Journal of Epidemiology and Global Health xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Journal of Epidemiology and Global Health

journal homepage: www.elsevier.com/locate/jegh

Prevalence of zoonotic tuberculosis and associated risk factors in Central Indian populations

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ARTICLE INFO

Article history:
Received 15 March 2017
Received in revised form 8 August 2017
Accepted 29 August 2017
Available online xxxx

Keywords:
Duplex PCR
Mycobacterium bovis
Tuberculosis
Zoonosis

ABSTRACT

In the present study, we aimed to estimate the occurrence of bovine tuberculosis (TB) and examine the determinants of distribution of the disease in three high-risk populations of Central India. A prospective cohort study was conducted in Central India between March 2014 and June 2015. Based on the requisite inclusion criteria, we recruited a total of 301 participants whose blood samples were subjected to polymerase chain reaction-based detection and differentiation of *Mycobacterium bovis* and *Mycobacterium tuberculosis*. *M. bovis* was detected in 11.4%, 8.9%, and 12.6% of the recruited participants belonging to three distinct population groups (Groups A, B, and C, respectively). The highest proportion of cases infected with *M. bovis* was observed in Group C, who lived in the high TB endemic region. Previous contact with active TB cases (odds ratio = 3.7; 95% confidence interval, 0.9612–14.4533) and raw milk consumption (odds ratio = 5.3472; 95% confidence interval, 1.9590–14.5956) were found to be important determinants of bovine TB in this population. The high incidence rates of bovine TB in the Central Indian populations indicate the substantial consequences of this disease for some population groups and settings. However, more research is necessary to identify the main transmission drivers in these areas.

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1. Introduction

Tuberculosis (TB) remains the foremost leading cause of death worldwide, affecting more than 9 million people every year. Although *Mycobacterium tuberculosis* is the most common cause of human TB, unknown proportions of TB cases are considered to be attributable to *Mycobacterium bovis* infection, which is also termed bovine TB [1]. The infection currently poses a major concern in human populations in developing countries, as humans and animals share the same microenvironment. It has been estimated that zoonotic transmission of *M. bovis* is responsible for 10–15% of new human TB cases in developing countries [2]. The disease in humans primarily occurs through close contact with infected cattle or consumption of improperly cooked beef and drinking of unpasteurized milk and milk products [3,4]. People in specific occupations such as veterinarians, farmers, and abattoir workers are considered to be more at risk [5].

The prevalence of bovine TB infection and associated risk factors have been insufficiently characterized in the Indian population that is considerably dependent on agriculture for its livelihood. It has been suggested that poverty, poor dietary habits, close physical contact between humans and animals, and inadequate disease control measures facilitate the transmission of zoonoses [6]; however, there is no substantial evidence in the Indian context to justify the hypothesis.

Currently available tests used for identification of *M. bovis* are based on bacterial isolation and biochemical tests, which are both time-consuming with low diagnostic accuracy [7]. Furthermore, TB caused by *M. tuberculosis* in humans is clinically and radiologically identical to TB caused by *M. bovis* [8]. These problems were overcome by molecular techniques to some extent; however, this technique could not identify the mycobacterial pathogens to the

Abbreviations: TB, Tuberculosis; M. *bovis*, *Mycobacterium bovis*; MTB, Mycobacterium tuberculosis; MTBC, Mycobacterium tuberculosis complex species; RD, Region of difference; PCR, Polymerase chain reaction; AFB, Acid fast bacilli; CIIMS, Central India Institute of Medical Sciences; PBS, Phosphate buffered saline; EDTA, Ethylenediaminetetraacetic acid; CTAB, Cetyl-trimethyl-ammonium bromide; BCG, Bacille Calmette Guerin; ESAT-6, 6 kDa Early Secretory Antigenic Target.

Peer review under responsibility of Ministry of Health, Saudi Arabia.

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http://dx.doi.org/10.1016/j.jegh.2017.08.007
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Please cite this article in press as: Bapat PR et al. Prevalence of zoonotic tuberculosis and associated risk factors in Central Indian populations. J Epidemiol Global Health (2017), http://dx.doi.org/10.1016/j.jegh.2017.08.007

species level. In the present study, with the purpose of detecting zoonotic transmission of *M. bovis* and simultaneous differentiation of members of *M. tuberculosis* complex species (MTBC), we targeted the regions of difference (RDs) through a duplex polymerase chain reaction (PCR) assay.

As there are limited reports on the occurrence of bovine TB in India, we aimed to estimate its incidence among participants belonging to three distinct population groups and settings based on assessment of risk factors and occupational exposure to animals. The participants for this study were recruited from Achalpur, Amravati, Pilkapar, and Nagpur, located in the Central Indian region of Maharashtra, India. A comparative analytical study was then carried out to evaluate the determining factors that may influence the distribution of the disease in each population.

2. Material and methods

2.1. Ethics statement

The study was approved by the Ethical Committee of Central India Institute of Medical Sciences, Nagpur. All clinical investigations were conducted according to the principles expressed in the declaration of Helsinki 1975, as revised in 1983. Written consent forms were obtained from all recruited participants after they were given a detailed oral explanation of the study.

2.2. Study design and participants

A total of 433 participants were enrolled from three different populations within Central India region through camps organized between March 2014 and June 2015. These population groups were approached with the help of a local health care practitioner. A survey using a standardized questionnaire investigating exposure to TB cases; consumption of meat, unpasteurized milk, and milk products; as well as other sociodemographic parameters such as age, sex, occupation, duration of exposure to animals, and type of animal reared along with clinical history was conducted (Table S1). Active TB was investigated in individuals with respiratory or systemic symptoms by acid fast bacilli (AFB) smear and culture of appropriate sputum samples and chest X-ray. The details of the recruited populations are described in the following subsections.

2.2.1. Group A: Farmers, dairy workers, and livestock keepers

Participants in this group included farmers from Achalpur and Pilkapar villages of the state of Maharashtra in Central India. Most of the population had agriculture as their major occupation. Some of them were also involved in dairy production and livestock keeping. The farmers or members of their family were personally involved in the maintenance of these animals. The animals reared included Indian breed of ox, cows, buffaloes, and calves. Milking and delivery of pregnant animals were also done by the members of the household.

2.2.2. Group B: Zookeepers and animal handlers

This population included zookeepers and veterinarians from a specific locality within the Nagpur district. The participants from this group were involved in activities such as guarding, cleaning, feeding, and maintenance of animals in the zoo. The veterinarians recruited in this group were involved in routine health checkup, semen analysis, treatment, and *post mortem* of deceased animals.

2.2.3. Group C: Residents of high TB endemic area

This population belonged to a specific locality of Nagpur district, in the Vidarbha region of Maharashtra. The majority of the population routinely included meat and other animal products in their diet. The endemic area had high crowding index with an average of six to eight individuals living in small poorly ventilated rooms. The majority of the population had poor socioeconomic status and living conditions characterized by lack of sanitation and poor hygiene. Some households also participated in cattle and goat rearing.

Of the 433 participants enrolled in this study, 84 participants were excluded based on their refusal to give blood. The remaining 349 participants who matched the inclusion criteria were selected for the study. Among these, pregnant women ($n = 8$), children below the age of 10 years ($n = 18$), and individuals with fungal or viral infections ($n = 22$) were also excluded from the study. Fig. 1 represents the inclusion/exclusion criteria adopted for recruitment of the study populations.

2.3. Sample collection

For DNA isolation, 2.5 mL blood was collected in 5-mL sterile syringe BD and dispensed in a sterile vacutainer with coagulant EDTA). Each sample was labeled with a code that corresponded to the study location and identification of the individual.

2.4. DNA isolation and quantification

DNA was extracted from blood samples using the phenol chloroform extraction method described by Deshpande et al. [9], in which 6 mL phosphate buffered saline (PBS) was added to 2 mL blood and mixed thoroughly. Next, 8 mL of blood + PBS was added slowly from the sides of the tube to 4 mL Histopaque and centrifuged at 2000 rpm for 10 min. The buffy coat was transferred to another tube, and an equal volume of PBS was added and the tube was centrifuged again at 2000 rpm for 10 min. The supernatant was discarded and the pellet was suspended in 500 μL PBS, 15 μL 10% SDS, and 3 μL proteinase K (20 mg/ml), then mixed and incubated at 55 °C for 1 and ½ hour. After incubation, 100 μL of 5 M NaCl and 80 μL of high-salt cetyl-trimethyl-ammonium bromide (CTAB) (containing 4 M NaCl, 1.8% CTAB) was added and mixed followed by incubation at 65 °C for 10 min. An approximately equal volume (350 μL) of phenol and of chloroform/isoamyl alcohol (24:1) was added, mixed thoroughly, and centrifuged for 10 min in a microcentrifuge at 12,000 rpm. The aqueous viscous supernatant was carefully decanted and transferred to a new tube. An equal volume of phenol/chloroform-isoamyl alcohol (1:1) was added, followed by a 10-min spin at 12,000 rpm. The aqueous layer was separated and then mixed with 30 μl of 3 M sodium acetate and 0.6 vol of isopropanol to obtain a precipitate. The precipitated nucleic acids were washed with 70% ethanol, dried and resuspended in 30 μL Tris–EDTA (TE) buffer, and then stored at −20 °C prior to use. DNA concentrations for all samples and strains used in this study were determined with the Quant-iT dsDNA HS assay kit using a Qubit fluorometer (Invitrogen).

2.5. Duplex PCR

For determination to the species level of the mycobacterial pathogens—namely, *M. tuberculosis*, *M. bovis*, and *M. bovis* bacille Calmette–Guerin (BCG)—two genetic regions RD4 and RD1 were amplified using a duplex approach. Primers used in this study are shown in Table 1. RD4 is an RD in the bovine lineage. The use of RD4 flanking primers ensured that the PCR products were formed only if the deletion was present [10]. The genes in the RD1 region belong to the *esat6* gene cluster. Early secretory antigenic target-6 (ESAT-6) is a potent stimulator of the immune system, and is an antigen recognized during the early stages of infection. The RD1 region of *M. tuberculosis* is considered to be

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