.9)	0.27
Smokers	44 (26.8)	17 (41.5)	23 (33.8)	4 (7.3)	P< 0.001
Children	38 (23.2)	11 (26.8)	18 (26.5)	9 (16.4	0.34
Who consume raw animal products	10 (6.1)	6 (14.6)	4 (5.9)		**
Others	9 (5.5)	2 (4.9)	2 (2.9)	5 (9.1)	0.32
Perceptions on tuberculosis					
Apart from modern medicine there is no alternative treatment for tuberculosis	42 (25.6)	12 (29.3)	17 (25.0)	13 (23.6)	0.81
I don't think I can get tuberculosis	35 (21.3)	9 (22.0)	14 (20.6)	3 (5.5)	0.03
Tuberculosis is no longer a problem in my area	31 (18.9)	10 (24.4)	11 (16.2)	10 (18.2)	0.56
Tuberculosis can't be prevented	14 (8.5)	1 (2.4)	9 (13.2)	4 (7.3)	0.14
In my community a person with tuberculosis may try traditional medicine	9 (5.5)	2 (4.9)	3 (4.4)	4 (7.3)	0.77
A person can't pass tuberculosis on to others	8 (4.9)	1 (2.4)	5 (7.4)	2 (3.6)	0.45
Tuberculosis can't be cured	4 (2.4)		1 (1.5)	3 (5.5)	**

***Some cells had expected values < 5; the analysis did not meet criteria.*

The reported high awareness of tuberculosis in our study corresponds well with findings from a study conducted in Ethiopia among cattle farmers [18]. It may be that the good awareness reported in our study community may have been acquired from long time experience in keeping cattle and continued sharing of information with peers on tuberculosis as a potential zoonotic disease. Although previous lessons show that awareness does not necessarily translate into knowledge, behavior and practice [18,20], our findings on awareness were important when considering the need to identify target areas, formulation and implementation of appropriate health interventions in agropastoral and similar communities. Previous studies done among pastoral communities reported that the communities were aware of basics about tuberculosis [21,22]. However, the respondents had limited information concerning bacteria as a causative agent of tuberculosis [21-24]. In the current study, although awareness on most of the assessed elements was good, we show that more patients than relatives and neighbours said tuberculosis was caused by animals, and that the diseases was caused by alcohol. Also, more relatives than patients and neighbours thought that tuberculosis can be prevented. These findings raise some questions on the appropriateness of the health education given when tuberculosis patients receive treatment. Poor awareness of the causes of the disease may have a negative impact on patients' attitude towards health-seeking behavior and preventive methods as most people with such beliefs may not visit health facilities.

Previous studies elsewhere reported that mass media (television, radio and newspapers) were common sources of health information [25,26]. Although more than half of our respondents were informed about tuberculosis through health care services, we found that teachers and newspapers were not common sources of information on tuberculosis. The purpose of effective communication is to increase awareness and knowledge on health issues in the community. These findings call for appropriate means through which health information on tuberculosis can reach the community with emphasis on causes, transmission and earliest symptoms of tuberculosis infection. Teachers unlike newspapers for example, are often available in almost all villages. Emphasis on my cobacterial diseases as part of school curriculum may be a better target to improve knowledge in the community.

Our findings on low knowledge on some aspects of tuberculosis are similar to those reported in other studies in Tanzania and elsewhere [0,27]. Studies conducted in Tanzania show that poor

knowledge on causes and transmission of tuberculosis among the Maasai people was associated with low level of schooling [28,29]. The importance of knowledge in reducing disease burden by promoting early diagnosis and adherence to treatment has been emphasized [30]. The reported low knowledge on causes and mode of transmission of tuberculosis in our study highlights current implementation gaps by health programmes in the study community. Appropriate interventions should be considered to improve knowledge about tuberculosis.

Previous studies have associated the low knowledge on tuberculosis with wrong perceptions and beliefs on the diseases [32,33]. The current study shows that a significant proportion of respondents perceived that microbes could cause tuberculosis and that transmission occurs during sexual intercourse. This may partly be explained on illness perception; e.g in South Africa a study revealed how patients constructed dichotomous identities associated with tuberculosis and HIV. The coming together of the two epidemics has rendered tuberculosis symbolic and symptomatic of HIV [33-35]. Misconceptions around most of the assessed elements were documented. These include undermining self risk from tuberculosis, transmission between individuals, possibility to prevent, prevention, treatment, and availability of alternative treatment including traditional healers or medicine. These misconceptions, together with poor knowledge, could potentially influence the transmission of tuberculosis in the study area. The insight obtained from these perceptions will help in planning an appropriate approach for a public health education package which may correct some misconceptions. This could also include strategies such as health promotion through community involvement.

This study has some limitations. As this was part of another study with another aim, the sample size was too small for analysis of sub groups, and the design was not favorable for testing associations. This has affected the precision of our estimates and may have affected some of our comparisons. There were more patients and neighbours selected in rural areas than urban, and this may have influenced the results and affected the comparisons. This has also affected the distribution of occupation among the respondents. Hence, any generalization must be done with caution.

5. CONCLUSION

Our study shows that many study participants were aware of tuberculosis but specific knowledge was generally low. Misconceptions were common. Schools and newspapers were not common sources of information about tuberculosis in the study community.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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9.0. APPENDICES

Appendix I: Ethics clearance certificate

THE UNITED REPUBLIC OF
TANZANIA

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NIMR/HQ/R.8a/Vol. IX/1009

24th September 2010

Dr Esther Ngadaya
NIMR Muhimbili
P O Box 3436,
DAR ES SALAAM

CLEARANCE CERTIFICATE FOR CONDUCTING MEDICAL RESEARCH IN TANZANIA

This is to certify that the research entitled: The Epidemiology and Diagnosis of Mycobacterial Disease in Tanzania: An Integrated study of Environmental Mycobacteria, Zoonotic Mycobacteria, and Mycobacterium tuberculosis complex (MTB) Disease in Rural-pastoral and Urban populations in Tanzania, (Ngadaya E *et al)*, has been granted ethics clearance to be conducted in Tanzania.
The Principal Investigator of the study must ensure that the following conditions are fulfilled:

1. Annual Progress report is submitted to the Ministry of Health and the National Institute for Medical Research, Regional and District Medical Officers.
2. Permission to publish the results is obtained from National Institute for Medical Research.
3. Copies of final publications are made available to the Ministry of Health & Social Welfare and the National Institute for Medical Research.
4. Any researcher, who contravenes or fails to comply with these conditions, shall be guilty of an offence and shall be liable on conviction to a fine. NIMR Act No. 23 of 1979, PART III Section 10(2).
5. Approval is for one year: 24th September 2010 to 23rd September 2011.

Name: Dr Mwelecele N Malecela

Signature
ACTING CHAIRPERSON
MEDICAL RESEARCH
COORDINATING COMMITTEE

Name: Dr Deo M Mtasiwa

Signature
CHIEF MEDICAL OFFICER
MINISTRY OF HEALTH, SOCIAL WELFARE

CC: RMO
DMO

Appendix II: The Union Ethics Advisory Group (EAG) clearance certificate

International Union Against Tuberculosis and Lung Disease
Promoting lung health in low- and middle-income countries

Ethics Advisory Group

Date: 11th April, 2012

To: Andrew Martin Kilale

Title of research project:

Performance of culture and drug sensitivity testing among tuberculosis re-treatment patients: trends from a ten year audit of programme data and reference laboratories in Tanzania

EAG number: 18/12

Investigators:
PI: Andrew Martin Kilale
Others:
Godfrey Mfinanga, National Institute for Medical Research
Bernard Ngowi, National Institute for Medical Research
Said Egwaga, National TB and Leprosy Program (NTLP)
Basra Doula, National TB and Leprosy Program, CTRL
Prof. Sven G Hinderaker,Centre for International Health (CIH) of the University of Bergen
Dr. Rony Zachariah, MSF
Prof. Anthony D Harries, The Union

Thank you for your application to the Ethics Advisory Group of the Union.
Your study has our formal approval.

- Please note that this approval is conditional on the **approval of your national or regional or relevant Ethics committee.** We require proof of this approval or an explanation about why you do not have it or cannot obtain it. If you have applied, but do not yet have the result, please ensure that you send a copy on receipt to eag@theunion.org
- Any changes to the approved protocol need to be sent to the EAG, using the form for **extension/modification** of proposals (to be found on the Union website under EAG).

We trust that your study proceeds well and that it will be productive.
With best wishes,

M.E. EDGINTON

Prof. Mary Edginton

Chairperson

Union Internationale
Contre la Tuberculose
et les Maladies Respiratoires

Unión Internacional
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