REVIEW ARTICLE

Bovine Tuberculosis in Endemic India: Epidemiology and the Evolving Role of Skin Testing in Disease Detection

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Abstract

Mycobacterium bovis conferring Tuberculosis in bovines, a member of *Mycobacterium tuberculosis* (Mtbc) complex, is a persistent major threat to bovines with significant zoonotic propositions. Tuberculosis in milch animals is a potential hazard to the Indian dairy industry as the disease consequents in mortality, morbidity and production decline leading to significant economic down-curves. Hence, the continuous monitoring is the option of choice to combat the situation including surveillance and disease control tests, *viz.*, cell-mediated immune (CMI) dependent assays (Tuberculin skin tests (TST): single intradermal test (SID) or comparative/double intradermal test (DIT)). But in contrast, estimation of true burden of the disease still remains unknown due to the lack of proper routine surveillance data. This review projects along with different aspects of bovine TB, summary of various studies performed in different parts of India covering 20 states and union territories where skin testing of the animals have been preferred as the major test inferring a view of the prevalence-picture of these areas. Furthermore, implementation of various control programs at both national and international level with acceleration in research velocity could lead in succeeding the present challenges.

Key words: Bovine tuberculosis, Endemicity, Intradermal test, *M. bovis*, Zoonosis.

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Introduction

The discovery of *Mycobacterium tuberculosis* in 1882 by Robert Koch had made a new revolution in the field of medical science as this had enhanced the traceability of the disease in respect of both morbidity and mortality by effective detection. Thereafter the pathogen had been grouped into the family *Mycobacteriaceae* under the genus Actinobacteria; in which various pathogenic species of the bacterium which are known to cause serious diseases in mammals like *Mycobacterium tuberculosis* Complex (MTBC), leprosy* *by M. leprae** etc (Pfyffer and Palicova, 2011). MTBC consists of several genetic homologous species, *viz.*, *M. tuberculosis*, *M. bovis, M. bovis* BCG (bacillus Calmette-Guérin), *M. africanum, M. caprae, M. microti, M. canettii* and *M. pinnipedii* (Homolka *et al.,* 2008). Approximately 6,000 years ago, certain sequences were shed from a progenitor strain of a human-adapted *M. tuberculosis* lineage which eventually gave rise to MTBC. In addition, according to genetic studies of modern animal and human tuberculosis (TB) strains around the world, *M. bovis* had reported to be evolved from the same. This could be linked to early farming and animal domestication between 10,000 and 15,000 years ago in evolutionary terms (Donoghue *et al.,* 2004). Recent research has expanded the understanding of the *Mycobacterium tuberculosis* complex (MTBC) in India, identifying *Mycobacterium orygis* as an emerging zoonotic pathogen that is increasingly recognized in both human and animal populations. A 2024 meta-analysis estimated that India's dairy sector currently harbors approximately 21.8 million infected cattle, a burden that significantly complicates the national 'One Health' strategy. Furthermore, 2025 case reports in Uttar Pradesh have highlighted the capability of *M. orygis* to cause severe, generalized systemic disease in cattle, matching the clinical severity of *M. bovis*.

The major threatening agent of bovine TB (bTB) is *M. bovis*, also *M. caprae* to a lesser extent, as the former causes

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the majority of the cases occurring across the globe with cattle being the major reservoir of infection. Close contact with the affected animals (mostly by inhalation of aerosals) and consumption of unpasteurized milk are the major factors responsible for the zoonotic transmission of the pathogens (Ayele *et al.,* 2004). Pathogenesis-analysis clearly implies that bovine tuberculosis is mostly transmitted through the respiratory system and requires transmission by infected aerosols (Cassidy, 2006). Whereas the disease development is very slow ranging from months to years leading to fatalities in morbid animals. Symptoms in bovines include progressive weight loss, loss of appetite, intermittent cough, swollen lymph nodes, weakness, low-grade fluctuating fever and diarrhea (Une and Mori, 2007). Infection also leads to milk yield reduction up to 10-20%, reduced fertility and reduced carcass value (Collins, 2006). Subsequently with the progress of clinical signs, transmission begins and continues to occur (Pollock and Neill, 2002; Good and Duignan, 2011).

M. tuberculosis is the most common member of the MTBC but *M. bovis* possesses wider host range and is the most common cause of TB in domestic and wild mammals (Rua- Domenech *et al*., 2006). The transmission of MTBC strains among animals and humans is termed as the zoonotic tuberculosis (zTB). The fraction of *M. bovis* isolates among the human cases is used to quantify zTB. Despite the fact that India has the highest number of human tuberculosis cases (very contagious too) and having the world's largest cattle population where bTB is also widespread, but ironically the burden of zTB is still unclear (Duffy *et al.,* 2020). Zoonotic tuberculosis is clinically and pathologically indistinguishable from tuberculosis caused by *M. tuberculosis*. Only sophisticated laboratory methods involving bacteriological culture of clinical specimens, typing of isolates based on growth characteristics, biochemical properties, routine resistance to pyrazinamide (PZA) and specific non-commercial nucleic acid techniques can distinguish between the causative organisms (Rua-Domenech *et al*., 2006). Despite minor morphological and biochemical variations, *M. bovis* and *M. tuberculosis* are extremely similar and the diseases they cause in humans are indistinguishable clinically, radiographically, pathologically and/or by direct smear microscopy (Rua-Domenech *et al*., 2006).

Global Scenario

Between the 18th and 19th centuries, TB outbreaks in humans spiked mortality rate at 900 per 100,000 of population. With the beginning of industrial revolution, the rural societies in Europe and America expanded into industrial-and-urban areas as the workers moved towards cities in search of work. Poverty, malnutrition, inadequate sanitation, poorly ventilated houses and overcrowding all contributed to the barb in tuberculosis deaths (Daniel, 2006; Frith, 2014). By the year 1914, it was agreed with most of the TB specialists that most of the human cases were transmitted as a result of consumption of infected milk. Whereas in 1917, *M. bovis* was found to be responsible of estimated cause of around 15,000 deaths in the US alone which are roughly three times the deaths caused by foodborne diseases in today's time (Atkins, 2000; Palmer and Waters, 2011).

The possibility of human-cattle-human transmission with routine isolation of *M. tuberculosis* (usually human agent) from cattle raises the concerns about the absence of effective eradication programs. Such observations laid the importance of adopting effective control and eradication programs for tuberculosis in both humans as well as animals (Ghodbane and Drancourt, 2013; Mittal *et al.,* 2014; Hlokwe *et al.,* 2017). Henceforth, the increasing threat of zoonotic TB paved the decision of eradicating *M. bovis* for the purpose of health and economic concerns in 1983 (Kleebergh, 1984).

In 1995, an estimated 8.8 million new tuberculosis cases were reported with 5.5 million (62%) in Southeast Asia and the Western Pacific and 1.5 million (17%) in Sub-Saharan Africa (Dolin *et al.,* 1994). According to a WHO report, worldwide average incidence rate of 128 out of 100,000 population per year were detected in 2010 (WHO, 2011). It is stated that approximately three people die only because of tuberculosis every minute even in the present calendar-time, despite the disease is considered to be preventable, treatable and even curable since 1950s. It continues to thrive irrespective of the fact that the causative agent only multiplies and propagates inside the host (Onyango, 2011; Levy, 2012). In fact, one-third of global population is considered to be TB infected primarily due to lack of advanced screening, diagnostics and treatment facilities. As a result, a death toll of 1.5 million people had been recorded recently in 2014 (WHO, 2015).

In contrast, around 51 million people were treated successfully in the countries adopting WHO strategy leading to saving of 20 million lives between the years 1995-2011. Though the progress of response towards multidrug-resistant TB (MDR-TB) remained comparatively slow. As a result, the number of cases in high-burdened MDR-TB countries is increasing which is a matter of concern (WHO, 2012). In the period of 2009 to 2012, majority of the tuberculosis cases with the maximum mortalities were reported from poorly and/or under developed areas including regions of Africa, China, India and Southeast Asia accounting at a rate of 20-40 per million people (Fitzgerald *et al.,* 2010; Small, 2012).

Indian Studies On Btb

Studies on bovine tuberculosis detection in India have been carried out since a long past which is mostly based on the tuberculin antigen application in skin. Tuberculin skin tests (TST) are based on a cell mediated immune response which is used in humans and animals for diagnosing preclinical infections (Monaghan *et al.,* 1994). These are in fact the recommended ante-mortem tests (international standard), prescribed by World Organization for Animal Health (OIE) for international cattle trade; and also endorsed in most of the developing countries for routine screening of cattle herds (Rua-Domenech *et al.,* 2006). Presently, two

types of tuberculin tests are in use, *viz.*, single intradermal test (SIT) using bovine tuberculin and single intradermal comparative tuberculin test (SICTT) using both bovine and avian tuberculin. Following is the table (Table 1) listing the prevalence of tuberculosis in various states and union territories of India performed by different researchers using different tuberculin tests (Srinivasan *et al.,* 2018; Refaya *et al.,* 2020).

Recent Studies

In a cross-sectional study of 525 animals across 10 districts of Punjab, Singh *et al.* (2020) noted that the prevalence of *M. bovis* reactors varied depending on the diagnostic methodology employed. Using the Comparative Intradermal Tuberculin Test (CITT), the prevalence was recorded at 9.3% (49/525). However, the Defined Skin Test (DST) yielded a slightly higher reactor rate of 11%. Kaur *et al.* (2021b) compared multiple diagnostic platforms on 230 dairy animals, finding that Gamma-IFN assay (21/230) and IS6110 PCR (23/230) identified more positive cases than the SICTT (17/230). Notably, PCR detected the *Mycobacterium tuberculosis* complex (MTC) in blood samples of all SICTT reactors and additional animals, suggesting higher sensitivity in subclinical cases. In a study across two districts of Punjab, India (Prajapati *et al.,* 2025), the comparative intradermal tuberculin test (CITT) revealed a 14.4% (12/83) prevalence of bovine tuberculosis (bTB). The study noted higher positivity in cows/heifers (16.17%) compared to bulls (6.66%) and highlighted increased susceptibility in indigenous breeds.

A cross-sectional study by Kaur *et al.* (2021a) in Tamil Nadu revealed an overall prevalence of 8.46% using the CIDT test. The highest prevalence within the state was recorded in Vellore (10.86%), followed by Kancheepuram (8.20%) and Thiruvallur (7.92%). Kalaiselvi *et al.* (2024) screened organized livestock farms and found a low positivity of 0.11% in cattle and buffaloes, though significantly higher rates were found in horses (8.1%) within the same environments.

Thaker *et al.* (2023) utilized ELISA techniques and reported an overall prevalence of 6.25% in cattle and buffaloes in Gujarat. Chaudhari *et al.* (2024) found that HF crossbred cattle demonstrated the highest positivity at 14.16% when tested with the SIT test in the Anand and Rajkot districts. In the same study, the Single Intradermal test (SIT) recorded a much higher positivity rate (10.62%) than the Comparative Intradermal test (CIT) which recorded 1.37% positivity, highlighting the impact of cross-reactivity with environmental mycobacteria in traditional testing.

Table 1: State wise prevalence of bTB obtained using different tuberculin tests by various researchers

State	Skin testing methods			No. of screened animals	Prevalence (%)	Reference
	SIT	SICT	DIT			
Punjab	-	√	-	121	14	Filia *et al.,* 2016
	-	√	-	202	19.8	Brahma *et al.,* 2019
	-	√	-	200	19	Sidhu *et al.,* 2020
Haryana	√	-	-	628	2.4	Bali and Singh, 1980
Himachal Pradesh	√	-	-	440	14.3	Aneesh *et al.,* 2010
J& K	√	-	-	40	37.5	Taggar & Bhadwal, 2008
Rajasthan	√	-	-	353	4.8	Shringi, 2004
Uttar Pradesh	√	-	-	245	14.3	Ashish *et al.,* 2014
	√	-	-	442	16.1	Thakur *et al.,* 2016
Uttarakhand	√	-	-	99	0	Thakur *et al.,* 2016
Gujarat	√	-	-	2310	2.3	Trangadia *et al.,* 2013
Maharashtra	-	√	-	2043	1.2	Bapat and Bangi, 1985
West Bengal	-	-	√	173	21.4	Das *et al.,* 2018
Bihar	-	-	√	169	4.7	Lall *et al.,* 1969
Telangana	-	-	√	426	1.9	Lall *et al.,* 1969
Madhya Pradesh	√	-	-	465	9	Sisodia *et al.,* 1995
Karnataka	√	-	-	2668	2.4	Phaniraja *et al.,* 2010
Kerala	-	√	-	302	8.9	Murti and Hazarika, 1982
Tamil Nadu	√	-	-	63	49.2	Nishanth & Ganesan, 2006
Odisha	√	-	-	670	3.4	Mishra *et al.,* 1997
Puducherry	-	√	-	41	51.2	Mukhopadhyay *et al.,* 2001
Assam	√	-	-	50	28.0	Konch *et al.,* 2017

SIT: single intradermal test, SICT: single intradermal comparative test, DIT: double intradermal test

Yadav *et al.* (2023) screened peri-urban dairy farms near Guwahati and found an animal-level prevalence of 10.55% and a herd-level prevalence of 50%.

National Overview

A large-scale analysis by Srinivasan *et al.* (2018) demonstrated that bovine tuberculosis (bTB) remains endemic across most Indian states, with a moderate but persistent national prevalence (~5-8%), translating to tens of millions of infected cattle. Prevalence varies widely by region and production system, reaching >15-20% in certain states and high-density settings such as gaushalas and intensively managed dairy farms. Cattle show higher prevalence than buffaloes, and cross-bred/exotic animals and organized or intensified dairy systems consistently exhibit higher risk than traditional rural systems. Overall, the findings indicate substantial heterogeneity across states, long-term persistence of bTB, and a clear association between dairy intensification and increased disease prevalence.

Challenges in India

With the goal of 'End TB' strategy, the aim of WHO is to reduce the incidence of tuberculosis by 90% till 2035 (WHO, 2014). With more than 2.6 million cases and 400,000 deaths reported in a single year (2019), India appeared as the largest burden of human TB globally (WHO, 2019). India possesses a huge cattle population with more than 300 million but it is estimated that around 21.8 million cattle are infected turning in an endemic bTB country (Srinivasan *et al.,* 2018; USDA, 2019). Various past studies inferred the prevalence of zoonotic tuberculosis to be around 10% in India (Prasad *et al.,* 2005; Shah *et al.,* 2006; Bapat *et al.,* 2017). A study was conducted during 2014-15 by Bepat *et al.* (2017) in three groups consisting human-sample size of 301 persons in central part of India. It was observed that the highest proportion of cases infected with *M. bovis* was observed in groups who lived in high TB endemic regions. Other two important determinants found were the previous contact with active infected cases and consumption of raw milk (Bapat *et al.,* 2017).

Despite being categorized as primary zoonosis, the epidemiology of bTB in India is not so well characterized that even the Revised National Tuberculosis Control Program (RNTCP) failed to distinguish between tuberculosis of human and animal origin (Sekar *et al.,* 2011; CTD, 2017). The risks of emergence and spread of bTB have the potential to overstate the advances in agriculture. Moreover, it exaggerates the practice of rearing high susceptible exotic animals typically in the peri-urban ecosystems where biosecurity, animal screening etc. are lagging behind (Humblet *et al.,* 2009; Narain *et al.,* 2013). However, in human cases, the success of anti-TB programs is hindered by both MDR-TB and XDR-TB as the cases of MDR-TB rose from 0.04% in 2005 to roughly 4 times, 0.15% in 2007 which is quite alarming (Sandhu, 2011).

Advancements in Techniques

Though the intradermal tests are enormously used but there lie several limitations as these inferred in variable test results. The purified protein derivatives (PPDs) used contain cross-reactive antigens which trigger hypersensitivity response of the delayed form resulting in false positive reactions. Efforts are being made for the development of novel skin antigen known as 'Defined Skin Test'. Antigen of the same has been used in countable research centers of India for trials. It is a cocktail of strains used which are the field ones (pathogenic strains) rather than the vaccine or immunogenic strains *i.e.,* BCG. This serves the purpose of both detection of affected animals and the differentiation of Infected from Vaccinated Animals (DIVA) (Whelan *et al.,* 2010; Vordermeier *et al.,* 2016; Srinivasan *et al.,* 2019; Kumar *et al.,* 2021).

As of February 2025, comparative performance trials have validated Defined Antigen Skin Tests (DSTs) utilizing peptide cocktails (ESAT-6, CFP-10, and Rv3615c) (Godfray *et al.,* 2025). These tests maintain 100% specificity in BCG-vaccinated calves, addressing the long-standing issue of false positives in traditional PPD-based testing. While global molecular diagnostic markets are shifting toward high-sensitivity CRISPR and qPCR platforms (targeting IS6110), the field-applicability of skin testing remains the primary screening tool for India's large-scale dairy herds due to its cost-efficiency.

Indian Approaches Towards Tuberculosis

The first step taken by Government of India in this regard was the launching of National Tuberculosis Control Program in 1962. But unfortunately, it was unable to fulfil the expected outcomes. Thereby it was revised and relaunched in 1993 under the name of 'Revised National Tuberculosis Control Programme'. By the year 2006, it achieved to cover the majority of Indian population, though countering many challenges. The National Strategic Plan (2012-17) was then launched under the program National Health Mission (NHM) which involved the introduction of new techniques like Computer aided diagnosis (CAD) and Cartridge based nucleic acids amplification test (CBNAAT) along with establishment of sentinel surveillance system with National Tuberculosis Research Institute, Bengaluru and two other National Reference laboratories. Also, the National TB elimination board been established to facilitate the process of development of policies and their implementation. Provision of incentives is initiated upon providing notification in TB reporting software, Nikshay. This initiative is considered notable to increase the affordability of treatment in private sectors and to improve the facilities related to TB care. The time period of National Strategic Plan (NSP) up to 2025 is of utmost importance with the hope of introduction of new drugs, regimens and diagnostics. The implementation-outcome of NSP can be evaluated in end

TB efforts contributing towards good public health (CTD, 2017; RNTCP, 2017).

Conclusion

TB has proved itself as a burning and endemic health issue in India. To combat the effects of it, awareness among people needs to be implemented across the country along with surveillance and monitoring programs. Nation based control strategies are essential keeping in pace with the increase in milk demand and dairy intensification. Hence, the relevant skin tests can be a valuable tool against bovine TB. The works are in progress to arrest the zoonosis by improving the sensitivity and specificity of the tests considered for employment. Hence, antigenic protein cocktails over single proteins are on the way to gain more reliability and tuberculosis detection measurability.

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