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# Community-based prevalence of undiagnosed mycobacterial diseases in the Afar Region, north-east Ethiopia

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#### ABSTRACT

Background: Information on the community-based prevalence of tuberculosis (TB) in different settings is vital for planning, execution and evaluation of strategies to control the disease. Objective: To assess community-based prevalence of undetected active pulmonary TB (PTB) in pastoralists of the Amibara District.

Methods: Between March and April 2010, a community-based cross-sectional survey of undiagnosed active PTB was conducted in the pastoralists of the Amibara District of the Afar Region, north-east Ethiopia. The study participants were interviewed for symptoms suggestive of PTB using a structured questionnaire. Sputum samples were collected and processed for smear microscopy and culture. Mycobacterium genus typing was performed using a multiplex polymerase chain reaction (PCR).

Results: Out of 222 individuals who had symptoms suggestive of PTB, 4 (1.8%) were found positive by smear microscopy, while mycobacterial growth was observed on 62 (27.9%) samples. Mycobacterium genus typing was carried out for 42 of these 62 samples; 39 (92.9%) gave a positive signal for the genus Mycobacterium. Of these, 23 (59%) isolates proved to be members of the Mycobacterium tuberculosis (Mtb) complex, while the remaining 16 (41.0%) were found to be members of non-tuberculous Mycobacteria (NTM) species.

Conclusion: Sputum culture is highly sensitive, and it is the gold standard for the bacteriological diagnosis of PTB, while smear microscopy is less sensitive to detect acid fast bacilli (AFB) in stained sputum smears. The findings of the present study warrant the strengthening of culture facility services in the study area. The study also provides important preliminary information on the status of NTM infection in the pastoral setting. Nevertheless, further investigations into the species identification of the NTM infections would be useful in the study area.

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# Introduction

Although TB is known to be an ancient human disease, it has come to the forefront as one of the major causes of morbidity and mortality in the sub-Saharan African and Southeast Asian countries since the mid-1980s mainly owing to the Human Immunodeficiency Virus (HIV) pandemic [1]. In 1993, the World Health Organization (WHO) declared TB to be a global

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health emergency that would claim over 3 million lives during the next decade unless immediate action was taken to control its spread. Consequently, directly observed treatment short course (DOTS) has been the internationally recommended strategy to combat TB globally [1]. Although the DOTS strategy is crucial for the control of TB, many community-based surveys of active PTB have revealed the existence of considerable numbers of undiagnosed individuals who would contribute to the transmission of the disease [2–7] indicating that the DOTS strategy alone will not be sufficient to achieve the targeted reduction in the global incidence rate by 2015 unless it is supported by active case detection.

Moreover, in most developing countries, the DOTS strategy uses smear microscopy for case detection, which is less sensitive than the culture method. Hence, a considerable number of PTB cases who can transmit the disease remain undiagnosed and untreated [6,8]. Similarly, diagnostic and treatment delay due to patient-associated reasons [9–12], especially a lack of knowledge of the early symptoms of TB (coughing), unless accompanied by other severe symptoms (like chest pain or hemoptysis) [13], could have a significant impact on the success of the TB control strategies.

Numerous studies in both developed and developing countries have shown that infection with NTM causes significant health problems in both HIV-infected and non-infected individuals [14–23]. However, in developing countries, where there are limited resources and laboratory facilities for the identification of NTM, infection with NTM can be misdiagnosed as infection with Mtb complex [22,24].

Ethiopia is among the 22 countries in the world with a high TB burden [25]. TB is one of the major public health problems among the Afar pastoralists [26]. However, the prevalence of undiagnosed active TB in the Afar pastoral communities is unknown. In this study, the community-based prevalence of undiagnosed active PTB and NTM infections in pastoralists in the Amibara District, Afar Region, was assessed using a questionnaire, sputum smear microscopy, sputum culture and Mycobacterium genus typing.

### Materials and methods

#### Study area and study population

A household-based cross-sectional survey of undiagnosed active PTB was conducted in the Amibara district, north-east Ethiopia, between March and April 2010. The region has a total population of 1,411,092, with an estimated area of 96,707 square kilometers [27]. The Amibara District is located in the Middle Awash valley, about 260 km to the north-east of Addis Ababa. The district has 18 kebeles (small administrative units), of which 4 are semi-towns, while the rest are pastoral kebeles. Detailed information on the study areas and the study population has been described elsewhere [28].

The aim was to include all 14 pastoral kebeles of the district in this survey. However, one kebele, which has a total population of 2331 (of whom 1231 are over 15 years of age), was not included because of financial constraints; hence, 13 kebeles with a total population of 34,428, of whom 18,192 (52.84%) were ≥15 years of age were included in this study. The excluded kebele is situated on the main road to Djibouti,

and there is a small town in that kebele of which about half of the residents are not pastoralists.

#### Data collection

A house-to-house survey of all households in the 13 kebeles of the district was conducted by trained community health workers under the supervision of the principal investigator. Although clinical symptoms are not the ultimate methods for screening for PTB in TB endemic settings [6,29] standardized questionnaires were used for screening symptoms suggestive of PTB [30].

After the aim of the survey had been explained, the heads of the households (husband or wife) or individuals aged over 18 years were asked if there was any family member (age ≥15 years) who had suffered from coughing for at least two weeks, expectoration, haemoptysis, chest pain, night sweats, breathlessness, loss of appetite/weight or fever. Individuals who had coughed for at least two weeks, with or without other symptoms, were interviewed using the structured questionnaire for common signs/symptoms of PTB, onset of illness, treatment sought, past history of TB, and contact with PTB patients. The respondent was also asked whether he/ she suspected his/her illness was TB. In the case of children younger than 18 years, the head of the household or other household members were interviewed. Information on socio-demographic characteristics of the individuals was also included in the questionnaire.

#### Sputum collection and examination

Following the interview, each individual suspected of PTB was instructed on how to expectorate a sputum sample. A coded sputum cup was then provided, and the individual was requested to submit the first on-the-spot sputum sample. After providing the first sample, the individual was given a new sputum cup and instructed to provide an early morning sputum sample. A third sputum sample was collected on the second day, when the individual submitted the early morning sputum sample.

Each sputum sample was transported to the main district health center (Melka Werer Health Centre) on the day of collection. At the health center, a direct smear was prepared from each specimen, processed using the Ziehl-Neelsen (ZN) staining technique, and microscopically examined for acid-fast bacillus (AFB) by two experienced technicians. A minimum of 100 oil immersion fields were examined before a negative result was declared. Positivity was confirmed when at least two out of the three smear results were positive for AFB [31].

The remainder of each specimen was stored at 4  $^{\circ}$ C pending transport to the Aklilu Lemma Institute of Pathobiology (ALIPB) TB laboratory under refrigeration. Upon reaching the laboratory, specimens from the same individual were pooled together, processed for culture as previously described [32] and incubated for eight weeks at 37  $^{\circ}$ C. Cultures were followed weekly for the growth of mycobacterial colonies, and positivity for AFB was confirmed by microscopy. The AFB positive colonies were collected, heat-killed in a water bath at

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