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Review Article

Mycobacterium bovis induced human tuberculosis in India: Current status, challenges & opportunities

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Tuberculosis (TB) caused by *Mycobacterium tuberculosis* is a leading cause of human deaths due to any infectious disease worldwide. However, infection of *Mycobacterium bovis*, primarily an animal pathogen, also leads to the development of 'human tuberculosis'. Infected animals have been considered the major source of *M. bovis* infection and humans get exposed to *M. bovis* through close contact with infected animals or consumption of contaminated milk, unpasteurized dairy products and improperly cooked contaminated meat. The information on the global distribution of bovine TB (bTB) is limited, but the disease has been reported from all the livestock-producing middle- and low-income countries of the world. In recent years, there is a renewed interest for the control of bTB to minimize human infection worldwide. In India, while the sporadic presence of *M. bovis* has been reported in domestic animals, animal-derived food products and human beings from different geographical regions of the country, the information on the national prevalence of bTB and transmission dynamics of zoonotic TB is, however, not available. The present article reviewed published information on the status of *M. bovis*-induced zoonotic TB to highlight the key challenges and opportunities for intervention to minimize the risk of *M. bovis* infection in humans and secure optimum animal productivity in India.

Key words Bovine tuberculosis - cattle - human - *Mycobacterium bovis* - *Mycobacterium tuberculosis* - zoonotic tuberculosis

Tuberculosis (TB) continues to be an important disease both for humans and animals since the origin of human civilization. Evidence of TB exists in 3000 year old Egyptian mummies and about 17,000 year old fossilized bison[1-3]. TB affects both humans and animals (domestic and wild ruminants), and *Mycobacterium tuberculosis* is the primary cause of human TB. *M. tuberculosis* survives under extreme adverse conditions in the host[4], infects nearly one-third human population[5] and is responsible for about 1.6 million deaths per year (including 0.3 million deaths in HIV-positive patients)[6]. *Mycobacterium bovis* has 99.5 per cent genomic similarity with *M. tuberculosis*[7], and the proportion of human TB cases caused by this bacillus in the world still remains to be defined. Although *M. bovis* is primarily of bovine origin, the bacilli can infect almost all the species of mammals including domestic livestock reared for food production[8,9]. In most of the species, *M. bovis* causes a slow debilitation of health, although it may remain asymptomatic for many years in infected animals[10].

The diagnosis of *M. bovis* infection in animals [bovine TB (bTB)] is difficult and there is a general belief that none of the single diagnostic tests can detect all infected animals in every stage of *M. bovis* infection[11,12]. The accurate diagnosis of bTB mainly relies on the clinical manifestation of disease, tuberculin skin test (TST) and isolation of *M. bovis* on artificial medium[13,14]. TST is the most widely used method for the diagnosis, but this assay has the limitations of sensitivity and specificity and requires cautious administration and expertise in the interpretation of results. The issues associated with TST were minimized by interferon-gamma release assays, but high cost and difficulties in test standardization[15] may limit its scope in middle- and lower-income countries including India. Currently, the microbiological culture of *M. bovis* on pyruvate-containing media is considered as the "gold standard test" for diagnosis of bTB. However, it also has certain limitations including a long turnaround time to get the result and lower sensitivity[16]. The use of molecular techniques to detect *M. bovis* provides a rapid alternative to culture. A number of polymerase chain reaction (PCR)-based methods have been developed and proposed for the rapid diagnosis of bTB in animals[17]. Shah *et al*[18] developed a multiplex PCR assay for the single-step detection and differentiation of *M. bovis* and *M. tuberculosis*. Other molecular assays, especially PCR-RFLP targeting 16S-23S rRNA, the insertion sequences *IS6110* and *IS1081* as well as genes coding for proteins specific for *M. tuberculosis* complex such as MPB70, MPB64 and *hsp65* have been developed and used for the diagnosis of bTB in animals[14,17,19-23]. Molecular typing techniques, such as spoligotyping and pulsed-field gel electrophoresis, have also been applied directly on clinical samples for rapid diagnosis of *M. bovis* in animals[24,25]. Molecular typing assays have the advantages of simultaneous detection of disease and transmission dynamics of infection in animal herds[25-27]. However, the use of molecular typing tools on paucibacillary samples has limitations regarding the sensitivity of the test[12,25].

Transmission of *M. bovis* from animals to humans (zoonosis) constitutes a major public health concern worldwide[13,28]. Infection primarily occurs through close contact with infected animals or by consumption of contaminated food of animal origin (*viz*., unpasteurized milk and undercooked meat)[29]. Although the exact underlying mechanism of *M. bovis* infection in humans is not well understood, it is believed that the alimentary route of infection leads to extra-pulmonary TB[30], whereas the aerosol route of *M. bovis* infection during close contact with animals results in pulmonary disease in humans[31]. Macrophages are among the first cells to encounter invading mycobacteria, and the fate of the disease mainly depends on the response of these macrophages to the incoming threat[32]. It has been established that *M. bovis* can alter the antimicrobial pathways of the macrophage and cause disease by replicating inside the host cells[33-36]. Recently, Mendum *et al*[37] identified a few novel genes of *M. bovis* BCG which are responsible for the specific interaction of the bacilli with the host's immune system for long-term survival in the host. *M. bovis* causes zoonotic bTB which is indistinguishable from TB caused by *M. tuberculosis* based on clinical and radiological features[38]. In developed countries, cases of zoonotic TB are uncommon. This may be due to the implementation of bTB control programmes and practices of pasteurization of raw milk used for making dairy products. Therefore, majority of cases of zoonotic bTB have been frequently reported from resource-limited developing countries where pasteurization is often inadequately done or control programmes are not in place[20,28].

Historically, bTB has been considered to be a rare disease in India[39]. However, in recent years, bTB has been frequently reported from domestic livestock species located in different parts of the country[40-49]. A recent meta-analysis revealed a moderate (7.3%) prevalence of bTB in domestic livestock in India[49]. Several researchers have reported the animal breed variation in resistance to bTB in animals[48,50]. In India, Das *et al*[48] reported a significantly higher prevalence of bTB in exotic crossbreed animals (P<0.001) as compared to indigenous cattle in West Bengal, India. The presence of *M. bovis* has also been reported in samples from the environment (soil and water) and animal-derived milk in India[43,51,52]. In view of the inadequate and sporadic control measures, cases of zoonotic transmission have been frequently reported in India[52,53]. The human population continues to be at risk of exposure to *M. bovis* through contact and food products. The present review is based on published information and an attempt to discuss the status of *M. bovis* infection in the human population and highlights key challenges in minimizing cases of *M. bovis*-derived human TB in India.

Status of *Mycobacterium bovis*-derived zoonotic tuberculosis (TB)

Infection of *M. bovis* in humans results in the disease identical to TB caused by *M. tuberculosis*. It has been estimated that *M. bovis* accounts only for one per cent of all cases of human TB in developed countries as compared to 10 per cent in the developing world[54]. The predominance of *M. bovis* infection has been reported in cases of extra-pulmonary TB as compared to pulmonary TB. As per the estimation of the World Health Organization (WHO)[6], about 1,47,000 people fell ill and around 12,500 people died due to zoonotic TB in 2016, mostly from African and South-East Asian countries. Cases of *M. bovis*-derived human TB are usually underreported due to clinical, radiographical and histopathological similarities between the cases of human TB caused by *M. tuberculosis* and *M. bovis*[55].

India has the highest number of TB patients in the world. The cases of *M. bovis*-induced human TB in the country were documented in the early years of the last century[14,56]. However, the disease has not received much attention and the country lacks data on the national prevalence of *M. bovis*-derived human TB. This may be due to the difficulties in differential diagnosis and the limited availability of laboratory infrastructure to isolate and differentiate *M. bovis* strains from *M. tuberculosis*. Species identification is important in guiding treatment, as the members of *M. tuberculosis* complex have important differences in response to anti-TB treatment[57-59]. The failure of distinction of *M. bovis* from *M. tuberculosis* complex can have fatal consequences in the management of TB patients[60].

In recent years, sporadic studies have reported variable prevalence of *M. bovis* infection in suspected TB cases from different geographical regions of India (Table). Recently, Bapat *et al*[65], using duplex PCR, studied the prevalence of *M. bovis* in three groups of high-risk human population from Central India – group 1: farmers, dairy workers and livestock keepers; group 2: zookeepers and animal handlers and group 3: residents of high-TB burden endemic area. They reported a higher prevalence (12.6%) in blood samples of participants belonging to group 3, followed by group 1 (11.4%) and group 2 (8.9%). The study also reported consumption of raw milk and previous contact with active cases of TB as important determinants for zoonotic TB in the human population residing in the area. Jain[61] screened 300 patients of extra-pulmonary TB (tubercular meningitis, tubercular ascites and tubercular lymphadenitis) using PCR targeting *hupB* gene and reported 85.7, 9.5 and 4.7 per cent of PCR samples positive for *hupB* gene for *M. tuberculosis* and *M. bovis* and co-infection of both, respectively. Kohli *et al*[62] screened 100 infertile women for tuberculous endometritis using PCR targeting *hupB* gene and reported 13 per cent as positive for extra-pulmonary TB. Of the 13 PCR-positive cases, 38.4 per cent (5/13) were positive for *M. tuberculosis*, 23.07 per cent (3/13) for *M. bovis* and 38.4 per cent (5/13) showed co-infection with both the *Mycobacterium* species[62].

Most of the data available on the presence of *M. bovis*-induced TB in India are based on observational studies conducted on a small number of suspected TB patients. Therefore, these studies may not be truly indicative of the general population and may under report the burden of *M.bovis*-induced TB in the country[65]. These studies indicate that *M. bovis*-induced cases of human TB may account for about 10-15 per cent of all the cases of extra-pulmonary TB in India deserving, therefore, more attention of public health officials in the country. Real-time prevalence frequency and clinical manifestations of *M. bovis*-induced TB and cases of co-infection with *M. tuberculosis* or non-tuberculous mycobacteria need to be investigated on priority for better management and planning for control of TB in the human population of the country.

Key challenges for effective management of zoonotic tuberculosis (TB)

The most formidable challenges for the control of zoonotic TB in India are as follows:

Limitations of routine diagnostic tests and limited laboratory infrastructure for the diagnosis of zoonotic tuberculosis (TB): The most commonly used laboratory tests for the diagnosis of TB do not differentiate *M. tuberculosis* from *M. bovis* and may potentially overlook zoonotic TB cases. Accurate diagnosis of *M. bovis*-induced zoonotic TB is complex and requires isolation and characterization of the bacilli from clinical samples. For this purpose, an equipped laboratory infrastructure is needed and currently, the country has 80 Revised National Tuberculosis Control Programme [renamed as National Tuberculosis Elimination Programme (NTEP)] certified culture and DST laboratories for the diagnosis of TB[66]. India has a huge number of TB patients (around 2.74 million), accounting for about 27 per cent of newly diagnosed TB cases in the world[6]. The NTEP mainly focuses on early detection and initiation of treatment with an

Table. Major reports on *Mycobacterium bovis* infection in human beings in India

Area of study	Study group	Number of patients/ samples	Sample	Test used	Number of positive, n (%)			Reference
					M. bovis	*M. tuberculosis*	Dual infection	
Gujarat	Persons working either in livestock farm or attending the OPD	64	Sputum	Culture (LJ medium with and without glycerol and/or sodium pyruvate)	0	6 (9.38)	-	Parmar *et al*[52]
		24	Throat swab	Do	0	1 (4.17)		
		62	Nose swab	Do	0	6 (9.68)		
New Delhi	Patients suspected for extra-pulmonary TB	192	Tissue biopsies	Nested PCR targeting *hupB* gene (*Rv2986c*) gene	8 (4.2)	49 (25.5)	14 (7.3)	Prasad *et al*[53]
		139	Body fluids	Do	26 (18.7)	3 (2.2)	44 (31.6)	
New Delhi	Anti-TB treatment responders, patients of tubercular meningitis, tubercular ascites and tubercular lymphadenitis	147	CSF, ascitic fluid and lymph node fine-needle aspirate	PCR targeting *hupB* gene	14 (9.52)	126 (85.71)	7 (4.76)	Jain[61]
New Delhi	Infertile women (positive by *hupB* PCR)	13	Endometrial samples	Nested PCR targeting *hupB* gene	3 (23.07)	5 (38.4)	5 (38.4)	Kohli *et al*[62]
New Delhi	Suspected patients of tubercular meningitis	112	CSF from adults	Multiplex PCR	19 (17.0)	3 (2.7)	15 (13.4)	Shah *et al*[63]
		100	CSF from children	Do	17 (17.0)	3 (3.0)	7 (7.0)	
Maharashtra	Farmers, dairy workers and livestock keepers	105	Blood	Duplex PCR targeting RD region	12 (11.4)	13 (12.4)	-	Bapat *et al*[64]
	Zookeepers and veterinarians	45	Blood	Do	4 (8.9)	7 (15.6)	-	
	Residents of high TB endemic area	151	Blood	Do	19 (12.6)	41 (27.2)	-	

M. tuberculosis, Mycobacterium tuberculosis; OPD, outpatient department; LJ, Löwenstein-Jensen; TB, tuberculosis; CSF, cerebrospinal fluid; RD, region of difference aim to control the spread of TB in the community. Due to the huge burden of pulmonary TB, most of the NTEP-culture laboratories are over-burdened and use non-pyruvate culture media for the isolation of mycobacterial species rather than pyruvate-containing media which are considered the media of choice for primary isolation of *M. bovis* from clinical samples[67]. Therefore, the strengthening of laboratory infrastructure and improved laboratory diagnostic tests are essential for better detection and control of zoonotic TB in India.

Lack of information on effective treatment and recovery of patients: Treatment of *M. bovis*-induced TB differs from the pulmonary TB by *M. tuberculosis* and is a major challenge for the recovery and effective management of the patient. *M. bovis* has been found to be intrinsically resistant to pyrazinamide, one of the most important first-line anti-TB drugs used in the treatment regimen[68,69]. In India, most of the healthcare providers initiate treatment of TB based on the presence of acid-fast bacilli in the clinical samples, without knowing the actual *Mycobacterium* species involved. With universal DST in place, treatment is modified based on the results of cartridge-based nucleic acid amplification test (CBNAAT or GeneXpert) which, however, cannot differentiate *M. tuberculosis* from *M. bovis* as both belong to *M. tuberculosis* complex. In such situations, patients with *M. bovis*-induced TB receive inadequate treatment which may lead to adverse consequences on the management of TB patients[60] and may even induce the emergence of drug resistance to anti-TB drugs. Hence, the differential diagnosis of *M. bovis* from *M. tuberculosis* and systematic checking of resistance to pyrazinamide for patients suspected for zoonotic TB should be ensured. Previous studies also reported co-infection of *M. bovis* and *M. tuberculosis*[63,70]. Therefore, clinical variations and treatment outcomes of co-infection need to be investigated for better management of patients in our country and in countries where bTB is endemic.

Population density and close interaction between humans and animals: India possesses about 2.4 per cent of the world's geographical area[71]. The country has the highest number of animals and ranks second where the human population is concerned. In the countryside, animals and humans live in close proximity to each other. bTB has been well documented in herds and flocks of domestic livestock and wild animals from all parts of the country[40-49]. The majority of livestock are owned by farmers in India, and more than 68.0 per cent of the Indian workforce depends on farming that is in close contact with domestic animals and poultry to earn their livelihood in India[72]. Aerosol route and ingestion of contaminated milk/milk products are two important means of transmission of *M. bovis* from animals to humans[73,74]. The humans can also acquire the *M. bovis* infection from direct contact with the wound[75]. Studies reported a higher risk of exposure to *M. bovis* in individuals working or living with infected animals[76]. TB patients may also transmit *M. bovis* or *M. tuberculosis* to animals or to the in-contact human population through aerosol route[77,78]. Infection of *M. tuberculosis* has been reported in domestic or wild animals, living in prolonged contact with humans in captive settings[79-84]. Prasad *et al*[53] reported mixed infection of *M. tuberculosis* and *M. bovis* in humans and animals, and highlighted the risk of transmission of two pathogens from humans to animals (reverse zoonosis) and *vice versa* (zoonosis). Ultimate elimination of human TB will first require control of bTB in animals. The strategies for the control of bTB in animals may include the development of facilities for animal quarantine of TB-infected animals and implementing legislation to control the marketing and movement of TB-infected animals in the country. Recent studies have revealed that the knowledge in context to TB among Indian farmers is mainly limited to human TB and majority of farmers are not aware about the transmission of TB from animals to humans or *vice versa*[85,86]. Therefore, awareness and educational campaigns for human TB would also need to highlight the risk factors and public health implications of zoonotic TB in India.

Infection of the immune-compromised individuals: Suppressed immune system is one of the most important risk factors for *M. bovis* infection in humans. Previous studies have shown that HIV-positive individuals are more susceptible to *M. bovis* infection and it influences mortality rate and clinical presentation of the disease[69,87]. Suppressed immunity also promotes interpersonal transmission of *M. bovis* as reported amongst immune-suppressed and even immune-competent persons in the UK[88]. Park *et al*[89] retrospectively reviewed 86 cases of HIV and TB co-infection and reported that *M. bovis* infection was present in 34.9 per cent of cases in San Diego, USA. India has a huge burden of HIV/AIDS (2.14 million)[90] and other immune-compromised (diabetes, organ transplant recipients, cancer, drugs user, *etc.*) patients[91]. Since, human beings acquire *M. bovis* infection mainly through ingestion of unpasteurized milk and dairy products[72,73,92];

the presence of *M. bovis* in these products in India[43,51,52] poses a great risk of exposure to *M. bovis* in immune-compromised patients in India. Presently in India, the status of *M. bovis* infection in HIV or other immune-compromised patients is not known, and therefore, this aspect needs to be investigated. In the current scenario, precautionary measures are needed to restrict the transmission of live *M. bovis* to humans. Counselling of immune-compromised patients about zoonotic TB and risk factors for the acquisition of *M. bovis* infection should be made compulsory at the healthcare facilities in India.

Sociocultural and religious values: In India, cultural diversity in day-to-day life has led to stratification of the society with respect to religious beliefs and practices and specific nutritional and healthcare requirements. In few communities, raw cattle milk has been considered healthy and is therefore a staple diet of some families and societies. An interesting outbreak of TB among the cattle and people on the dairy farm due to consumption of raw milk has been reported in 2009[93]. Hence, the traditional practice of boiling of milk before consumption, as practiced in our society since time immemorial, should be revived and propagated by increasing awareness in the rural and tribal communities. Recently, Srinivasan *et al*[49] estimated that 6.3 per cent of cattle in the country are infected with bTB. As infected cows can transmit *M. bovis* infection to other animals and to human beings, these are likely to increase the bioburden of *M. bovis* in India. Development of quarantine facilities for infected cattle and initiation of bTB control programme will require joint efforts of the State and Central Governments in India.

Conclusions

Despite being declared a priority disease by the WHO as early as in the 1950s, zoonotic TB has not received due attention in India. This may be due to several reasons including a lack of exhaustive surveys on bTB in animals and zoonotic TB in humans. The available evidence sufficiently indicated that bTB is an 'insidious problem' among domestic livestock species in India and the human population carries the risk of exposure to *M. bovis*. A surveillance programme is urgently needed to estimate the prevalence and economic losses caused by *M. bovis*-induced TB. This information as well as other specific steps (Box) can be crucial for the strengthening of control strategies of bTB and for minimizing the transmission of *M. bovis* infection to the human population in India.

Box. Suggested steps to deal with the zoonotic tuberculosis in India

a) Zoonotic TB needs to be prioritized in the national health agenda in India. The official guidelines for the prevention, diagnosis and treatment of zoonotic TB need to be established at the national level.

b) Need for expansion of facilities for the identification and characterization of *M. bovis* infection in human and animal populations at the regional level.

c) National programmes on surveillance and monitoring of *M. bovis* are needed to estimate the true bioburden of zoonotic TB both in suspected TB patients and high-risk human populations (abattoir workers, animal healthcare workers, farmers, veterinarians, *etc*.) in the country.

d) The key population and high-risk pathways for the transmission of *M. bovis* from animals to humans and *vice versa* need to be identified.

e) Inspections of animal-derived food products should be made mandatory to reduce the transmission of *M. bovis* to the human population.

f) A national surveillance programme is required to estimate the burden of *M. bovis* infection in domestic livestock species in India.

g) Eradication of bovine TB in food animals should be a long-term objective to eliminate the risk of transmission of *M. bovis* to the human population.

h) Initiate awareness programmes about the impact of zoonotic TB in farmers and high-risk population to stimulate the interest in prevention and control strategies.

i) Encourage close collaboration between human and veterinary health professionals at the regional level to bridge the existing gap between public health and zoonotic TB awareness.

j) Database on 'zoonotic TB research' at the national level will improve the quality and consistency of the published information and will be helpful for the development of policies in the country.

k) The government and relevant agencies should enforce occupational health safety measures including the use of personal protective equipment (PPE) in high-risk group population.

The key international health organizations (WHO, World Organization for Animal Health, the Food and Agricultural Organization and the International Union against Tuberculosis and Lung Disease) have advocated for the 'One Health' approach to combat zoonotic infections in animals and humans[94]. The spillover of TB from animals to humans or *vice versa* may jeopardize the efforts of TB eradication programmes in high-endemic countries like India. The existing TB elimination programme in the country (NTEP) can adopt comprehensive policies to tackle human as well as zoonotic TB under the 'One Health' umbrella. The implementation of this approach will be helpful towards the speedy reduction of human TB and achieving the ambitious goal of TB elimination in India by 2025.

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