
ADDIS ABABA UNIVERSITY, SCHOOL OF
GRADUATE STUDIES

MSC RESEARCH THESIS

TITLE:

**PREVALENCE OF TUBERCULOSIS AMONG DAIRY FARM WORKERS
IN ADDIS ABABA AND ITS SUBURBS**

By

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May, 2011

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SUBURBS**

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A THESIS SUBMITTED TO THE SCHOOL OF GRADUATES STUDIES,
ADDIS ABABA UNIVERSITY, FOR PARTIAL FULFILLMENTS OF THE
REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE (MSC) IN
MEDICAL MICROBIOLOGY

May 2011

ACKNOWLEDGEMENTS

My great appreciation goes to Addis Ababa University, Department of Microbiology, Immunology and Parasitology for giving me the chance to be a student in the department and for the contribution to the budget in this study.

I want to give my kind regards to all of my advisors - Dr Solomon Gebre-Selassie, Dr Abraham Aseffa and Ato Tadesse Awoke for guiding me through each of the steps in the thesis process.

I would like to thank Armauer Hansen Research Institute for funding my work and the BTB project team for sharing their knowledge and skills during my work.

I also want to thank Ato Kassahun Alemu for his support during field works and for providing me with valuable references.

My deepest gratitude goes to the study participants without whom this work was impossible.

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ACRONYMS

AAU:	Addis Ababa University
AHRI:	Armauer Hansen Research Institute
AFB:	Acid Fast Bacilli
BP	Base pair
BTB:	Bovine tuberculosis
AOR:	Adjusted Odds Ration
COR:	Crude Odds Ratio
CSA:	Central Statistics Agency
DFW:	Dairy farm worker
DM	Diabetes Mellitus
DMIP:	Department of microbiology, Immunology and Parasitology
DNA:	Deoxyribonucleic acid
DR:	Direct repeat region
HIV:	Human Immunodeficiency virus
L-J:	Lowenstein-Jensen
MDR:	Multidrug resistant
MTC:	<i>Mycobacterium tuberculosis</i> complex
NAHDIC:	National Animal Health Diagnostic and Investigation Centre
NTM:	Non-tuberculous <i>mycobacteria</i>
PCR:	Polymerase chain reaction
RD:	Regions of difference
WHO:	World Health Organization

ABSTRACT

Background: Dairy farm workers (DFWs) are at the interface between the inter-transmission of human tuberculosis (TB) to cattle and zoonotic TB to humans. DFWs in and around Addis Ababa work in settings where the overall individual animal and herd prevalence of TB are reported to be 33% and 57% respectively.

Objective: The main objective of the study was to determine the prevalence of TB and associated factors among DFW's in Addis Ababa and the surrounding suburban areas.

Methods: A cross-sectional study was conducted among DFWs in Addis Ababa and the 5 main suburban districts by using a structured and pretested questionnaire to collect the data. Individuals with symptom complex of TB were subjected to meticulous physical examination, radiographic and laboratory investigations. Samples were collected for bacteriologic analysis and inoculated on to Lowenstein Jensen culture media followed by genus typing, all at Armauer Hansen Research Institute. Field and laboratory data were double entered, cleaned and verified using Access, 2007; analysis was done using SPSS software version 16. We computed the adjusted odds ratios, the 95% CI and p-values at a significance level of <0.05 by using logistic regression. Ethical clearance and written informed consents were obtained before data collection.

Results: A total of 256 voluntary participants were included. Out of these, 12 TB suspects and 3 DFW TB cases were identified. The prevalence of TB among DFWs was found to be 1.172 %. DFWs who consumed raw animal products were about 4 times more likely to manifest the symptom complex of TB than those consuming cooked products (AOR= 3.8, 95 % CI: 1.08-13.29, P=0.03). Only 20.3% DFWs knew the main routes of transmission of TB between humans and cattle. DFWs who had knowledge about the routes of transmission of TB were found to be 3.7 times more likely to have the symptom complex of TB compared to those who do not know the routes of transmission of TB (AOR=3.7, 95 % CI: 1.12 - 12.23, p=0.03).

Conclusions and Recommendations: Our analysis revealed the prevalence of TB among DFWs in Addis Ababa and its suburbs to be 2.4 times higher than the national average; policy makers and stakeholders need to design a program aiming early DFW TB case detection and prevention of spread of TB to all susceptible hosts.

Key words: Dairy farm, *M. tuberculosis*, *Mycobacterium tuberculosis* complex, *M. bovis*, Tuberculosis.

1. INTRODUCTION

1.1 STATEMENT OF THE PROBLEM

Dairy farm workers are at the interface between the inter-transmission of human tuberculosis to cattle and zoonotic tuberculosis to humans. They have an occupational risk of acquiring tuberculosis of animal origin in addition to the already existing risk of acquiring tuberculosis from infectious fellow humans. Dairy farm workers experiencing contagious forms of active tuberculosis are themselves critical sources of infection to all susceptible hosts with whom they have a close contact, such as humans and cattle (Thoen and Steele, 1995).

Livestock production constitutes a very important component of the agricultural economy of developing countries. Some 62 % of the estimated 600 million poor livestock keepers live in Sub-Saharan Africa and South Asia. Ethiopia is one of the countries listed as having high density of poor livestock keepers in Sub-Saharan Africa (Thornton *et al*, 2003).

In countries that are highly affected by the HIV epidemic, the tuberculosis load had consequently increased over the past decades, making TB an important public health priority (Cosivi *et al.*, 1998).

Nearly 40 million people are living with HIV infection worldwide and as many as one-third are co-infected with TB. The dual epidemics of TB and HIV are particularly pervasive in Africa, where HIV has been the single most important factor contributing to the increasing incidence of TB over the last ten years (HIV/TB Coinfection: Basic Facts, 2007). This not only poses a risk for other humans but it could also result in cows being exposed to far higher levels of *M. tuberculosis* and other Mycobacteria.

Infection with *Mycobacterium tuberculosis* of animals living in close contact with humans has been reported (Fetene *et al*, 2011) in Ethiopia as well as from different parts of Africa.

The occurrence of human TB of animal origin caused by *M. bovis* is becoming increasingly worrying in third-world countries, given the frequent opportunities for transmission. In the Ethiopian situation, the proximity between farmers and their cattle, the customs of consuming raw milk and meat, the cultural use of insufficiently heated or sour milk and milk products and

the virtual absence of meat inspection services in slaughterhouses constitute a high risk of transmission to humans, especially to the agricultural community (Regassa *et al*, 2008; Ameni *et al*, 2002).

It is believed that for people who are frequently exposed to either livestock infected with bovine tuberculosis or infected products such as unpasteurized milk, their risk of contracting zoonotic tuberculosis increases considerably if there is a concomitant immune-suppression induced by HIV (Raviglione *et al.*, 1995). Human to human transmission of *M. bovis* has been reported in recent studies, hence making zoonotic TB a serious public health threat to persons at risk in settings where conditions favor zoonotic transmission to humans.

M. bovis is almost always resistant to pyrazinamide. As a result, tuberculosis due to *M bovis* needs a longer treatment duration compared to that of *M. tuberculosis* patients (LoBue and Moser, 2003). Generally, TB due to *M. bovis* has higher mortality rates during treatment compared to *M. tuberculosis*. Also, as with *M. tuberculosis*, multi-drug resistance is beginning to be detected in *M. bovis* strains, and this is a significant concern for HIV patients in developing countries that are still exposed to *M. bovis* through risky activities.

Hence, in a country such as Ethiopia, where there is a high human TB prevalence, documented zoonotic tuberculosis and high HIV burden, the epidemiology of tuberculosis in dairy farm workers needed to be studied.

1.2 LITERATURE REVIEW

Tuberculosis is a disease of poverty affecting mostly young adults in their most productive years, with an estimated global incidence rate of 137 cases per 100 000 population, according to the latest World Health Organization (WHO) report. In 2009 alone, 1.7 million people died from TB at a rate equal to 4,700 deaths a day. In the same year, there were 9.4 million new TB cases (including 3.3 million women and 1.1 million cases among people with HIV) (WHO, 2010).

Tuberculosis is caused by the genetically related members of the *Mycobacterium tuberculosis* complex (MTC) organisms. The two major pathogenic species in this complex are *M. tuberculosis* and *M. bovis*, the causative agents of TB in humans and cattle, respectively. However, non-tuberculous *mycobacteria*, which are widely distributed in nature have also been observed to be associated with disease in humans. *M. avium*, which is common in the environment, is regarded as an opportunistic infection to both cows and humans. *M. avium* is now considered to be a major opportunistic infection agent in immunocompromised humans (Fetene *et al.*, 2011; Coetzer *et al.*, 2005).

About 2 billion people (roughly one-third of the world population) are infected with *Mycobacterium tuberculosis* (Raviglione *et al.*, 1995). Infection with *M. tuberculosis* has also been sporadically reported in domestic and wild animal species, most frequently in animals living in prolonged, close contact with humans (Alfonso *et al.*, 2004). Among domestic animals, infection with *M. tuberculosis* has been most frequently identified in cattle of Ethiopia (Berg *et al.*, 2009), as well as from the neighboring Sudan (Boulahbal *et al.*, 1978). A slaughterhouse study from Ethiopia indicated that around 7% of isolates from TB infected cattle were *M. tuberculosis* (Berg *et al.*, 2009). Another study conducted among grazing cattle in central Ethiopia reported that approximately 27% of isolates were *M. tuberculosis* (Ameni *et al.*, 2010).

M. bovis, which affects a wide variety of animals including humans, is a zoonosis with highly significant socio-economic and public health impacts. Global prevalence of human TB due to *M. bovis* is estimated at 3.1% of all human TB cases, of which 2.1% are pulmonary infections and 9.4% extra pulmonary (Cosivi *et al.*, 1998). However, the proportion of *M. bovis* in Africa and within the TB–HIV complex is unknown.

A report published in 2006 indicated that *M bovis* is responsible for 0.5–7.2% of human tuberculosis cases in industrialized nations and is estimated to be responsible for 10–15% of new cases in the developing world (De la Rúa-Domenech *et al*, 2006).

Literature reports indicate that approximately 85% of the cattle and 82% of the human population of Africa are in areas where bovine TB is either partly controlled or not controlled at all. Pastoral and traditional milk harvesting is far more common than the commercial system in developing countries (Cosivi *et al*, 1998) thus increasing the risk of zoonotic tuberculosis to humans having the exposure.

Ethiopia is one of the countries in the world with high TB burden having an estimated prevalence rate of 480 cases per 100,000 populations in 2009 alone (WHO, 2010). Available reports show that the prevalence of bovine TB in Ethiopia ranges from 3.4% in smallholder dairy farms to 50% in intensive dairy production systems. A study conducted between 1999 and 2001 reported an overall individual animal prevalence of 46.8% and a herd prevalence of 91.7% among randomly selected dairy farms in Ethiopia (Ameni *et al*, 2001).

The overall prevalence of bovine TB, as judged by the number of skin test-positive cows, was 13.5% in dairy cattle reared in the central highlands of Ethiopia (Ameni and Erkihun, 2007; Berg *et al* 2009). Another study conducted in Southern Ethiopia reported a herd and individual animal prevalence of BTB as 48.7% and 11.6% respectively (Regassa *et al*, 2008).

Mycobacterium bovis as an etiology in humans from Ethiopia is reported mainly from extrapulmonary sites of TB infection. Kidane *et al*. (2002) reported *M. bovis* as a cause for 17.2% (6/35) of the cervical lymphadenitis cases from rural Ethiopia.

The pathogenic potential of non-tuberculous *mycobacteria* (NTM), as a cause of pulmonary and nonpulmonary infections, has been recognized since the beginning of the last century (Marks and Jenkins, 1971). The most frequent agents of non-tuberculous mycobacterioses belong to the *Mycobacterium avium* complex (MAC). Members of the *Mycobacterium avium* complex (MAC) are ubiquitous bacteria that can be found in water, food, and other environmental samples. They are considered opportunistic pathogens for numerous animal species, mainly birds and pigs, as well as for humans. In particular, *M. avium* subsp. *hominissuis* is a frequent agent of human mycobacterioses. Literatures indicate that *M. avium* subsp. *hominissuis* is also capable of

infecting a wide range of animal species including cattle, deer, wild boars, goats, and horses (Falkinham, 1996; Throel *et al*, 1997).

The etiologic role of non-tuberculous *mycobacteria* in Ethiopian cattle owners with tuberculosis and from milk of their cattle has been described by a number of studies (Fetene *et al*, 2011; Berg *et al*, 2009).

TB is transmitted primarily by inhalation of infectious droplets produced during coughing, sneezing, laughing or shouting, by persons with pulmonary or laryngeal disease. The main route of transmission between cattle is also through inhalation of infected aerosol droplets from another animal or dried secretions in dust (Rodgers *et al*, 2007). In fact, TB in cattle is principally a pulmonary disease, where only 1% of tuberculous cows excrete tubercle bacilli in their milk (Grange and Yates, 1994). However, it can be transmitted between cattle percutaneously, by oral ingestion, venereally and through the teat canal. The congenital route is also important and calves may be born with bovine tuberculosis. Humans with open tuberculosis caused by *M. bovis* can transmit it to cattle by the aerogenous route, spitting, coughing or urinating (Coetzer *et al*, 2005).

M. bovis is usually transmitted to humans via infected milk, although it can also spread via aerosol droplets. All tuberculin positive cattle are regarded as 'open' cases of TB potentially capable of transmitting infection to other animals and humans (O'Reilly and Daborn, 1995). Although, it is considered that human-to-human transmission of *M. bovis* is not of major importance in immunocompetent persons, Mandal *et al* (2011) have demonstrated the possibility of human to human transmission of *M. bovis* strains (Mandal *et al.*, 2011), thus making *M. bovis* infection a serious public health threat (Daborn and Grange, 1993).

Humans suffering from active TB are the most probable source of *M. tuberculosis* in animals, with infection spread via sputum, and rarely urine or faeces (Thoen and Steele, 1995).

A recent publication has identified a high risk practice in Central Ethiopia among farmers in the Selalle region of chewing ground, baked tobacco and discharging the juice directly into the oral cavity of cattle (Ameni *et al*, 2010). A study conducted in Western Gojam, Ethiopia, indicated that the individual and herd prevalence of BTB was higher in cattle owned by TB patients than in those owned by tuberculous free individuals (Fetene *et al*, 2011).

Unfortunately, the majority of Ethiopians living in rural parts of the country are not well aware of the risk of zoonotic TB. In a study carried out in central Ethiopia (Biffa *et al*, 2010), only 18.1% of the interviewed cattle owners knew that meat may be a vehicle for *M. bovis* transmission although 90% of the interviewees reported that they consumed both raw and cooked meat.

The potential zoonotic risk of *M. avium subsp. hominissuis* strains is supported by the isolation of identical genotypes in common between pigs and human patients. Moreover, these mycobacteria can be found in environmental sources such as water, biofilms, soil, aerosols and phagocytic protozoa and amoebae, all of which can act as common sources of infection for animals and humans (Falkinham, 1996; Thorel *et al*, 1997).

One of the well studied risk factors for mycobacterial disease is immunosuppression. TB is the major co infection in HIV patients. In 2009, an estimated 11–13% of incident cases of TB were HIV positive. In the same year, TB caused 0.38 million deaths among HIV positive people (WHO, 2010).

Other risk factors include chronic lung disease, silicosis, renal and cardiac transplantation and neoplasms. Patients with diabetes mellitus are at increased risk of contracting tuberculosis (Teo *et al*, 1992; Collins *et al*, 1989; Restrepo, 2007).

Consumers of unpasteurized milk and poorly heat-treated meat are at higher risk for zoonotic tuberculosis. A study in central Ethiopia indicated that cattle owners who consumed raw milk were at greater risk of having active tuberculosis than those who consumed boiled milk (Regassa *et al*, 2008). Ameni *et al*. (2002) showed 52.1% (49 of 94) household heads had the habit of raw milk consumption in Central Ethiopia (Ameni *et al*, 2002). In the same study it is described that less than half (38.3%; 36 of 94) of the respondents knew about BTB and only 30.8% (29/94) of the respondents were conscious of its transmission from cattle to humans.

Animal farm workers, veterinarians, abattoir workers and farmers have an occupational risk to zoonotic tuberculosis. The zoonotic transfer of *M. bovis* via unpasteurized milk and droplet inhalation from coughing cows is well known (Cosivi *et al*, 1998; Grange *et al*, 1994).

Rates of zoonotic diseases among various human populations are largely unknown owing to the lack of epidemiological data and to misdiagnoses. In particular, there is a notable lack of data on prevalence of tuberculosis among dairy farm workers in developing areas of the world such as Ethiopia.

Available reports in Ethiopia describe the strains of genus *Mycobacteria* that were isolated from humans in close contact with animals. A study by Fetene *et al* (2011) in Western Gojam areas of Ethiopia, report the isolation of *M. tuberculosis*, *M. bovis* and atypical mycobacteria from sputum and fine needle aspiration specimens of tuberculosis patient cattle owners.

A study conducted to characterize the *Mycobacteria* isolates from cervical lymphadenopathies found both MTC and NTM as etiologies in the pastoral area of Karamoja in Uganda. They documented that *M. avium*, *M. tuberculosis* and *M. bovis* caused cervical lymphadenitis in humans in pastoral communities in the Karamoja region of Uganda (Oloya *et al*, 2008).

Diagnosis of tuberculosis depends on the history, physical examination and radiographic evidence or the presence of acid fast bacilli (AFB) in acid fast smears (Powel and Hunt, 2006). Acid fast staining (AFS) is the main diagnostic means for developing countries, where the conventional method of Ziehl Neelsen staining of smears for detection of AFB has been frequently used for the diagnosis of tuberculosis (Truffot-Pernot *et al*, 2006). However, among patients with active pulmonary TB, only an estimated 50 to 60% of infections are detected by sputum microscopy. This technique has very limited sensitivity in patients with paucibacillary forms of pulmonary and extra-pulmonary tuberculosis (EPTB) (Dye *et al*, 2005). Chest radiography is often used to aid in the diagnosis of pulmonary tuberculosis when none or only one of the sputum smears examined is positive for AFB or to evaluate concomitant lung pathology in a patient with EPTB.

Culture remains the gold standard for the definitive diagnosis of tuberculosis as it allows drug susceptibility testing. In addition, isolates obtained from cultures can be used for mycobacterial species identification, detection of drug susceptibility and molecular epidemiology. It has a higher specificity and sensitivity than smear microscopy; however the slow growth of most pathogenic mycobacteria results in delays in definitive diagnosis. As few as 10 to 100 viable bacilli per ml may be detected, although the sensitivity of culture varies substantially depending

on the specimen-processing method, the culture medium used and the amount of bacilli present in the sample to be cultured. Culture on solid media, especially Löwenstein-Jensen and its modified version, is the most widely used technique. However, it has disadvantages owing to the length of time required (4 to 8 weeks) for growth of mycobacteria on a solid medium. This can affect treatment by either delaying (Schirm *et al*, 1995) it or causing inappropriate empiric treatment for subjects without mycobacterial infections or with atypical mycobacteria.

The species identification of members of the *Mycobacterium tuberculosis* complex is also critical to the timely initiation of both appropriate antibiotic therapy and proper public health control measures. In addition, the treatment strategy for tuberculosis and other mycobacterioses is different. The clinical presentation and radiological appearance of the pulmonary disease due to *M. bovis* may be like tuberculosis due to *M. tuberculosis* (Grange, 2001). Hence, isolation of these mycobacteria from representative specimens and their rapid identification is very important.

Different molecular tools based on nucleic acid amplification have been developed in recent days. Nucleic acid amplification constitutes a rapidly evolving improvement in the detection and identification of *M. tuberculosis*. Bacterial DNA (or ribosomal RNA transcribed into DNA) is enzymatically amplified and detected with an appropriate reading system via a signal generating probe. Several enzymatic amplification processes have been developed and introduced into commercial products (Parsons *et al*, 2002).

Tests based on nucleic acid amplification are usually highly specific for *M tuberculosis* (98 % to 100%), although some commercial products require a two-step diagnostic procedure (initial test for mycobacterial genus, followed by tests which differentiate *M tuberculosis* from non-tuberculous *mycobacteria*). Positive results can be obtained with less than 10 bacteria/ml; therefore sensitivity is much better than smear microscopy. The sensitivity is greater than 95% in smear positive cultures and 60% to 70% in smear negative cultures. Currently, nucleic acid tests are used primarily for confirmation of smear positive culture results or for primary case detection together with other methods (Toman and Frieden, 2004).

Polymerase chain reaction (PCR) is one of the most widely used nucleic acid amplification techniques. Deletion typing is a PCR based amplification technique used for differentiating

between MTC groups of organisms from other *Mycobacteria* species such as NTM. It utilizes the fact that the mycobacteria grouped in the *Mycobacterium tuberculosis* complex are characterized by 99.9% similarity at the nucleotide level and identical 16S rRNA sequences but differential hybridization arrays identified 14 regions of difference (RD1–14), as illustrated in Fig.1 (Brosch *et al*, 2002). It is a rapid and simple means of differentiating members of MTC based on differing deletion regions between strains within the same lineage (Parsons *et al*, 2002).

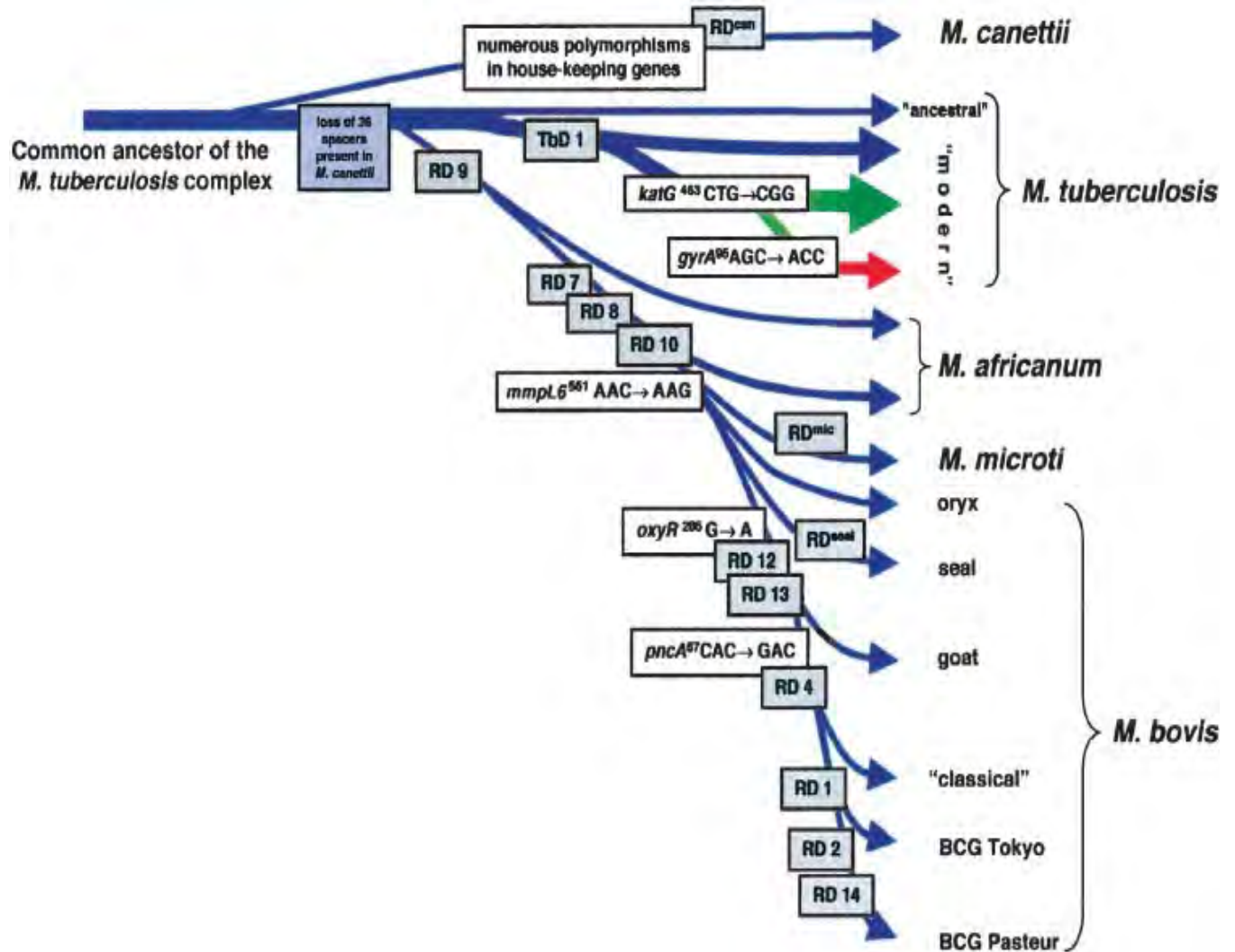


FIGURE 1. EVOLUTIONARY PATHWAY OF TUBERCLE BACILLI (BROSCH *ET AL*, 2002)

Spoligotyping is a new method for simultaneous detection and genotyping of *M. tuberculosis* complex. This method is based on PCR amplification of a highly polymorphic direct repeat locus in the *M. tuberculosis* genome. This technique targets a direct repeat (DR) region located in the chromosomes of members of MTBC (Driscoll, 2009).

Molecular typing methods have proved useful for the identification of different NTM. That is because some NTM isolates have special requirements, making growth on ordinary media such as Lowenstein-Jensen, Middlebrook and Dubos Broth/Agar difficult. These days, the amplification of 16 S rRNA followed by sequencing is being used for rapid identification of clinical isolates of NTM (Katoch, 2004).

1.3 SIGNIFICANCE OF THE STUDY

The high TB prevalence in both humans and livestock in the HIV era where there are many vulnerable groups is a special situation that calls for a baseline epidemiological study to be undertaken among DFWs in Ethiopia.

In the absence of effective formal bovine tuberculosis (BTB) control measures under the Ministry of Agriculture of Ethiopia, dairying is a common practice in livestock farms found in Addis Ababa and its suburban regions. Also, the fact that eating raw or undercooked meat is one way of contracting BTB has great implications for importance of BTB as a zoonotic disease in Ethiopia in particular, since raw meat and/or milk consumption is a local cultural habit.

Even though Ethiopia is one of the countries in Africa with a high prevalence of BTB in cattle, the amount of information about the genotypic characteristics of MTC strains infecting the occupationally at risk dairy farm worker communities is relatively limited.

However, data about the MTC strains circulating among cattle in and around Addis Ababa has been compiled by a cattle morbidity study conducted at Armauer Hansen Research Institute (AHRI) and the National Animal Health Diagnostic and Investigation Center (NAHDIC) from December 2008 and December 2011. This study aimed to determine prevalence of BTB from randomly selected dairy farms located in Addis Ababa and the 5 main suburban districts, namely, Holeta, Sululta, Sendafa, Debre Zeit and Sebeta. Suspect TB lesions from cattle were processed and cultured for molecular typing of AFB positive isolates. They reported an overall individual animal prevalence of 33 % and an overall herd prevalence of 57%. However, the results depended on the size farms that were studied whereby large farms were found to be more affected than small or medium farms (Firdessa et al, unpublished data).

The presence in Ethiopia of a large population of HIV infected people and these results of high BTB prevalence in and around Addis Ababa inspired the design of this current study. It was imperative to do the assessment of the TB status of humans working in these dairy farms because the data that would be generated could give an overview to scale of TB among humans occupationally exposed to individual cattle and herd affected by a high rate of zoonotic TB. Plus, molecular studies from the in-contact human workers with TB of these same dairy farms could

help make molecular epidemiological inferences, since the isolates of MTC infecting cattle in the farms from the above listed areas were characterized.

Therefore, in view of the global importance of TB and the zoonotic importance of MTC strains, the present study was conducted to assess the burden of tuberculosis amongst dairy farm workers, who were occupationally in close contact with livestock that were also susceptible to infection by organisms of *M. tuberculosis complex* or NTM.

1.4 HYPOTHESIS

- The prevalence of tuberculosis among dairy farm workers in Addis Ababa is not different from the national average.
- The most common *Mycobacterium* species causing tuberculosis in dairy farm workers in Addis Ababa and its suburbs is *M. bovis*.

2. OBJECTIVES

2.1 GENERAL OBJECTIVE

To assess the epidemiology of tuberculosis among dairy farm workers in Addis Ababa and its suburbs

2.2 SPECIFIC OBJECTIVES

- To determine the prevalence of tuberculosis among dairy farm workers in Addis Ababa and its suburbs
- To molecularly characterize the *Mycobacterium tuberculosis* complex isolates causing tuberculosis among dairy farm workers in Addis Ababa and its suburbs
- To identify associated factors for contracting tuberculosis among dairy farm workers in Addis Ababa and its suburbs

3. METHODS AND MATERIALS

3.1 STUDY DESIGN :

Cross-sectional

3.2 STUDY AREA AND PERIOD

This study was carried out in dairy farms that were located at Addis Ababa and its five main suburban regions, namely: Sendafa, Sululta, Debre Zeit, Sebeta, and Holeta. Addis Ababa is the capital city of Ethiopia, having a status of both a city and a state. It has a population of 2, 739, 551 (CSA, 2007). This city lies at the foot of mount Entoto, at an altitude of 2300 meters. Having a subtropical highland climate, Addis Ababa is located at 9°1'48"N 38°44'24"E. The other farms were located within a distance of 45 Km from the capital, i.e., Sululta-13.5Km, North; Sebeta-20Km, South west; Holota-25 Km, West; Sendafa-35.5Km, North East; and Debrezeit-45Km, South East. It was conducted from February 2010 to February 2011.

3.3 SOURCE POPULATION

The source populations were dairy farm workers employed at private and government owned commercial dairy farms in Addis Ababa and the 5 main suburban districts.

3.4 STUDY POPULATION

The study populations were dairy farm workers working in the dairy farms included in the cattle morbidity study.

3.5 SAMPLE SIZE

The sample size was calculated using the single proportion formula by taking assumptions of a proportion of 50% as there was no similar study conducted in the study areas before, 95% CI and 5% margin of error. The formula was:

$$n = \frac{z^2 \cdot p(1-p)}{D^2}$$

, where n= the sample size, z is the value from the normal curve at 95% confidence interval (1.96), P is the proportion of TB patients, and D (the margin of error) as 0.05.

Therefore, the calculated sample size was:

$$n = 1.96 \times (0.5) (0.5) / (0.05)^2$$

$$n = 384.$$

As the total number of dairy farm workers was assumed to be <10,000, the final sample size was calculated using reduction formula as follows:

$$Nf = 384 / (1 + 384/N), \text{ where } N = \text{estimated total dairy farm workers } (=900)$$

Thus, the total number of dairy farm workers that needed be assessed in this study was 269.

3.6 SAMPLE SELECTION PROCEDURE

The dairy farm selection process was conducted by the cattle morbidity study whereby a stratified random sampling technique was used to ultimately select 90 dairy farms representing the dairy farming practices in and around Addis Ababa. From each of the 6 study areas, 15 farms (5 small, 5 medium and 5 large farms) were included.

3.7 OPERATIONAL DEFINITIONS

Case of tuberculosis. A patient in whom TB has been bacteriologically confirmed or diagnosed by a clinician. **Note.** Any person given treatment for tuberculosis should be recorded as a case. Incomplete "trial" tuberculosis treatment should not be given as a method for diagnosis (WHO, 2003).

Definite case of tuberculosis. A patient with positive culture for the *Mycobacterium tuberculosis* complex. (In countries where culture is not routinely available, a patient with two sputum smears positive for acid-fast bacilli (AFB) is also considered a "definite" case) (WHO, 2003).

Medium sized farms. Dairy farms containing 11 to 50 cattle (Adopted from the definition of Firdessa *et al*, unpublished work, for consistency).

Large dairy farms. Dairy farms containing more than 50 cattle (Adopted from the definition of Firdessa *et al*, unpublished work, for consistency).

Small dairy farms. Dairy farms containing less or equal to 10 cattle per farm (Adopted from the definition of Firdessa *et al*, unpublished work, for consistency).

Tuberculosis suspect. Any person who presents with symptoms or signs suggestive of TB, in particular cough of long duration (more than 2 weeks) (WHO, 2003).

3.8 INCLUSION AND EXCLUSION CRITERIA

All dairy farm workers from the preselected dairy farms who were willing to sign on the written informed consent sheets were included in the TB screening stage of this study. Underage participants who could bring their parents/guardians for consent were also included.

Dairy farm workers with symptom complex of tuberculosis were selected to provide adequate amount of sputum samples for molecular analysis.

As patients from other dairy farms were excluded by the sample selection procedure, no exclusion criteria were applied and all voluntary participants working in the preselected dairy farms were considered eligible for this study.

3.9 VARIABLES

Dependent / Outcome variable:

- The occurrence of tuberculosis among dairy farm workers was the outcome variable in this study.
- Because of prior interest in symptoms that could indicate tuberculosis, the symptom complex of tuberculosis was also taken as an additional outcome variable.
- To see the role of different variables on the knowledge of dairy farm workers (DFWs) on the routes of transmission of tuberculosis, the latter is also taken as an outcome variable.

Independent /Exposure variables:

Explanatory variables such as age, sex, religion, educational status, duration of employment consumption of raw animal products and sleeping in the farm as part of professional obligation were used as independent variables.

3.10 DATA COLLECTION METHODS

QUESTIONNAIRE:

A structured and pretested questionnaire was used to collect the data. The questionnaire comprised of the questions to categorize the types of factors associated with tuberculosis:

- We assessed respiratory exposure from animal origin or grain dust indirectly by responses concerning the presence in the farm of PPD reactive cattle and whether the worker sleeps with the cattle in the barn on the job.
- We also assessed exposure via consumption of raw or undercooked livestock products such as milk and/or meat, to address the well established route of transmission of bovine tuberculosis from animal to human hosts.
- Knowledge of DFWs about the routes of transmission of tuberculosis, with a focus on BTB, was assessed. Participants were asked to list possible routes of TB transmission; those who were able to mention the two major routes of TB transmission, i.e., aerosol and gastrointestinal routes, were categorized as having knowledge about the transmission of TB.
- The presence in the same family of diagnosed tuberculosis patient: ‘yes’ response indicating a possible source of tuberculosis from a sick fellow human.
- Other factors known to affect the vulnerability to tuberculosis such as smoking, HIV, DM and the like were also assessed.

LABORATORY PROCEDURES (METHODS)

Individuals with chronic respiratory symptoms were subjected to meticulous physical examination, radiographic and laboratory investigations to identify possible tuberculous lesions.

Tuberculous suspect DFWs with chronic productive cough were instructed to produce and provide a minimum of 10 ml of sputum in to marked sterile sputum cups in triplets (i.e., spot, morning and spot) (WHO, 2003). All sampled specimens from respective patients were kept at 4°C in universal containers and transported on ice to the AHRI laboratory in Addis Ababa within 24 hours of collection.

SPUTUM DIGESTION AND DECONTAMINATION PROCEDURE

Sputum digestion and decontamination was carried out by the Sodium Hydroxide method, suspension according to the Mycobacteriology Culture SOP at AHRI.

First, an equal amount of 4% NaOH solution was added to the specimens in each labeled tube. The specimens were allowed to stand for 10 minutes with occasional vigorous shaking. After adding sterile distilled water, the tubes were centrifuged to separate the sediments from the supernatants. The supernatant was then poured off through a funnel into a waste container. Indicator phenol red (0.2%) was added to the remaining sediments. Finally, neutralization of the sediments was done with 2N HCl (Sterile) solution.

The pellets were re-suspended with sterile distilled water using a sterile disposable transfer pipette. A few drops (about 50 μ l) of processed sample were inoculated on Lowenstein-Jensen (L-J) media with and without pyruvate and incubated at 37°C for a period of up to 8 weeks.

Cultures were considered negative if no visible growth was detected after 8 weeks of incubation.

IDENTIFICATION OF MYCOBACTERIA

Preliminary identification of mycobacteria was done by Ziehl-Neelsen staining followed by inspection by light microscope, before culturing the specimen. Acid fast staining by the same method was also done for confirmation of the presence of AFB from the growths of culture positive specimens. AFB positive cultures were prepared as 20% glycerol stocks and stored at -80°C until species level identification of cultured AFB was done with molecular techniques.

MOLECULAR ANALYSIS

Molecular analysis was done at AHRI, Addis Ababa.

Heat-killed cells of each isolate were prepared by mixing 2 loopfuls of cells (~20 μ l cell pellet) in 500 μ l 1 X TE buffer followed by heating, where the tubes are submerged in water at 80°C for 1 hour, and then cooling to room temperature.

Heat-killed AFB positive samples were investigated by multiplex PCR for genus typing with the primers listed in Table 1 to ascertain whether the isolates were a member of genus *mycobacterium* or not and if so whether NTM or not. The primers used for deletion typing for further mycobacterial characterization are also listed in Table 1. The quality control samples for this multiplex PCR was included and interpretation of the unknown acid fast positive culture isolates was made according to presence or absence of the genus specific PCR product and the respective species-specific PCR product.

TABLE 1. OLIGONUCLEOTIDE PRIMERS USED FOR MOLECULAR TYPING OF ACID FAST POSITIVE CULTURE ISOLATES AND SIZES OF THE EXPECTED PCR PRODUCTS

Primer Name	Primer Sequence	Present	Interpretation
100 μ M MYCGEN-F	5'-AGA GTT TGA TCC TGG CTC AG-3'	1030bp	Genus Mycobacteria
100 μ M MYCGEN-R	5'-TGC ACA CAG GCC ACA AGG GA-3'		
100 μ M MYCAV-R	5'-ACC AGA AGA CAT GCG TCT TG-3'	180bp	NTM
100 μ M MYCINT-F	5'-CCT TTA GGC GCA TGT CTT TA-3'		
100 μ M TB1-F	5'-GAA CAA TCC GGA GTT GAC AA-3'	372	MTC
100 μ M TB1-R	5'-AGC ACG CTG TCA ATC ATG TA-3'		
RD9_ FlankF	5'-AAC ACG GTC ACG TTG TCG TG-3'		
RD9_ FlankR	5'-CAA ACC AGC AGC TGT CGT TG-3'		
RD9_ InternalR	5'-TTG CTT CCC CGG TTC GTC TG-3'		

3.11 DATA QUALITY MANAGEMENT

Five per cent of the questionnaires were pretested and subsequent changes made to modify the questionnaire. Equipment necessary for the processing and storage of samples coming from this study were identified and functionality monitored. The quality control of the LJ media was made

by streaking LJ tube-with *M. gordonae*, an overnight incubation at 37 ° C and observation for growth of the fast growing mycobacteria. Appropriate PCR controls were included in each run and band size verified after amplification.

3.12 DATA PROCESSING AND ANALYSIS

Data were double-entered, cleaned and verified using Microsoft Access 2007; then the data was exported to SPSS software version 16 for analysis.

First, we performed descriptive analysis for the age, sex, educational status, religion and the duration of employment. The mean and standard deviations for age and duration of symptom complex of tuberculosis was also calculated.

Tables and graphs were used to explore the data.

We assessed the relationship between the exposure variables and the outcome variables using binary logistic regression analysis; we computed the crude odds ratio (COR), 95% CI and $p < 0.05$. Also, we used multiple logistic regression to avoid confounders and to identify the predictors by calculating adjusted odds ratio (AOR), 95% CI and a significance level of $P < 0.05$.

3.13 ETHICAL CONSIDERATIONS

Ethics review and approval was obtained from AHRI, Addis Ababa Regional health bureau and DMIP at AAU. Data from Sululta and Sebeta areas was collected based on ethical clearance from AHRI/ALERT Institutional Review Board. Administrative support letter was obtained from the Oromia Regional health bureau, Addis Ababa, Ethiopia. Permissions to conduct the study in the dairy farms were obtained from the respective owners/managers of each of the dairy farms included in this study. Written informed consents were obtained from the dairy farm workers or guardians of child contacts before data collection. Treatment service was facilitated for patients diagnosed with tuberculosis in collaboration with the local health centers. Confidentiality was ensured.

4. RESULTS

In total, 256 dairy farm workers participated in this study. This gives a respondent rate of 95.2%. The proportion of participants from dairy farms in Addis Ababa 43 (16.8%), Sebeta 43 (16.8%) and Holota 48 (18.8%) areas was nearly similar. While the majority 67 (26.2%) came from Debre Zeit area, the number of participants from Sendafa and Sululta areas was 20 (7.9%) and 35 (13.7%) respectively as depicted in Fig.2.

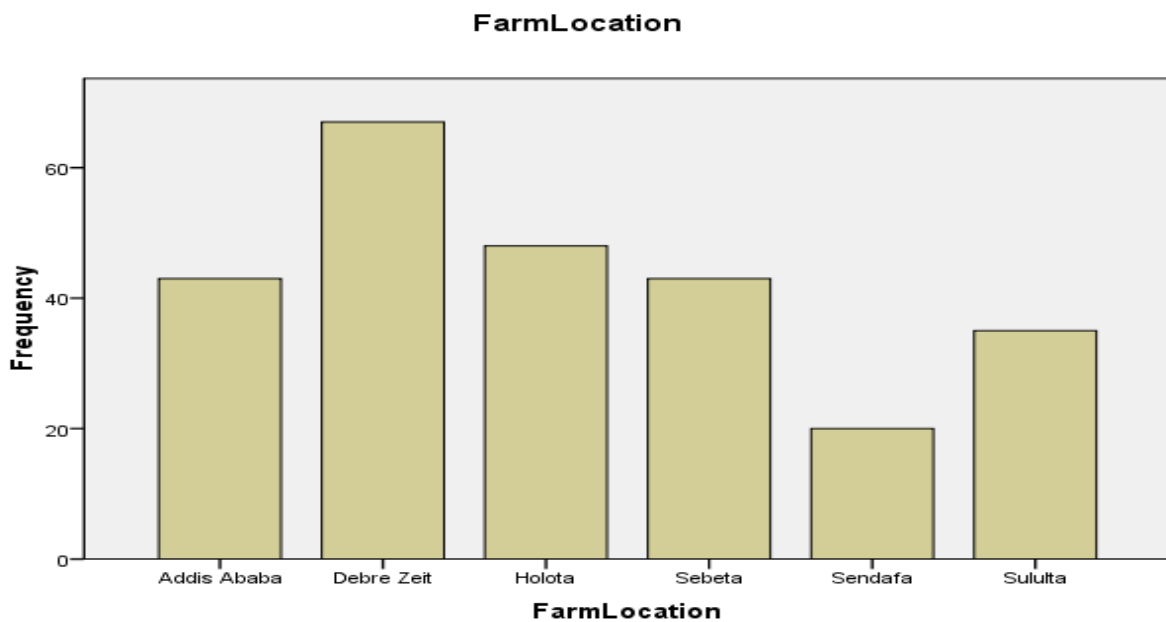


FIG. 2. NUMBER OF DFW PARTICIPANTS BY PLACE OF WORK IN ADDIS ABABA AND ITS SUBURBS, 2011

Out of the total 88 dairy farms which took part in the cattle morbidity study, DFWs working in only 48 dairy farms (54.5%) participated in this study. TB screening from the rest of the dairy farms proved impossible because the owners and/or managers of 31 dairy farms (35.3%) refused to let their employees participate, by denying the investigator direct access to the DFWs to ask individual consent for participation. The main reason provided to justify their uncooperativeness was their disappointment about the cattle TB results, which they had just received, and concern for their business as they feared human cases might be found amongst their employees. DFWs from 9 dairy farms (10.2%) were not included because of inconveniences to the PI, such as bad road condition, difficulty of contacting the owners to get permission and lengthy bureaucracy.

Among the participants, 198 (77.3%) worked in dairy farms harboring cattle that were PPD reactive. Majority of the DFWs 189 (73.8%) worked in large dairy farms, i.e., farms containing more than 50 cattle. Small (≤ 10 cattle per farm) and medium sized farms (having 11 to 50 cattle) contributed to 32 (13.5%) and 35 (12.5 %) of the participants.

4.1 Socio-demographic variables of participants

As depicted on table 2, the majority of DFWs who participated in this study were males (78.1%). Age ranged between 2 years and 90 years, with a mean of 30.4 years (\pm SD=13.1). Ethiopian Orthodox religion was exercised by 89.8% of the participants. The largest proportion of the dairy farm workers (38.7%) had primary education only.

In this study, 16 (6.2%) participants were owners of the dairy farms; out of the employees, 91.8% worked as animal attendants; veterinary professionals accounted for 1.2 % of the total (3/256).

4.2 Tuberculosis suspects

Amongst all screened dairy farm workers, 4.7 % (12/256) manifested chronic cough associated with sputum production and profuse night sweating, all lasting for more than 2 weeks. The average duration of these chronic symptoms was 25.5 weeks (\pm -SD=15.4 weeks). No participant complained of neck lump, discharging wounds or chronic skin lesions associated with symptom complex of tuberculosis.

On physical examination, lung findings such as decreased air entry, bronchial breath sounds and crepitations were observed in 2 out of 12 (16%) of the suspected TB patients. In the remaining patients manifesting symptom complex of tuberculosis, there was no abnormality detected on systematic physical examination.

4.3 DFW TB Cases

A total of 4 patients were identified as PTB cases at the end of clinical, radiology and bacteriologic workup. No case of extra-pulmonary TB was identified.

Cases 1 and 2: These two patients were diagnosed with PTB at local health facilities; they were on treatment already and had current TB treatment identification cards as a confirmation of ongoing treatment.

TABLE 2. SOCIO-DEMOGRAPHIC CHARACTERISTICS OF DFWs IN ADDIS ABABA AND ITS SUBURBS, 2011 (N=256)

Variable	n (%)
Age Categories	
<=24	84 (32.8 %)
25-34	100 (39.1%)
35-44	43 (16.8%)
>=45	29 (11.3%)
Sex	
Male	200 (78.1%)
Female	56 (21.9%)
Religion	
Orthodox Christian	230 (89.8%)
Muslim	7 (2.7%)
Protestant	18 (7%)
Other	1 (0.4%)
Educational status	
Illiterate	56 (21.9%)
Read and write only	16 (6.2%)
Primary school	99 (38.7%)
Junior & Secondary High school	68 (26.5%)
Higher education	15 (5.9%)
Relation to farm	
Owners	16 (6.2%)
Employees	226 (88.3%)
Household members	14 (5.5%)
Types of Job	
Animal attendants	235 (91.8%)
Veterinary professionals	3 (1.2%)
Supportive staff	18 (7%)
Categories of duration of Employment	
< 1year	85 (33.2%)
1 -3 years	49 (19.1%)
>3 years	122 (47.7%)

The diagnosis of the remaining two patients, i.e., cases 3 and 4, was made by the principal investigator.

Case 3: The WHO case definition (WHO, 2003) (ANNEX I) was used to come to the conclusion of TB diagnosis in this patient. The patient had characteristic clinical presentation consistent with TB and a suggestive chest X-ray which was commented by a radiologist. Two sets of 3 sputum specimens collected at a one month interval were negative for AFB on both occasions. With the culture results pending, the patient was given a 10 days long antibiotic treatment with

Erythromycin, adult dose. However, there was not any improvement of the symptoms and the signs after this treatment with broad spectrum antibiotic. Thus, based on the patient history, clinical examination findings, results of patient treatment with antibiotics, radiographic clues and the results of bacteriologic analyses, the consequent high index of clinical suspicion made the patient be categorized as a clinical case of tuberculosis.

Case 4: The results of sputum culture were used to make the TB diagnosis of the second patient classified as TB patient by the principal investigator. Even though the patient had manifested symptoms of TB, there was no pertinent physical finding. The patient's chest X-ray was not suggestive for TB per radiologist's comments.

4.4 Culture and microscopy

In this study, sputum was the only kind of specimen collected from TB suspects for bacteriological analysis by microscopy as well as by culture. A total of 11 out of 12 patients (91.7%) with chronic respiratory manifestations were willing to give sputum for tuberculosis work up. One participant out of the 12 TB suspects (8.4%) refused to give sputum for analysis.

Except for the patients who were already on treatment, all the other participants with suspected tuberculosis gave their sputum for the first time after the onset of their clinical condition; this was done as aid to patient diagnosis. Nevertheless, those who were already on treatment had given sputum samples to the local health facilities at the time of diagnosis, where only AFS was possible.

None of the sputum samples collected from the patients in this study was AFB positive after Ziehl Neelson staining and observation under light microscopy.

The culture result was positive for only one patient from Sebeta area, i.e., case 4. This only one culture positive result belonged to the second of the two patients diagnosed as TB by the principal investigator.

All the rest of the specimens from 10 patients, including those who were on anti-tuberculous chemotherapy were negative for mycobacterial growths. Thus culture result for the patient diagnosed according to the WHO algorithm, i.e., case 3, was also negative; the culture results for the remaining of the participants with chronic respiratory manifestations were also negative.

4.5 Molecular characterization

Genus typing of the unknown isolate from the acid fast positive culture gave a result consistent with the negative control samples. There was no any PCR product with a length of 1030 bp with the primers MYCGEN-F/MYCGEN-R. The positive controls for *M. avium*, *M. intracellulare*, *M. bovis* and *M. tuberculosis* gave the genus specific and the respective species specific product. There was no PCR product with the primers for RD9 testing by deletion typing either.

Therefore, at the end of the molecular work ups, the final number of TB cases identified in the study was 3. See Table 3.

TABLE 3. SUMMARY OF DEMOGRAPHIC AND DIAGNOSTIC FINDINGS FOR TB CASES AMONG DFWs IN ADDIS ABABA AND ITS SUBURBS, 2011 (N=4)

Parameter	Case 1	Case 2	Case 3	Case 4
<u>Age (years)</u>	22	38	42	48
<u>Sex</u>	Male	Male	Male	Male
<u>Profession</u>	Veterinary professional	Milk Salesman	Animal attendant	Animal attendant
<u>Location</u>	Holota	Holota	Sebeta	Sebeta
<u>Symptoms of TB</u>	Yes	Yes	Yes	Yes
<u>AFS before culture</u>	Positive ¹	Negative	Negative	Negative
<u>Culture</u>	Negative	Negative	Negative	Positive
<u>AFS after culture</u>	Not Applicable ²	Not applicable ²	Not applicable ²	Positive for AFB
<u>Chest X ray</u>	Not suggestive of TB	Suggestive of TB	Suggestive of TB	Not suggestive of TB
<u>Treatment status</u>	On treatment	On treatment	Refused treatment	Refused treatment
<u>Strain type</u>	Not Applicable ³	Not Applicable ³	Not Applicable ³	Not genus <i>mycobacteria</i>
<u>Final conclusion</u>	TB Case	TB Case	TB Case	Not a TB Case

¹done at a local health facility.

²Culture is negative and no AFS isolate identified.

³Culture is negative and no MTC isolated.

4.6 Measures taken after patient diagnosis

For the two patients diagnosed by the principal investigator, the result was communicated to them individually, soon after diagnosis was made based on clinical, radiologic and culture data. Counseling about the advantages of chemotherapy, nutrition and prevention of transmission to other close contacts discussed. Referral sheets were obtained from St Peter's hospital.

Despite these efforts, the patients refused treatment; they reasoned that sputum tests in private clinics of their preference were not suggestive of TB. Therefore, it was impossible to know the nature of treatment response for these two cases. Nevertheless, the first two cases who were on treatment already (i.e., cases 1 and 2) completed treatment and were declared free of TB on a follow up examination at the local health facilities.

Appropriate treatment was also given for all other TB suspects who did not qualify to be TB cases.

4.7 Prevalence of TB among DFW in AA and its suburbs

In this study, 3 patients out of 256 participants were finally diagnosed as tuberculosis patients, based on combined clinical, radiologic, culture and molecular biologic findings.

This makes the prevalence of tuberculosis among DFWs in Addis Ababa and its suburbs to be 1.172 % or 1172 per 100,000 populations.

4.8 Associated factor assessment

As part of their profession 41.4% of the animal attendants (106 out of 256), sleep in cattle barns in addition to working in the farms during the day time. As illustrated in Fig. 3, nearly 89 % of the DFWs who spent nights in barns on duty worked in large sized dairy farms. The odds of developing symptom complex of tuberculosis for dairy farm workers sleeping in cattle barns as part of their profession is found to be 0.7 (95% CI: 0.2-2.37, p=0.56).

From all of the participants, 35.9% (92 out of 256) confessed to consuming raw/undercooked dairy products and meat as part of their routine feeding habits. Those DFWs who consumed raw animal products were found to be about 4 times likely to develop the symptom complex of

tuberculosis compared with those who consumed cooked products (AOR= 3.8, 95 % CI: 1.08-13.29, P=0.03).

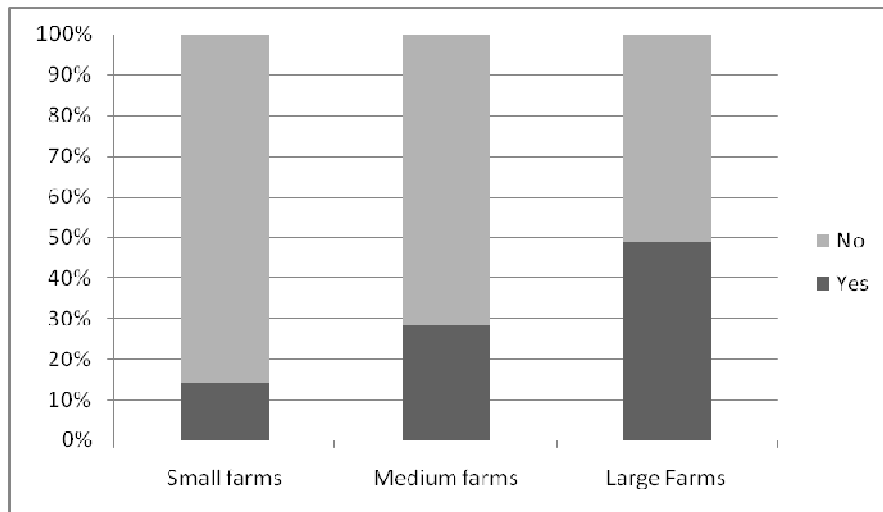


FIG. 3. PROPORTION OF PARTICIPANTS SPENDING NIGHTS IN DAIRY FARMS IN ADDIS ABABA AND ITS SUBURBS BY FARM SIZE, 2011

An assessment of the awareness of DFWs about the zoonotic BTB revealed that 62.9% of them (161 individuals out of 256) never heard of BTB before this study. Overall, 76.6% (196 out of 256) of the DFWs who participated in this study do not know how BTB is transmitted. Only 20.3% (52 out of 256) DFWs knew the routes of transmission of tuberculosis between humans and cattle.

DFWs who had knowledge about the routes of transmission of TB were found to be about 3.7 times more likely to manifest the symptom complex of tuberculosis compared with those who do not know the routes of transmission of TB (AOR=3.7, 95 % CI: 1.12 - 12.23, p=0.03).

The type of contact that DFWs had with the cattle and the duration of employment of the DFWs did not have a significant association to the occurrence of symptom complex of tuberculosis, as depicted on table 4.

The knowledge of the respondents about BTB transmission from cattle to humans and vice versa was assessed for any association with the gender, religion, educational background and the duration of employment of DFWs. See table 5.

Compared with the illiterate DFWs those who undertook higher education were found to be about 10 times more likely to have the knowledge about the routes of transmission of tuberculosis (AOR=9.6, 95 % CI 3.46-39.33, p=0.001).

TABLE 4. FACTORS ASSOCIATED WITH THE OCCURRENCE OF SYMPTOM COMPLEX OF TB AMONG DFWs IN ADDIS ABABA AND ITS SUBURBS, 2011 (N=256)

Variables	Symptom complex of TB		COR	p- value	95%CI	AOR	95% CI	p-value
	Yes	No						
Direct contact								
No	1	24	1.00	-	-			
Yes	11	220	1.20	0.86	0.15-9.70	-	-	-
Sleeping with cattle								
No	8	142	1.00	-				
Yes	4	102	0.70	0.56	0.20-2.37	-	-	-
Duration of employment								
< 1 year	3	82	1.00	-	-			
1-3 years	1	48	0.57	0.63	0.06-5.63	-	-	-
> 3 years	8	144	1.92	0.35	0.49-7.45			
Consuming raw products								
No	4	160	1.00	-	-			
Yes	8	84	3.8	0.03	1.12-13.02	3.79	1.08-13.29	0.03
Knowledge of TB transmission								
No	6	190	1.00	-	-			
Yes	6	46	4.13	0.01	1.27-13.40	3.70	1.12-12.23	0.03

Also, those DFWs aged 35 or above were found to be more likely to have knowledge about the routes of transmission of tuberculosis, compared to the age group ≤ 24 . Those aged ≥ 45 were found to be about 7 times more likely to know about the routes of transmission of TB than those aged ≤ 24 (AOR=7.2, 95% CI: 1.79-28.33, p=0.005).

In comparison between those consuming cooked animal products and those consumed raw/undercooked products, those who utilize cooked products were found to be about seven times more likely to have the knowledge about the routes of TB transmission of TB (AOR=6.5, 95% CI: 2.3-18.32, p=0.001).

TABLE 5. FACTORS ASSOCIATED WITH THE KNOWLEDGE OF DFWs ABOUT TRANSMISSION OF TB, AT ADDIS ABABA AND ITS SUBURB, 2011 (N=248)

Covariates	Knowledge about TB transmission		COR	p-value	95%CI	AOR	p-value	95%CI
	Yes	No						
Sex: female Male	7 45	44 152	1.00 1.86	0.16	0.78-4.42	-	-	-
Religion Orthodox Muslim Others	47 4 2	175 14 6	1.89 2.00 1.00	0.56 0.57 -	0.23-15.66 0.19-21.43 -	-	-	-
Education: Illiterate Primary Higher edu	6 12 34	66 81 49	1.00 1.38 7.88	- 0.56 0.001	1.00 0.47-4.09 2.56-19.6	1.00 1.89 9.6	- 0.89 0.001	- 0.59-6.01 3.46-39.33
Age: <=24 25-34 35-44 >=45	7 22 14 9	69 78 29 20	1.00 2.78 2.76 4.44	- 0.028 0.002 0.008	- 1.12-6.91 1.74-3.01 1.74-13.41	1.00 2.25 3.5 7.12	- 0.12 0.04 0.005	- 0.77-6.59 1.05-11.72 1.79-28.33
Consuming raw products: No Yes	30 22	133 63	1.00 1.55	- 0.17	- 0.83-2.90	1.00 1.55	- 0.25	- 0.73-3.30
Consuming cooked products: No Yes	5 7	81 115	1.00 6.62	1.00 0.001	1.00 2.52-17.38	1.00 6.50	- 0.001	- 2.30-18.32
Duration of employment < 1 year 1-3 years > 3 years	13 11 28	72 37 87	1.00 1.65 1.78	- 0.27 0.12	- 0.67-4.03 0.86-3.69	1.00 1.34 0.72	- 0.59 0.50	- 0.46-3.86 0.27-1.89

5. DISCUSSION

5.1 Prevalence of Tuberculosis among DFWs

In this study, the prevalence of tuberculosis among dairy farm workers in Ethiopia was described. In the majority of studies from Ethiopia, the general population was in focus. This special group of hosts was not the center of attention of many other TB studies.

The prevalence of tuberculosis among dairy farm workers was found to be about 1.172%. Compared to figures pertaining to the general population in Ethiopia, this prevalence of 1.172 % so identified in this study is about 2.4 times higher than the latest figures for prevalence of TB in Ethiopia, i.e., 0.48% as the best estimate of the prevalence of TB in 2009 (WHO, 2010).

To our knowledge, there is no literature that could be used for assessment on the consistency of this finding with that of other studies done on the same study subjects. However, some investigators believed that the prevalence of TB in dairy farm workers is expected to be higher than in the general population, as is true with other risk groups to TB (Fetene *et al*, 2011; Cosivi *et al*, 1998; Grange *et al*, 1994; Regassa *et al*, 2008).

One possible reason to explain the higher prevalence of TB among DFWs compared to national average is the high TB prevalence in the livestock of the study areas (Ameni and Erkihun, 2007; Berg *et al* 2009; Firdessa *et al*, unpublished data).

Another possible reason to the comparatively high TB prevalence of TB among DFWs in Addis Ababa and its suburban districts is the presence of a high number of DFWs who do not know how BTB is transmitted. We found out that the majority of DFWs do not know how TB is transmitted. It was only 20.3 % of the animal attendants in dairy farms included who knew how TB is transmitted. Ameni *et al* (2002) described that only 30.8 % of cattle rearing household heads were conscious of BTB transmission from cattle to humans. Under the circumstances where there are a lot of risky behaviors among the Ethiopian dairy farmers (Ameni *et al*, 2010), the investigators believe that this lack of knowledge about the disease might lead to the lack of implementation of self-protective measures and delayed diagnosis of TB cases thus favoring the inter-species transmission of TB between cattle and humans.

The absence of an effective BTB control program in Ethiopia (Cosivi *et al*, 1998) and in the study areas in particular could have an effect on the prevalence of TB. This is because a higher number of sources of infectious cattle could be allowed to continue living and breeding in the working environment of the DFWs. As a consequence, it is possible that these the dairy farm workers could have been exposed to the infectious agents to a greater extent and frequency than in the general population. Hence, the DFWs in and around Addis Ababa might have developed tuberculosis at a rate higher than the national average.

The defiant tendency of dairy farm owners and some employees towards the zoonotic importance of TB to DFWs so observed in this study showed their lack of initiative to control and prevent animal TB. Even though participation in this study was based on voluntariness for ethical reasons, nearly half of the dairy owners whose farms participated in the cattle morbidity study refused to let this study be conducted among their employees. The main reason forwarded to substantiate their uncooperativeness was their fear of possible discovery of TB patients among their employees with the consequent causal association to their livestock, as they were informed about the results of the cattle tests; some dairy farm workers withdrew from the study for fear of possible stigmatization if diagnosed as TB. Attempts to build the confidence of such apprehensive dairy farm owners and employees by discussion about the advantages of the study and confidentiality were not fruitful. Nevertheless, the tendency of dairy farm owners protecting their firms instead of public interest deters the grass root employees from being aware of the risks associated with working in areas with high BTB herd prevalence; consequently, universal precautionary measures the DFWs would not be exercised with the possibility of unparalleled exposure with the infected herd and individual animals. As one of the critical stakeholders, this negative attitude of dairy farm owners could have an indirect impact on the prevalence of TB partly, possibly contributing to the high figures identified in this study.

5.2 Factors associated with outcome variables

In this study, sleeping in the barn with cattle was assessed as an exposure variable, exercised by 41.4% of the DFW participants. However, this variable was not significantly associated with the occurrence of symptom complex of tuberculosis. This could be because the majority of DFWs identified to sleep in the barns were working in large dairy farms. Since large dairy farms are

usually built for profit purposes, the quality and design of these barns differ from those of small dairy farms. Thus, the wider space and the more ventilation found in such farms could explain the above finding because the role of natural ventilation for preventing the transmission of airborne infection of TB is well documented (Escombe *et al*, 2007).

In this study, we found less than half of the participants (35.9%) to have the habit of consuming raw dairy products. This figure is less than what was found from the observation among cattle rearing household in Wuchale Jida area of Central Ethiopia. Based on the results of the interview of such household heads, 52.1% of the respondents consumed raw dairy products (Ameni *et al*, 2002). The difference could be because the current study was conducted in Addis Ababa and its suburban areas, which are more civilized than Wuchale Jida area. Thus, the observed difference could be related to the possible modification of traditional feeding practices to modern less risky styles in urban and semi-urban areas compared to rural residence settings.

Those who consumed raw products were found to be about 4 times more likely to have symptom complex of TB than those who consumed cooked products (AOR=3.8, 95% CI: 1.08-13.29, p=0.03). This finding was consistent with the findings of another study in central Ethiopia which indicated that cattle owners who consumed raw milk were at greater risk of having active tuberculosis than those who consumed boiled milk (Regassa *et al*, 2008). This finding stresses the importance of the gastrointestinal route of TB transmission among DFWs with access to dairy products in and around Addis Ababa where a high BTB prevalence is documented.

Another independent variable found to be significantly associated with the occurrence of symptom complex of tuberculosis among DFWs was having the knowledge about the routes of transmission of tuberculosis. DFWs who had the knowledge about TB transmission were found to be about four times more likely to have symptom complex of TB than those who did not know the routes of TB transmission (AOR=3.7, 95%CI, 1.12-12.23, p=0.03).

This finding contradicts the logic favoring those having the knowledge about the routes of TB transmission from acquiring the infection. However, this situation needs to be seen from the stand point that multiple factors are involved in the transmission of tuberculosis. Knowledge can influence only some risky behaviors such as the tendency of consumption of cooked products instead of raw/undercooked products, whose role in BTB transmission is low (Grange and Yates,

1994). In concert with this idea, we found that those DFWs who utilize cooked animal products were about seven times more likely to have the knowledge about the routes of TB transmission of TB (AOR=6.5, 95% CI: 2.3-18.32, p=0.001).

The main route of transmission of tuberculosis is aerosol route (Rodgers *et al*, 2007), which is rather mainly influenced by the presence of external factors such as environmental sanitation programs, animal and human TB control programs, economic status of individuals and availability and accessibility of protective materials. Even if the individuals know how TB is transmitted between humans and cattle, or within each specific species, the financial constraints and scarcity of trained professionals might affect the use of protective materials such as masks, gloves and disinfectants. Thus, those DFWs with the knowledge about the routes of TB transmission but not exercising the appropriate protective measures due to inability to afford for the protective equipments could get infected and develop the infection following repeated exposures.

When treated as one outcome variable, we found that knowledge of the DFWs was significantly associated with the age and educational status of the participants. Compared to the age group ≤ 24 , those DFWs aged 35 or above were more likely to have knowledge about the routes of transmission of tuberculosis. This could be explained by the increased levels of career maturity and experience related to adulthood which influence individual observation and knowledge. Also, the knowledge of DFWs about the routes of transmission of TB was found to be influenced by the educational background of individual DFWs. Those who undertook higher education were found to be about 10 times more likely to have the knowledge about the routes of transmission of tuberculosis (AOR=9.6, 95 % CI 3.46-39.33, p=0.001) compared with the illiterate DFWs. This was expected because those who underwent higher levels of formal education would be introduced to TB and its routes of transmission through nutrition and health education facts from formal or informal media.

The above factors which were found to affect the knowledge of the DFWs about the routes of transmission of TB could also have affected the type of job and the intensity of exposure such more educated DFWs could have. Certain job descriptions such as working as certified or traditional veterinary professionals to attend sick or delivering cattle demand individuals to have more frequent exposures or fewer but more intensive exposures. These conditions may lead to

the increased exposure of the DFWs to the infectious agents and if the conditions were favorable, the DFWs might develop the symptom complex of tuberculosis. Thus knowing about the routes of TB transmission may not always be protective.

Assessing the statistical significance of each variable in this study to that of TB disease was not possible because of small number of TB patients identified in the study.

5.3 *Mycobacteria* Isolates from TB cases

The TB diagnostic procedures that were exercised in this study were as per the WHO guidelines (See Annex I). The diagnoses made at the local health centers were accepted to be correct because diagnosis in Ethiopian health facilities is made as per the national guideline which is generally similar to that of the WHO recommendations (WHO, 2003). The purpose of collection of sputum for the two known TB patients in this study was to follow up the response to treatment and identify any culturable organisms for molecular characterization. Thus, the lack of growth from samples of these patients could be attributed to the anti-tuberculous chemotherapy that they were taking, hence, indicating good response to treatment.

However, the absence of growth from case 3, a clinically diagnosed TB patient, was discrepant based on the expectations from the clinical and radiologic findings. However, this failure to get growth from samples from a clinically diagnosed TB patient could be explained in a number of ways.

One reason is related to the limited sensitivity of mycobacterial culture on LJ media. Thus, due to the possibility that not all patients with TB yield isolates on culture using LJ media. However, mycobacterial culture generally has a high sensitivity associated with low negative predictive value; hence, ruling out tuberculosis from this patient based on the negative culture result is difficult (Toman and Frieden, 2004).

Another microbiologic reason for the occurrence of culture negatives from tuberculous patients is the fact that not all species belonging to genus mycobacteria grow on LJ media. A study (Katoch, 2004) indicated that some NTM bacteria having special growth requirements do not grow on ordinary LJ media. Thus, it is possible that the patient had culture negative mycobacterial disease consistent with the clinical diagnosis.

Excess decontamination of the sputum leading to the killing of the mycobacteria and the possibility of unrepresentativeness of the sputa samples taken are also theoretically possible reasons for the culture negativity in this case; nevertheless, the fact that 3 sets of purulent sputa were collected and processed on two occasions at an interval of one month make them unlikely explanations for the culture negativity for Case 3.

The absence of growth in samples taken from these TB cases could imply that *M. bovis* was not probably the etiology to the illnesses so identified. That is because, had it been the case, the mycobacteria could have persisted despite the antituberculous treatment so administered due to the documented inherent resistance to pyrazinamide of *M. bovis* isolates (LoBue and Moser, 2003), thus recovery of the isolates could have been possible. The absence of any EPTB cases in this study was also taken as an indirect evidence to the possibility that *M. bovis* was not the etiology among DFWs with PTB in and around Addis Ababa; that is because *M. bovis* in humans is frequently associated with EPTB (Kidane *et al*, 2002; Cosivi *et al*, 1998). Therefore, the investigators infer that *M. bovis* was not the commonest cause of tuberculosis among dairy farm workers in and around Addis Ababa.

5.4 Molecular Work up

The application of strain-specific markers for differentiating *M. tuberculosis* strains is a useful tool for epidemiological studies of tuberculosis. At present the most extensively used method for differentiating among MTC strains at the Armauer Hansen Research Institute are deletion typing and spoligotyping. Genus typing has also been applied in settings where it is necessary to confirm isolates at the genus level. These techniques were applied in the detection of an MTC strains from PPD positive cattle in farms in and around Addis Ababa.

In this study, deletion typing and genus typing were applied to only a single culture positive sample, i.e., case 4. The absence of the genus specific PCR product with the MYCGENF/MYCGEN-R on two attempts by two different technicians had led to the conclusion that the organism did not belong to the genus *Mycobacterium*.

Although culture has a high specificity, indicating a high probability that the acid fast organism which was culture positive was *mycobacterium*, incidents where non-mycobacterial members of family Actinomycetales (such as *Corynebacteria*, *nocardia* species) growing on LJ media and

appearing acid fast on Ziehl Neelsen staining (e.g., *Rhodococcus equi* occurring as acid fast coccobacilli) are not rare (Halpern *et al*, 1994). Because of their growth on commonly used fungal media (e.g., Sabouraud dextrose agar) as well as some mycobacterial media (e.g., Lowenstein Jensen media), many nocardia samples may be misdirected to the mycology or mycobacteriology sections of clinical laboratory for identification (Text book of pediatric infectious diseases, vol 1). This situation is best explained by possible nocardiosis whose clinical presentation can mimic tuberculosis, carcinoma of the lungs or even fungal pneumonia.

Nucleic acid amplification tests have a specificity of 98% to 100% as well as a high sensitivity (Toman and Frieden, 2004). Thus, the probability that acid fast positive culture isolate does not belong to genus mycobacterium is very high, because such tests also have high negative predictive value.

Therefore, the investigators conclude that the culture positive isolate was not a member in the genus *mycobacterium*, even if the patient had chronic respiratory manifestations consistent with TB.

However, due to the absence of sequencing procedures for other organisms at AHRI, it was not possible to identify the exact nature of the non-mycobacterial isolate which appeared culture positive and acid fast positive.

Overall, the molecular section of this work could not give positive findings, unlike the parallel BTB study. Fetene *et al* (2011) and Oloya *et al* (2008) have reported the role of *M. bovis*, *M. tuberculosis* as well as NTM as etiologies in humans in close contact with livestock. The inconsistency of our findings with these studies could be due to the final small number of TB cases and the antiTB chemotherapy that we found from the study. Consequently, the molecular work up from this study could not provide a full picture of the MTC strains that cause TB among dairy farm workers in Addis Ababa and its suburban districts.

5.5 . Limitations of the study

- The small number of tuberculosis patients and culture positive isolates was a limitation, which made molecular characterization of the isolates and making statistical associations with all outcome variables impractical.
- Lack of sequencing techniques to identify the exact nature of the acid fast positive culture isolate from one of the patients was a limitation which made it difficult to ascertain the nature of the organism causing culture positive chronic respiratory condition.
- Refusal of some dairy farm workers to participate in the study was a limitation because it affected the response rate to be less than optimal. Screening and treatment of TB patients in dairy farms should be a regular activity as part of bovine TB control in humans and milk hygiene. However, since our study was based on voluntary informed consent of farm owners and individual farmers, we were not in a position to influence study participation.

7. CONCLUSIONS AND RECOMMENDATIONS

7.1 Conclusions

- The prevalence of tuberculosis among dairy farm workers in Addis Ababa and its suburban districts is more than two fold higher than the national average.
- *M. bovis* was not the most common cause of TB among DFWs in Addis Ababa and its suburbs.
- Consumption of raw animal products and having knowledge about the routes of transmission of TB are found to be significantly associated with the occurrence of symptom complex of tuberculosis.

7.2 Recommendations

To policy makers:

- The Ethiopian MOH, MOA and dairy farm owners need to design a national program that focuses on early identification, prevention and mitigation of hazards to protect workers
- Periodic tuberculosis screening to DFWs need to be demanded by law to ensure healthy dairy farm workers and to protect cattle herd as well as the public that consumes dairy products.
- Farmers and other occupationally at-risk individuals should be required to adopt appropriate measures to minimize exposure of employees and farm visitors to infections that can be transmitted to humans from animals and vice versa.
- Up-to-date molecular biological tools need to be part of the routine TB diagnosis program in Ethiopian hospital laboratories.
- Continued collaboration between animal and public health programs.
- Routine livestock surveillance should be established to identify possible hazards to the public

To Regional Health Bureaus:

- Education/training workers and dairy farm investors about the routes of transmission of TB, importance of timely diagnosis, prevention and control methods;
- Human exposure/contact investigations
- Latent infection treatment
- Raising the awareness of DFWs to avoid high exposure risk practices

Dairy Farm Workers

- Avoid high risk exposure practices
- Use of masks in the work place

Future Research:

- Large scale DFW studies need to be conducted.

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ANNEX: I (WHO, 2003)

TREATMENT OF TUBERCULOSIS: GUIDELINES FOR NATIONAL PROGRAMMES

CASE DEFINITION

Although culture is useful to diagnose TB, it is not as important as smear microscopy for TB control. Culture facilities are not universally available and the results take several weeks or months, which is too late to monitor progress. Smear-negative, culture-positive patients are less infectious and, except in immunodepressed individuals, have fewer bacilli. In general, the treatment regimens are the same for culture-positive and culture-negative patients.

The flow chart in Annex 1 shows the recommended diagnostic procedure for suspected pulmonary TB. The following definitions are used:

- Pulmonary tuberculosis, sputum smear-positive (PTB+)
 - a. two or more initial sputum smear examinations positive for AFB, or
 - b. one sputum smear examination positive for AFB plus radiographic abnormalities consistent with active PTB as determined by a clinician, or
 - c. one sputum smear positive for AFB plus sputum culture positive for *M. tuberculosis*.
- Pulmonary tuberculosis, sputum smear-negative (PTB-)

Case of PTB that does not meet the above definition for smear-positive TB. This group includes cases without smear result, which should be exceptional in adults but are relatively more frequent in children.

Note. In keeping with good clinical and public health practice, diagnostic criteria for PTB-should include:

- at least three sputum specimens negative for AFB, and
- radiographic abnormalities consistent with active PTB, and
- no response to a course of broad-spectrum TB antibiotics, and
- decision by a clinician to treat with a full course of antituberculosis chemotherapy.

Under programme conditions, when microscopy laboratory services are available and diagnostic criteria are properly applied, PTB smear-positive cases represent at least 65% of the total of PTB cases in adults, and 50% or more of all TB cases. Note that these proportions may be lower in high HIV-incidence populations.

It is apparent from the above definitions that in the absence of culture, standard chest radiography is necessary to document cases of smear-negative PTB. Fluoroscopy examination results are not acceptable as documented evidence of PTB.

ANNEX II: QUESTIONNAIRE

English version Questionnaire for assessing tuberculosis among dairy farm workers.

Questionnaire number _____ Date of interview _____

Interviewer name _____ Signature _____

Answer the questions in absolute number/text or mark the number of the correct option(s).

I. Description of the Farm

- Location: 1. AA 2. DZ 3. Holeta 4. Sebeta 5. Sendafa 6. Sululta
- Farm Identification code: _____
- Size: 1. Small (< or = 10 cattle) 2. Medium (11-50 cattle) 3. Large (>50 cattle)
- Farm type: 1. Intensive 2. Extensive
- Was tuberculin test done in your farm by the BTB project? 1. Yes 2. No
If yes, was there a reactive result to any one of the tested livestock?
1. Yes 2. No 3. I do not know

II. Farm worker or household member's description

- Case Identification code: _____
- Sex: 1. Female 2. Male
- Age (in years): _____
- Religion: 1. Orthodox 2. Catholic 4. Protestant 5. Muslim 6. Other
- Educational status:
1. Illiterate 2. Read and write only 3. Primary school
4. Junior secondary school 5. High school 6. Higher institutions 7. NA
- Relationship to farm: 1. Employee 2. Household member 3. Owner
- If household member, what is your relation to employee or owner?
1. Spouse 2. Child of owner or employee 3. Relative living with employee
4. House employee 5. Other (state _____)

III. Job and type of contact with cattle

- Do you have regular contact with livestock? 1. Yes 2. No
- Do you have a direct contact with cattle? 1. Yes 2. No
- What is your responsibility?
 - Animal attendant 1. Yes 2. No
 - Dairy farm worker 1. Yes 2. No
 - Butcher 1. Yes 2. No
 - Veterinary Professional 1. Yes 2. No
 - Other 1. Yes 2. No (If yes, state _____)
- Do you have an indirect contact with cattle? 1. Yes 2. No
- If yes, check all that apply
 - Sleep in the same house with cattle 1. Yes 2. No
 - Consumer of raw milk and/or meat 1. Yes 2. No
 - Consumer of cooked milk and/or meat 1. Yes 2. No
 - Other 1. Yes 2. No (If yes, state _____)
- For how long have you been involved in contact with cattle in this farm or elsewhere?
 - </= 1yr 2. 1yr-3yrs 3. >/=3 yr

- g. Have you heard about bovine tuberculosis before? 1. Yes 2. No 3. NA
- h. Do you know how BTB is transmitted? (Check 'yes' if the participant listed the two major routes of transmission of TB.) 1. Yes 2. No 3. NA

IV. Current status of the individual

1. Do you have any of the following symptoms for two weeks or longer?

Check all that apply and state duration of symptoms (actual weeks)

- | | | | |
|---------------------------|---------------------------------|--------------------------------|------------------------|
| Cough | 1. Yes <input type="checkbox"/> | 2. No <input type="checkbox"/> | If yes, duration _____ |
| Unexplained fever | 1. Yes <input type="checkbox"/> | 2. No <input type="checkbox"/> | If yes, duration _____ |
| Chest pain | 1. Yes <input type="checkbox"/> | 2. No <input type="checkbox"/> | If yes, duration _____ |
| Sputum production | 1. Yes <input type="checkbox"/> | 2. No <input type="checkbox"/> | If yes, duration _____ |
| Coughing up blood | 1. Yes <input type="checkbox"/> | 2. No <input type="checkbox"/> | If yes, duration _____ |
| Shortness of breath | 1. Yes <input type="checkbox"/> | 2. No <input type="checkbox"/> | If yes, duration _____ |
| Night sweats | 1. Yes <input type="checkbox"/> | 2. No <input type="checkbox"/> | If yes, duration _____ |
| Loss of appetite | 1. Yes <input type="checkbox"/> | 2. No <input type="checkbox"/> | If yes, duration _____ |
| Unexplained weight loss | 1. Yes <input type="checkbox"/> | 2. No <input type="checkbox"/> | If yes, duration _____ |
| Unexplained Fatigue | 1. Yes <input type="checkbox"/> | 2. No <input type="checkbox"/> | If yes, duration _____ |
| A lump on the neck/axilla | 1. Yes <input type="checkbox"/> | 2. No <input type="checkbox"/> | If yes, duration _____ |

2. Does the individual have symptom complex of tuberculosis? 1. Yes 2. No

3. If the person has any one of the above symptoms, do physical examination. Mark all that apply.

- | | | | |
|---------------------------|---------------------------------|--------------------------------|-------------------------|
| Cervical or axillary lump | 1. Yes <input type="checkbox"/> | 2. No <input type="checkbox"/> | If yes, describe: _____ |
| Lung findings | 1. Yes <input type="checkbox"/> | 2. No <input type="checkbox"/> | If yes, describe: _____ |
| Gibbus | 1. Yes <input type="checkbox"/> | 2. No <input type="checkbox"/> | If yes, describe: _____ |
| Chronic osteomyelitis | 1. Yes <input type="checkbox"/> | 2. No <input type="checkbox"/> | If yes, describe: _____ |
| Lupus vulgaris | 1. Yes <input type="checkbox"/> | 2. No <input type="checkbox"/> | If yes, describe: _____ |
| Others | 1. Yes <input type="checkbox"/> | 2. No <input type="checkbox"/> | |

(If yes, describe _____)

V. Assessment of risk factors to TB infection

1. Have you ever had contact with a person known to have active tuberculosis?

1. Yes 2. No

2. Have you lived with a diagnosed TB patient in your family during the last 2 years?

1. Yes 2. No

3. How often do you consume raw meat/milk?

1. 3 days or less per week 2. 4 or more days per week 3. Occasionally

4. Have you been diagnosed with a condition that may make you susceptible to TB (impair your immune system?) 1. Yes 2. No

If yes, please state: _____

5. Have you ever been treated for TB disease? 1. Yes 2. No

If yes, how long did you take the medication? _____

VI: Laboratory workup for DFW TB suspects in Addis Ababa and its suburbs

1. Patient code _____
2. AHRI code _____
3. Type of sample collected from the patient:
1. Sputum 2. FNA 3. Wound discharge 4 other (state-----)
4. Acid fast staining done 1. Yes 2. No
If yes,
Sample 1: Date Given: (d/m/y) ___ / ___ / ___ Result: 1. Positive 2. Negative
Sample 2: Date Given: (d/m/y) ___ / ___ / ___ Result: 1. Positive 2. Negative
Sample 3: Date Given: (d/m/y) ___ / ___ / ___ Result: 1. Positive 2. Negative
Interpretation: 1. Positive 2. Negative
5. Culture done 1. Yes 2. No
If yes,
Date culture done: (d/m/y) ___ / ___ / ___
Date of culture report (d/m/y) ___ / ___ / ___
Result: 1. Positive 2. Negative
6. Confirmatory AFS (from the colony) done 1. Yes 2. No
Result: 1. Positive 2. Negative
7. Sample stored for molecular characterization 1. Yes 2. No
8. Molecular work up from the growth
 - a. Genus typing 1. Yes 2. No
If yes, interpretation: MTC NTM
 - b. Deletion typing 1. Yes 2. No
If yes, the result of the deletion typing is:
 - i. RD 9 1. Present 2. Deleted 3. Negative
 - ii. RD 4 1. Present 2. Deleted 3. Negative
 - iii. Interpretation: *M. tuberculosis* *M. bovis* Other
 - c. Spoligotyping 1. Yes 2. No
If yes, spoligotype international type (SIT) _____
9. Comparison with cattle strains from the same farm done 1. Yes 2. No
If yes, the finding is
 - a. Similar MTC/NTM species as in cattle 1. Yes 2. No
 - b. Different MTC/NTM species from cattle 1. Yes 2. No
 - c. If similar MTC/NTM species are identified, were the spoligotype patterns compared?
1. Yes 2. No
 - d. If yes to the above question, were the spoligotype patterns of the DFW and cattle strains similar? 1. Yes 2. No
 - e. If the spoligotype patterns of the cattle and human strains is similar, was VNTR typing done? 1. Yes 2. No

f. If VNTR typing was done, please state the identified type: _____

Name of lab technician _____

Signature _____ Date of final report _____

VII. Supportive workups:

a. X-ray 1. Yes 2. No

If yes,

Type of X-ray _____ Date x-ray taken(d/m/y) ___/___/___

Result: 1. Suggestive of TB 2. Not suggestive of TB

b. Other investigations 1. Yes 2. No

i. WBC and differential 1. Yes 2. No

If yes,

Date done : (d/m/y) ___/___/___ Result: _____

ii. ESR 1. Yes 2. No

If yes,

Date done : (d/m/y) ___/___/___ Result: _____

VIII. Final conclusion

1. Does the individual have tuberculosis disease? 1. Yes 2. No

If yes,

a. What is the diagnosis? 1. PTB 2. EPTB 3. Disseminated TB 4. Other

b. What measures are taken following the diagnosis of disease?

1. Referred to the nearest health facility with the results 1. Yes 2. No

2. Sample labeled and stored. 1. Yes 2. No

3. Other measures taken 1. Yes 2. No

If yes, state _____

Remarks:

DECLARATION

I, the undersigned, declare that this MSc research thesis is my original work. It has not been for a degree in any other university. False statements cause the invalidation of this research thesis and may lead to other administrative or legal actions.

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Signature: _____

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