

## A High Prevalence of Tuberculosis among Dairy Farm Workers in Addis Ababa and its Surroundings

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### Abstract

**Background:** Dairy farm workers constitute the interface for inter-transmission of human tuberculosis to cattle and zoonotic tuberculosis to humans. Animal tuberculosis is endemic in Ethiopia, where human tuberculosis is also a large concern.

**Objective:** The main objective of the study was to determine the prevalence of tuberculosis and associated factors.

**Methods:** A cross-sectional study was conducted among dairy farm workers, between February 2010 to January 2011, in Addis Ababa and the surrounding districts. Individuals with symptom complex of tuberculosis were subjected to physical examination, radiographic and laboratory investigations. Sputum samples were collected and processed by culture on Lowenstein Jensen media. Data were entered, cleaned and explored using SPSS version 16. Logistic regression was fitted; odds ratios, 95% confidence interval and p-values were calculated to test associations between variables. Ethical clearance and written informed consents were obtained.

**Results:** Out of the total 256 voluntary participants, there were 12 tuberculosis suspects and 3 tuberculosis cases. The prevalence of tuberculosis was found to be 1.17%. Participants who consumed raw animal products were about 4 times more likely to manifest the symptom complex of tuberculosis than those consuming cooked products (Adjusted OR=3.8, 95% CI: 1.08-13.29). Only 20.3% of the participants knew the main routes of tuberculosis transmission. Those who had knowledge about the routes of transmission were 3.7 times more likely to have the symptom complex of tuberculosis compared to those who did not know the routes of transmission (Adjusted OR=3.7, 95% CI: 1.12-12.23).

**Conclusion and recommendation:** The prevalence of tuberculosis among dairy farm workers in Addis Ababa and surrounding areas is higher than the national average. Policy makers and stakeholders are encouraged to design a program aiming for early case detection and prevention of spread of tuberculosis to all susceptible hosts including animals.

**Keywords:** Dairy farm workers; Mycobacterium tuberculosis complex; Tuberculosis; Ethiopia

### Introduction

Tuberculosis (TB) is a disease of poverty affecting mostly young adults in their productive years [1]. Ethiopia is one of the countries in the world with high TB burden at an estimated prevalence rate of 480 cases per 100,000 populations in 2009 alone [1]. Livestock production constitutes an important component of the agricultural economy of Ethiopia, in spite of the fact that the country harbors a high animal prevalence of TB among cattle [2-4]. Ethiopia is also one of the countries in Sub Saharan Africa listed as having high density of poor livestock keepers [5].

TB is primarily transmitted by inhalation of infectious droplets produced from ill persons or cattle during coughing, sneezing, laughing or shouting [6,7]. Humans with contagious forms of active TB caused by *M. tuberculosis* or *M. bovis* can transmit the bacilli to all susceptible hosts with whom they have a close contact [8,9].

One of the well studied risk factors for mycobacterial disease (including zoonotic TB) is immunosuppression [10]. Animal farm workers, veterinarians, abattoir workers and farmers have an occupational risk to zoonotic TB [7,11]. Additional potential risk behaviors prevalent in Central Ethiopia include consumption of raw/unpasteurized milk [4] and mouth-to-mouth feeding of tobacco by

humans to cattle [12], where people feed cattle chewed-up tobacco leaves/juice directly from their mouth to the mouth of the cattle with close physical intimacy.

Unfortunately, the majority of Ethiopians living in rural parts of the country are not well aware of the risk of zoonotic TB [13,14]. Therefore, in view of the global importance of TB and the zoonotic importance of *M. tuberculosis* complex (MTBC) strains, the present study was conducted to assess the burden of TB amongst dairy farm workers (DFWs), who have professional obligation to get in close contact with livestock that are also susceptible to infection by organisms of the MTBC or non-tuberculous mycobacteria (NTM).

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## Methods and Materials

### Study area and period

This study was conducted between February 2010 and January 2011 in Addis Ababa and the five main surrounding districts, namely: Sendafa, Sululta, Debre Zeit, Sebeta, and Holeta.

### Design and source population

A cross-sectional study design was used. The source populations were DFWs employed at private and government owned commercial dairy farms in the study areas.

### Sample size and sampling procedure

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. As the total number of DFWs was assumed to be <10,000, a final sample size of 269 was calculated using the reduction formula. The participants of this study were voluntary owners and/or employees working in dairy farms included in a preceding cattle morbidity study [15]. All precautions were taken to prevent the introduction of any potential unintended bias for the present study.

### Data collection methods

A structured questionnaire was used to collect clinical and laboratory data. The questionnaire comprised of questions to assess possible routes of exposure to TB and factors that affected vulnerability to TB. In order to assess the knowledge of DFWs about the routes of transmission of TB, participants were asked to list possible routes of TB transmission; those who were able to mention the two major routes, i.e., aerosol and gastrointestinal routes, were categorized as having knowledge about the transmission of TB. TB suspects with productive cough were requested to provide sputum in triplets, i.e., spot, morning, spot [16] for work up.

### Laboratory procedures

Sputum digestion and decontamination was carried out by the sodium hydroxide method. In order to aid preliminary diagnosis, sputum analysis with Ziehl Neelsen staining and smear microscopy was done for all specimens before culture on LJ media. Acid fast staining by the same method was also done to confirm the presence of Acid-Fast Bacilli (AFB) from the growths of culture positive specimens. Species level identification of AFB positive cultures and confirmation of isolates was carried out by multiplex PCR for genus typing and deletion typing.

### Data quality management

Five percent of the questionnaires were pre-tested and functionality of equipments necessary for the processing and storage of samples was monitored. The quality control for the LJ media was implemented. Appropriate PCR controls were included in each run and band size verified after amplification.

### Data processing and analysis

Data were double-entered, cleaned and verified by Microsoft Access 2007. Analysis was done using SPSS software version 16, where the relationship between variables was assessed by binary logistic regression analysis and multiple logistic regressions; crude odds ratio (COR), adjusted odds ratio (AOR), 95% CI and p-values were calculated. P-value less than 0.05 was considered as the level of significance.

## Operational definitions

**Case of TB:** A patient in whom TB has been bacteriologically confirmed OR diagnosed by a clinician OR any person given treatment for TB [16].

**Small dairy farms:** Farms harboring less than 11 cattle per farm;

**Medium sized farms:** Farms harboring 11 to 50 cattle;

**Large dairy farms:** Farms harboring more than 50 cattle; (Adopted from the definition of Firdessa et al. [15]).

**Tuberculosis suspect:** Any person who presents with symptoms or signs suggestive of TB, in particular cough of long duration (more than 2 weeks) [16].

**Ethical considerations:** Ethical approval was secured from Armauer Hansen Research Institute. Written informed consents were obtained from the DFWs or guardians of underage participants. Confidentiality was ensured.

## Results

A total of 256 dairy farm workers, i.e., equivalent to 95% of the initially calculated sample size, participated in this study. The proportion of participants located in Addis Ababa, Sebeta and Holeta sites was nearly similar, with about 17% from each. However, the majority (26.2%) came from Debre Zeit area while participants from Sendafa and Sululta areas accounted for 7.9% and 13.7% of the study subjects, respectively.

The majority of DFWs in this study were males (78.1%). Mean age was 30.4 years ( $\pm$  SD=13.1). The largest proportion of the DFWs (38.7%) had primary education only.

Most of the DFWs, i.e., 189 (73.8%), worked in dairy farms of medium or large size and contributed to 32 (13.5%) and 35 (12.5%) of DFWs, respectively.

### TB suspects

Chronic cough associated with sputum production and profuse night sweating was observed in 12 (4.7%) of the study subjects. The average duration of these symptoms was 25.5 weeks (SD=15.4 weeks). On physical examination, lung findings (such as bronchial breath sounds or hyper-resonance to percussion) were observed in 2 out of 12 (16%) of the TB suspected patients. There was no abnormality detected for the remaining.

### Smear microscopy results

As no participant had neck lumps, discharging wounds or chronic skin lesions associated with symptoms of TB, no case of extra-pulmonary TB was identified. Sputum from TB-suspects was the only kind of specimen collected for analysis (Table 1). None of the sputum samples processed before culturing were positive for AFB after Ziehl Neelsen staining.

### Sputum culture results

Culture on LJ media was positive for one patient, although specimens from all TB suspects were cultured. Genus typing of the isolate from this acid fast positive culture gave a result consistent with the negative control samples. 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 by deletion typing.

Variable	Number (%)
<b>Age Categories</b>	
<=24	84 (32.8%)
25-34	100 (39.1%)
35-44	43 (16.8%)
>=45	29 (11.3%)
<b>Sex</b>	
Male	200 (78.1%)
Female	56 (21.9%)
<b>Religion</b>	
Orthodox Christian	230 (89.8%)
Muslim	7 (2.7%)
Protestant	18 (7%)
Other	1 (0.4%)
<b>Educational status</b>	
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%)
Not Applicable*	2 (0.8%)
<b>Relation to farm</b>	
Owners	16 (6.2%)
Employees	226 (88.3%)
Household members	14 (5.5%)
<b>Types of Job</b>	
Animal attendants	235 (91.8%)
Veterinary professionals	3 (1.2%)
Supportive staff	18 (7%)
<b>Categories of duration of Employment</b>	
<1 year	85 (33.2%)
1-3 years	49 (19.1%)
>3 years	122 (47.7%)

\*Educational status of two young children could not be assessed as they had not gone to school yet although they were working in the farms

**Table 1:** Socio-demographic characteristics of DFWs in Addis Ababa and its suburbs, 2011 (n=256).

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

**Table 2:** Summary of Demographic and Diagnostic findings for TB cases among DFWs in Addis Ababa and its suburbs, 2011 (n=3).

### TB cases

**Cases 1 and 2:** Two patients were on treatment already at the time of examination, as confirmed with their TB treatment identification cards, and hence considered as 'TB cases' based on the WHO TB case definition [16].

**Case 3:** This patient had characteristic clinical presentation consistent with TB and a suggestive chest X-ray; he was treated with a 10 days course of erythromycin to which he did not respond; two sets of 3 sputum specimens were collected from this patient and examined at a one month interval (before and at the end of antibiotic treatment), but AFB were negative on both occasions for Ziehl Neelsen staining and culture. This patient is considered as a 'TB case' in accordance with the WHO TB case definition [16].

### Prevalence of TB

At the end of the clinical, radiologic, culture and molecular investigations, the final number of TB cases identified in the study was 3 out of 256 participants. As a result, the prevalence of TB among DFWs in Addis Ababa and its surroundings was calculated to be 1.172% or 1,172 per 100,000 populations (Table 2).

### Factors associated with TB

As part of their job obligation, 106 of the animal attendants (41.4%) slept in cattle barns in shifts as guards despite working in the farms during the day time. Nearly 89% of the DFWs who spent nights in barns worked in large sized dairy farms.

Out of the participants, 92(35.9%) claimed to consume raw/undercooked dairy products and meat as part of their routine feeding habits. This group of DFWs was found to be about 4 times likely to develop the symptom complex of TB compared with those who had the habit of consuming cooked products (AOR=3.8, 95% CI: 1.08-13.29).

An assessment of the awareness of DFWs about the zoonotic TB revealed that 161 of them (62.9%) never heard of bovine TB (BTB) prior to this study. Overall, 196 (76.6%) of the participants did not know of the risk factors for contracting BTB. Only 52 (20.3%) knew the routes of transmission of TB 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 TB compared with those who do not know the routes of transmission of TB (AOR=3.7, 95% CI: 1.12-12.23), as depicted on Table 3. 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 TB (Table 3).

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

**Table 3:** Factors Associated with the Occurrence of Symptom complex of TB among DFWs in Addis Ababa and its suburbs, 2011 (n=256).

The knowledge of the respondents about BTB transmission from cattle to humans and vice versa was assessed for any association with the gender, religion and the duration of employment of DFWs, although no associations were noticed. Compared with the illiterate DFWs, those who attended higher education were found to be about 10 times more likely to have the knowledge about the routes of transmission of TB (AOR=9.6, 95% CI 3.46-39.33).

Also, DFWs aged 35 or above were found to be more likely to have knowledge about the routes of transmission of TB, 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).

In comparison with those who consumed raw/undercooked products, those who utilize cooked products were found to be about seven times more likely to have had knowledge about the routes of TB transmission of TB (AOR=6.5, 95% CI: 2.3-18.32) (Table 4).

## Discussion

In this study, the prevalence of TB among DFWs in Addis Ababa and surrounding areas was described; this is unlike other TB studies from Ethiopia where the general population was in focus instead. Compared to national figures, a TB prevalence of 1.172% result identified among DFWs was about 2.4 times higher than the figures for prevalence of TB reported for the general population in Ethiopia [1].

One reason to explain the higher prevalence of TB among DFWs in Addis Ababa and the surrounding districts is the high TB prevalence in the livestock in the study areas [3,14,15]. The finding that most of the participants did not know the routes of transmission of TB is also another factor which may have a role. Under the circumstances where there are risky behaviors among the DFWs [12], lack of knowledge about TB transmission might lead to the lack of implementation of self-protective measures and delayed observation of the TB disease, thus favoring the inter-species transmission of TB. Compared with the finding of Ameni et al. [17], where about 30.8% of their cattle harboring participants were conscious about BTB transmission from cattle to humans, the DFW participants in the relatively more urban and semi

urban settings from this study had lower knowledge about routes of TB transmission.

The absence of an effective BTB control program for cattle in Ethiopia [11] and in the study areas in particular could also have an effect on the prevalence of TB among DFWs reported from this study. 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, the DFWs could have been exposed to the infectious agents to a greater extent and/or to a higher frequency than the general population. Hence, the DFWs might have developed TB disease at a higher rate due to this as an additional factor.

Lower figures, for consumption of raw dairy products, were observed in this study compared to reports from rural areas in Central Ethiopia [17]. The observed difference could be related to the modification of traditional feeding habits to modern more hygienic and less risky practices in urban and semi-urban areas included in this study.

The finding indicating that higher likelihood of manifestation symptom complex of TB by those who consumed raw dairy products than those using cooked ones was consistent with the reports of another study from central Ethiopia [4]. This finding stresses the importance of the gastrointestinal route of TB transmission among DFWs, who have easy access to dairy products in high BTB prevalence settings [3,4,14].

An independent variable found to be significantly associated with the occurrence of symptom complex of TB was having the knowledge about the routes of transmission of TB. Our finding that DFWs who had the knowledge about TB transmission were more likely to have symptom complex of TB than those who did not have this knowledge was interesting. This is because knowledge about the routes of transmission of a given infectious agent is expected to be protective for the subjects can implement protective or control measures accordingly.

In the case of tuberculosis however, knowledge can influence only some of the factors in the transmission of TB, such as tendency of consumption of cooked products instead of raw/undercooked products, whose role in BTB transmission is low [7]. The main route of transmission of TB is the aerosol route [6], which is particularly influenced by the presence of external factors such as environmental sanitation programs, ventilation [18], animal and human TB control programs, economic status of individuals and availability and accessibility of protective materials. Thus, those DFWs with the knowledge about the routes of TB transmission but not exercising the appropriate protective measures due to financial constraints, for example, could get infected and develop the infection following repeated exposures. It seems clear from the previous statements that participant individuals are catching knowledge the hardest way through their experience with the disease itself and not through education and awareness. However, given the small number of positive cases from this study, which may affect the overall result, this association is perhaps an overestimation of the reality.

Increased levels of career maturity and experience related to adulthood could influence individual observation and knowledge. Also, passing through higher levels of formal education introduces individuals to formal or informal media teaching nutrition and health education facts. However, this may not be protective as certain job descriptions may demand individuals to have more frequent exposures OR fewer and more intensive exposures. Where the conditions favor, such DFWs might develop tuberculosis disease.

Variable	Knowledge about TB transmission		COR 95%CI	AOR (95%CI)
	Yes	No		
<b>Education:</b>				
Illiterate	6	66	1.00	1.00
Primary	12	81	1.38 (0.47-4.09)	1.89 (0.59-6.01)
Higher educ	34	49	7.88 (2.56-19.6)	<b>9.6 (3.46-39.33)</b>
<b>Age:</b>				
<=24	7	69	1.00	1.00
25-34	22	78	2.78 (1.12-6.91)	2.25 (0.77-6.59)
35-44	14	29	2.76 (1.74-3.01)	<b>3.5 (1.05-11.72)</b>
>=45	9	20	4.44 (1.74-13.41)	<b>7.12 (1.79-28.33)</b>
<b>Consuming raw products:</b>				
No	30	133	1.00	1.00
Yes	22	63	1.55 (0.83-2.90)	1.55 (0.73-3.30)
<b>Consuming cooked products:</b>				
No	5	81	1.00	1.00
Yes	7	115	6.62 (2.52-17.38)	<b>6.50 (2.30-18.32)</b>

**Table 4:** Factors associated with the knowledge of DFWs about transmission of TB, at Addis Ababa and its suburb, 2011 (n=256).

Anti-tuberculous chemotherapy that was being taken by some tuberculosis suspects and the limited sensitivity of mycobacterial culture on LJ media [19] can be explanations to the low culture positivity in this study. Excess decontamination of the sputa and under-representativeness of the sputa samples taken are also additional theoretically possible reasons.

In this study, deletion typing and genus typing were applied to the only culture positive sample. The absence of the genus specific PCR product with the MYCGENF/MYCGEN-R on two attempts by two different technicians 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* and *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 [20].

Because of their growth on commonly used fungal media (e.g., Sabouraud dextrose agar) as well as some mycobacterial media (e.g., LJ media), many nocardia samples may be misdirected to the mycology or mycobacteriology sections of clinical laboratory for identification.

Nucleic acid amplification tests have a specificity of 98% to 100% as well as a high sensitivity [19]. 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.

For these reasons, the investigators concluded that the culture positive isolate was not a member in the genus mycobacterium, even if the patient had chronic respiratory manifestations consistent with TB. The situation of this patient was best explained by possible nocardiosis whose clinical presentation can mimic TB. However, due to the difficulties of doing molecular techniques for identification of *Nocardia* species, it was not possible to correctly identify the pathogen.

In this study, the small number of TB patients and culture positive isolates was a limitation. This made molecular characterization of the isolates and making statistical associations with all outcome variables impractical.

We conclude that the prevalence of TB among DFWs in Addis Ababa and its suburban districts was higher than the national average. Hence, we recommend that the Ethiopian Ministries of Health and investors design a program targeting awareness creation, prevention, early identification and treatment of TB to protect workers. Periodic TB screening of DFWs needs to be demanded by law; farmers and other occupationally at risk individuals should also be required to adopt appropriate protective measures to minimize exposure to infectious agents. Further studies that improve the understanding of the interspecies transmission of TB and their role for TB disease among DFWs are recommended.

## Authors Contribution

MT wrote the proposal, participated in data collection, analyzed the data and drafted the paper. WM was involved in the processing of laboratory samples. TA and SG approved the proposal with some revisions, participated in the analysis. AA, LY and SB supervised data collection, processing and overall conduct of the study. All authors participated in the preparation of the manuscript and approved the final manuscript.

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