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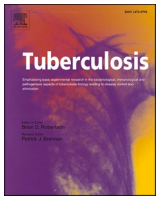

Review

Reverse zoonosis in bovine tuberculosis: The neglected threat of *Mycobacterium tuberculosis* infection in cattle

Vaishnavi Vivekanandan [a], Arun K [a], Sindhu Hasini Doredla [a], Harini Ramanujam [a], Ranjani Singaraj [a], Kannan Palaniyandi [a,b,*]

[a] Department of Immunology, ICMR-National Institute for Research in Tuberculosis, Chetpet, Chennai, India
[b] Faculty of Medical Research, Academy of Scientific and Innovative Research (AcSIR), India

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ABSTRACT

Tuberculosis (TB), caused by members of the *Mycobacterium tuberculosis* complex (MTBC), remains a significant global health concern. While zoonotic transmission of *M. bovis* from cattle to humans is well documented, reverse zoonotic transmission of *M. tuberculosis* from humans to cattle has received far less attention. This review provides the first comprehensive synthesis of *M. tuberculosis* infections in cattle, drawing on evidence from farms, households, and slaughterhouses where human–animal contact is most intense. Available data indicate that such spillover events are uncommon compared with *M. bovis* infection, and that infectious humans—via aerosols or sputum-contaminated feed or environments—represent the primary, and likely exclusive source of infection for cattle, as no sustained cattle-to-cattle transmission has been reported. Experimental *M. tuberculosis* infection in cattle consistently demonstrates an attenuated phenotype, with mild pathology and low bacterial loads. However, the identification of multidrug-resistant and pre–extensively drug-resistant *M. tuberculosis* strains in cattle raises a potential future concern regarding cross-species transmission, despite the fact that transmission back to humans has not been observed yet. Enhancing routine molecular diagnostics is vital for precise MTBC differentiation and a better grasp of cross-species transmission dynamics. A unified One Health strategy, which combines human, animal, and environmental monitoring, is essential to track and address this emerging threat.

1. Introduction

Tuberculosis (TB) remains a major global health issue, significantly impacting both morbidity and mortality rates worldwide. As of 2023, it is estimated that TB affects about a quarter of the world's population, resulting in approximately 10.8 million people contracting the disease and 1.25 million deaths each year [1]. The disease is caused by the *Mycobacterium tuberculosis* complex (MTBC), a group of species with high genetic similarity. Within this group, *M. tuberculosis* is the main pathogen causing TB in humans. However, other species within the MTBC, such as *M. bovis*, *M. caprae*, *M. orygis*, *M. pinnipedii*, *M. microttii*, *M. suricattae*, and *M. mungi*, are adapted to animals and primarily affect non-human hosts [2]. Although MTBC members are generally considered to be host-adapted, there is growing evidence of their ability to cross species barriers. This interspecies transmission leads to TB cases not only in humans but also in various domestic and wild animal species, underscoring the importance of TB in both public and veterinary health, as shown in (Fig. 1). The phenomena of zoonosis, where TB is transmitted from animals to humans, and reverse zoonosis or zooanthroponosis, where transmission occurs from humans to animals, have been reported in several countries [3]. Within the MTB complex, *M. bovis* is traditionally associated with bovine tuberculosis (bTB), a chronic infectious disease mainly affecting cattle [4]. Bovine TB poses both an economic challenge, due to decreased productivity and increased mortality in livestock, and a public health concern, especially in low- and middle-income countries(LMICs) where close human-livestock interaction and the consumption of unpasteurized dairy products are common [5].

Nonetheless, emerging research indicates that *M. tuberculosis* can indeed infect and cause disease in cattle, challenging the long-standing belief of its strict host specificity. Although the zoonotic transmission of bTB from cattle to humans, primarily attributed to *M. bovis*, has been well recognized for more than a century, the reverse transmission of *M. tuberculosis* from humans to cattle has received comparatively limited

* Corresponding author. Department of Immunology, ICMR-National Institute for Research in Tuberculosis, #1, Mayor Sathiyamoorthy Road, Chetpet, Chennai, 600 031, India.
E-mail address: palaniyandi.k@icmr.gov.in (K. Palaniyandi).

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attention. *M. tuberculosis* is only sporadically detected in cattle, as represented in (Fig. 2). The cases that do occur, particularly in high-risk settings such as farms, household cattle environments, and slaughterhouses, demonstrate that this pathogen can play a meaningful role in the epidemiology of TB in cattle populations[6–8]. Historically, infections of animals with *M. tuberculosis* have been considered accidental spillover events from infected humans, with no evidence of sustained animal-to-animal transmission [9]. Reverse zoonotic transmission may occur through direct exposure to infectious humans via aerosols or contamination of feed and water with sputum. These risks are particularly heightened in settings where humans and cattle coexist in proximity and biosecurity measures are inadequate [10]. Remarkably, the emergence of drug-resistant *M. tuberculosis* strains in the bovine population is a matter of considerable concern, as it complicates diagnostics and control measures and raises the potential future risk of re-transmission to humans through occupational exposure or contaminated animal products. Although no cattle-to-human spillover events have been reported, such scenarios could perpetuate a continuous cycle of infection across species [11,12]. As illustrated in (Fig. 3), the global documentation of *M. tuberculosis* infection in cattle is notable and warrants careful attention due to its implications for the global End TB Strategy.

These emerging trends highlight the importance of adopting a One Health strategy, which combines the efforts of human, animal, and environmental health sectors to efficiently track, diagnose, and manage TB across different species [13]. The presence of MTBC species that infect both humans and animals, especially in regions with high TB prevalence, complicates surveillance efforts and poses diagnostic challenges due to the difficulty in differentiating between infections caused by *M. bovis* and *M. tuberculosis*. The limited ability of standard diagnostic tools to distinguish between MTBC species further complicates the situation, often leading to underreporting or misidentification of reverse zoonotic cases [14]. In response to these issues, this review seeks to critically explore the role of *M. tuberculosis* in bovine tuberculosis, evaluate its potential for reverse zoonosis, summarize current knowledge of its molecular characteristics, and emphasize the diagnostic challenges that hinder effective detection and control.

2. Documented cases of *M. tuberculosis* infection in bovine populations

Instances of *M. tuberculosis* infections in cattle have been recorded on multiple continents, including nations such as Ethiopia, Nigeria, Egypt, Sudan, Tanzania, Ghana, South Africa, Rwanda, India, Mexico, China, Spain, Poland, Turkey, Croatia, and Slovenia, as detailed in Table 1. This table also indicates that *M. bovis* is the main cause of bTB in most cases, whereas *M. tuberculosis* is found at lower rates. Importantly, the detection of *M. tuberculosis*, often alongside *M. bovis*, has been primarily reported in areas with a significant human TB prevalence, such as Ethiopia, Nigeria, Tanzania, and India. This reflects the close interaction between humans and animals and the overlapping TB burden in pastoral and smallholder farming communities[15–17].

Ethiopia stands out as a significant area of concern, being a top livestock producer with more than 70 million cattle, which are vital for the survival of both rural and urban families. The economic repercussions of bTB are significant, with herd productivity estimated to drop by 7.2 % [18]. Despite this, bTB remains widespread, worsened by insufficient abattoir-based monitoring and unrestricted animal movement. Consistent research from Ethiopia has identified *M. tuberculosis* in cattle associated with TB-positive households, with samples obtained from both milk and tissue [19,20]. Additionally, in Selalle, a central Ethiopian region, cultural practices like giving cattle chewed tobacco juice have been suggested as possible transmission routes [21]. Beyond Ethiopia, a study from Nigeria identified *M. tuberculosis* in a heifer exhibiting strong tuberculin reactivity, concurrent with a TB-positive farm worker, thereby suggesting human-to-cattle transmission. Despite limited direct evidence, at that time, humans with active TB were widely considered the primary source of *M. tuberculosis* infection in cattle [22]. Furthermore, a crucial study in Nigeria's Ebonyi State discovered genetic similarities between *M. tuberculosis* strains isolated from cattle and several pastoralists, highlighting the ongoing cross-species circulation of human-adapted strains within African livestock systems [8]. In Tanzania, environmental monitoring among pastoralists and villagers in the Iringa district detected *M. tuberculosis* in about 7 % of cattle fecal samples during both dry and wet seasons, with one cow co-infected with *M. bovis*. Additionally, 1.3 % of boma soil samples tested positive, all

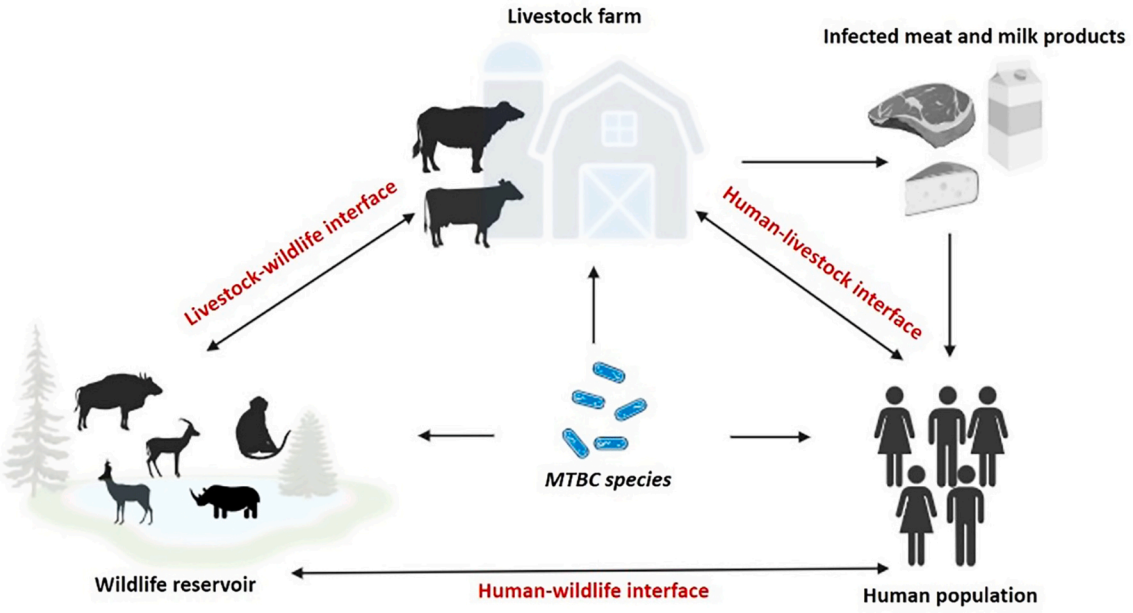

Fig. 1. Transmission chain of tuberculosis across human, livestock, and wildlife interfaces.
The diagram illustrates potential routes of *Mycobacterium tuberculosis* complex (MTBC) transmission, including direct contact and indirect exposure through contaminated environments or animal-derived products across the human-animal interface.

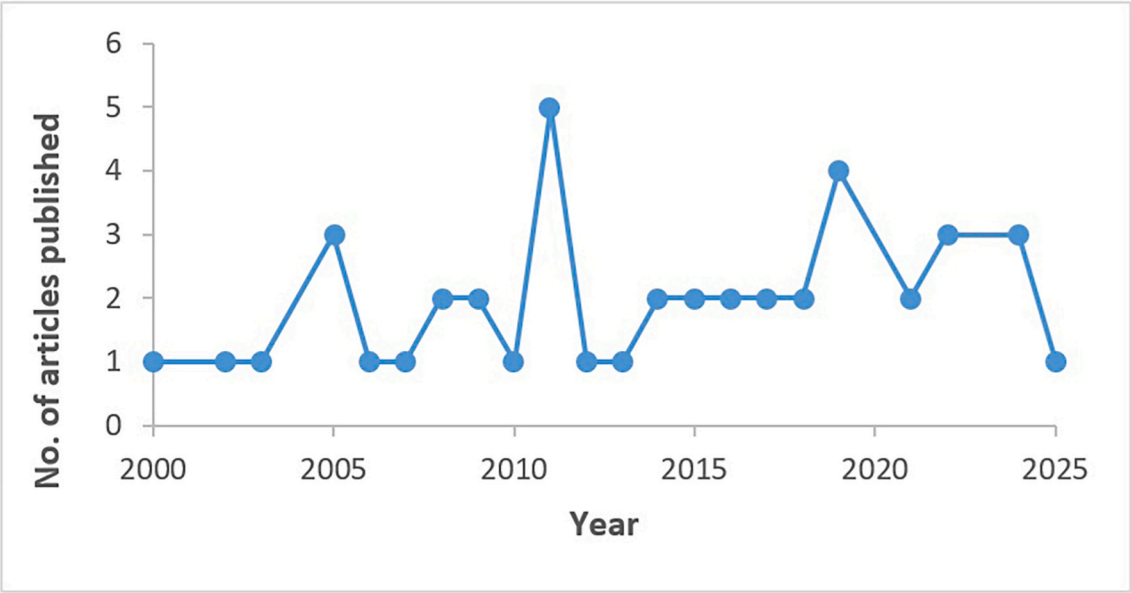

Fig. 2. Annual trend of *M. tuberculosis* cases in cattle (2000-2025), illustrating the sporadic reporting of its detection over the past two decades, as documented in case reports and surveillance studies.

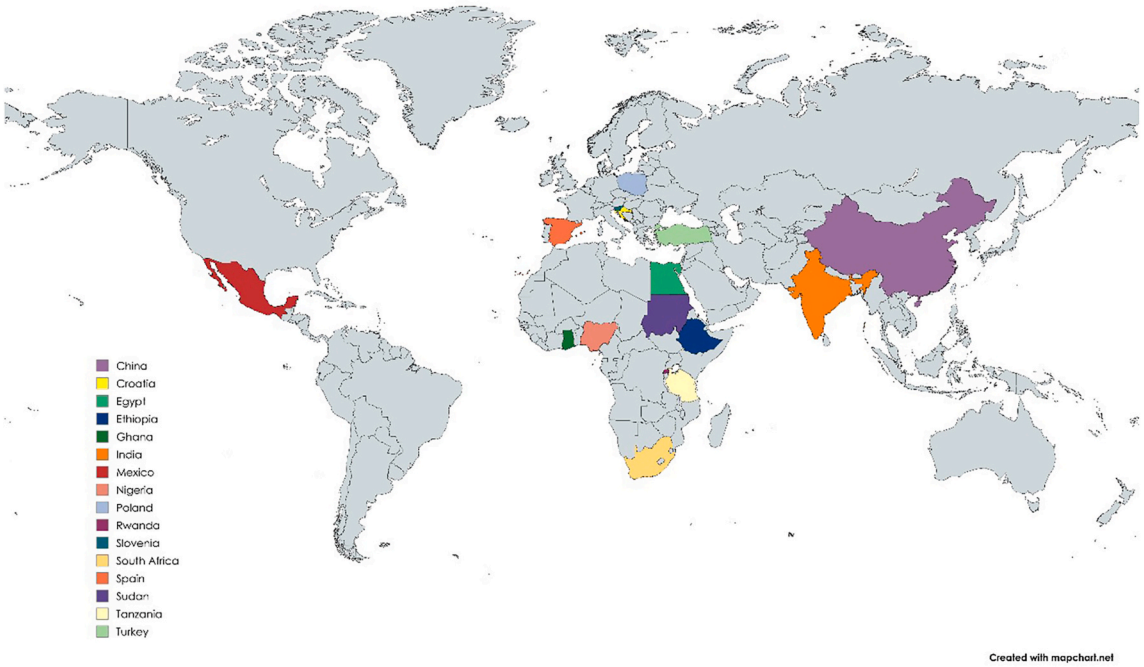

Fig. 3. Global distribution of Tuberculosis in Cattle Caused by *M. tuberculosis*
This map highlights countries where *M. tuberculosis* infections have been reported in cattle, based on published case reports and surveillance studies. The coloured regions represent confirmed detections across diverse geographic settings, predominantly in areas with close human–animal interactions and a high human TB burden.

from households with infected cattle, suggesting ongoing human-to-cattle transmission in shared environments [17]. Overall, although cases are sporadic worldwide, most *M. tuberculosis* recoveries from cattle have been reported in African countries, with additional isolated cases from other regions listed in Table 1.

To comprehend why these infections frequently go unnoticed, it is essential to consider their slow progression in cattle populations. Typically, TB infection in cattle develops slowly over several months to years, with many animals showing no symptoms while experiencing a decline in productivity due to decreased milk production, reduced fertility, and carcass losses. When clinical symptoms do manifest, whether from *M. tuberculosis* or *M. bovis*, they usually indicate an advanced stage of the disease, characterized by ongoing weight loss, diminished appetite, sporadic coughing, mild fever, swollen lymph nodes, and diarrhea. In more severe cases, enlarged or ruptured lymph nodes may block essential vessels or organs, resulting in breathing difficulties, bloating, or constipation [23]. To distinguish between these infections more effectively, researchers have utilized experimental bovine models to compare *M. tuberculosis* and *M. bovis*. In a study by Whelan et al. (2010), cattle were infected with 10^6 CFU of either

Table 1
Year-wise case reports and epidemiological studies on TB caused by *M. tuberculosis* in cattle worldwide.

Year	Sampling location	Screening test	Total no. of animal specimens screened	*M. tuberculosis* positive	Sample type: Tissue/ milk/blood/nasal swab	Comments	References
2000	20 dairy farms, Dar es Salaam Region, and 39 bomas, Lugoba Area, Tanzania	SIT Post-mortem examination Biochemical analysis Culture Spoligotyping	2632	2	Blood and tissue samples	Between 1995 and 1997, a study in Tanzania tested cattle for bovine TB using the SIT test. Two SIT-positive cows were slaughtered, and *M. tuberculosis* was confirmed by Spoligotyping. Notably, the owners of these cows had a family history of TB, suggesting possible reverse zoonotic transmission from humans to cattle.	Weinhäupl 2000 [28]
2002	Slaughterhouses in Khartoum (central Sudan), Gedarif and Kassala (eastern Sudan)	Microscopy Biochemical analysis Culture PCR targeting IS*6110* insertion sequences 16S rRNA gene sequencing	120	4	Tissue samples	Out of 120 caseous lesions collected from slaughtered cattle in Sudan, 64 (53.3 %) were positive for AFB. Culture and molecular analyses confirmed the presence of *M. bovis* in 25 samples and *M. tuberculosis* in 4 samples.	Sulieman 2002 [29]
2003	Cattle farm, Poland	–	–	9	–	*M. tuberculosis* was isolated from 9 cattle during the year between 1990 and 1999.	Pavlik 2003 [30]
2005	Single cattle herd, India	Culture N-PCR assay, targeting the *hupB* gene of *M. tuberculosis* (*Rv2986c*) and *M. bovis* (*Mb3010c*) to differentiate these closely related species	56	9	Tissue sample and fluids	Fifty-six cattle from a single herd were grouped into TB (n = 29) and Non-TB (n = 27) based on clinical signs. Culture identified pathogenic mycobacteria in 17 animals: 9 with *M. tuberculosis*, 7 with *M. bovis*, and 1 mixed infection from a non-TB animal.	Prasad 2005 [31]
2005	Cattle farm, Slovenian capital, Ljubljana	CIDTT Post-mortem examination Pathology Microscopy Biochemical analysis Culture GenoType MTBC Assay IS*6110* RFLP analysis	78	1	Tissue samples	In the year 2000, a 2-year-old cow tested positive for *M. tuberculosis* during routine tuberculin testing, with molecular analysis (IS*6110* RFLP) confirming 100 % strain similarity with a farm worker previously treated for TB, providing the first confirmed evidence of human-to-cattle transmission of *M. tuberculosis*	Ocepek 2005 [32]
2005	Military farms under the jurisdiction of the Central Military Veterinary Laboratory (CMVL), Meerut, India	Culture PCR-RFLP for the *hupB* gene N-PCR for the C-terminal portion of *hupB* DNA to differentiate *M. tuberculosis* and *M. bovis* Standard biochemical analysis	Samples (n 753) collected from 144 heads of cattle	7	Fine needle aspirate (FNA) from the prescapular lymph node (PSLN), citrated blood, milk, pharyngeal swab, urine, rectal pinch, and fecal sample	Fifty-six isolates were derived from collected samples, out of which, using PCR RFLP assay, thirty-four isolates were identified as *M. bovis*, 7 as *M. tuberculosis*, and 15 as NTM.	Mishra 2005 [33]
2006	Private resident's cattle herd, and a major abattoir in the city of Ibadan, Nigeria	Post-mortem examination Microscopy Culture RD deletion analysis Spoligotyping VNTR analysis	170	1	Tissue samples	Out of the seventeen MTBC isolates recovered from cattle, two showed non–*M. bovis* spoligotypes and were further examined using RD deletion typing. One isolate displayed the RD deletion pattern characteristic of a modern *M. tuberculosis* strain, although its VNTR profile could not be conclusively determined due to inconsistent allele calls. The other isolate exhibited a spoligotype typical of *M. africanum*, indicating an additional non–*M. bovis* MTBC strain likely introduced	Cadmus 2006 [34]

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Table 1 (*continued*)

Year	Sampling location	Screening test	Total no. of animal specimens screened	*M. tuberculosis* positive	Sample type: Tissue/ milk/blood/nasal swab	Comments	References
						through human-to-cattle transmission.	
2007	Small-scale dairy farms, Adama Town, central Ethiopia	CIDTT Biochemical and drug susceptibility test Microscopy Culture	524	3	Milk samples	Out of 524 cattle screened, 44 reactor cows were further tested. Bacilli grew in 16/44 of the milk samples, and 11/16 were acid-fast. Biochemical testing confirmed 6 isolates as *M. bovis*, 3 as *M. tuberculosis*, and 2 as other mycobacteria. The detection of both *M. bovis* and *M. tuberculosis* highlights the potential for zoonotic and reverse zoonotic transmission.	Ameni 2007 [15]
2008	Cattle farm of Central Military Veterinary Laboratory (CMVL), Meerut Cantonment, Uttar Pradesh, North India	SICCT Post-mortem examination Microscopy Culture Species-level identification by standard biochemical tests	Samples (n 768) collected from 161 cattle	14	Heparinized or EDTA-containing blood, fine needle aspirates from prescapular lymph gland, milk, pharyngeal swab, rectal pinch, and fecal sample.	During 1999 to 2001, from CMVL, among 54 MTBC isolates obtained, biochemical profiling identified 40 as *M. bovis* and 14 as *M. tuberculosis*, based on characteristic reactions to niacin, nitrate reduction, 2-thiophene carboxylic acid hydrazide (TCH), catalase, Tween hydrolysis, and arylsulfatase.	Srivastava 2008 [35]
2008	Cattle owned by human TB cases and non-TB patients, Central Ethiopia	CIDTT Milk culture Microscopy Biochemical tests Drug susceptibility tests	1041	2	Milk samples	Between October 2004 and April 2005, a case-control study in central Ethiopia showed higher bTB prevalence in cattle owned by TB-positive farmers compared to those without TB. Of 11 cattle isolates, 18 % were *M. tuberculosis*, 46 % *M. bovis*, and 36 % atypical mycobacteria, indicating possible interspecies transmission.	Alemayehu 2008 [36]
2009	Abattoirs in the rural areas of Gonder, Woldiya, Gimbi, Butajira, and Jinka in Ethiopia	Microscopy Culture Multiplex PCR to detect the presence or absence of RD4, RD9, and TbD1 Spoligotyping	32800	8	Tissue samples	During the years 2006–2008, out of ~32,800 cattle tissues inspected from abattoirs, 4.7 % had suspected TB lesions; cultures revealed *M. bovis* in 58 samples, NTM in 53 samples, and *M. tuberculosis* in 8 samples, all lacking the TbD1 region. Spoligotyping matched seven isolates to known SIT patterns, with most belonging to the Euro-American lineage. Two isolates were identified as Beijing strains based on SIT1 and the RD105/RD181 deletions	Berg 2009 [37]
2009	Single cattle herd, China	CIDTT IFN-ɣ in vitro release test ELISA Post-mortem examination Histopathology Culture Multiplex PCR for species differentiation Standard biochemical assay MIRU VNTR Spoligotyping	130	6	Tissue samples	Of 38 cows that tested positive by TST and IFN-γ assay, six were confirmed as *M. tuberculosis,* and the rest as *M. bovis*. Spoligotyping identified the bovine *M. tuberculosis* isolates as Beijing family strains, and 16-loci MIRU-VNTR analysis showed identical profiles, indicating a common source and a potential epidemiological link between cow and human infections.	Chen 2009 [38]
2010	56 Cattle herds in Addis Ababa City, the Capital of Ethiopia	CIDTT Post-mortem examination Microscopy	1132	3	Tissue samples	A cross-sectional study of 1132 dairy cattle revealed *M. bovis* (8) and *M. tuberculosis* (3)	Tsegaye 2010 [39]

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Table 1 (*continued*)

Year	Sampling location	Screening test	Total no. of animal specimens screened	*M. tuberculosis* positive	Sample type: Tissue/ milk/blood/nasal swab	Comments	References
		Culture Spoligotyping VNTR RD-based PCR				isolated from 11 reactor animals.	
2011	Central Ethiopia	Post-mortem examination Culture 16S rDNA sequence analysis Multiplex PCR RD typing Spoligotyping	–	6	Tissue samples	In this study, 52 mycobacterial isolates were recovered from cattle—30 from an intensive-production farm (Holeta) and 22 grazing cattle from Selalle. All 30 Holeta cattle were infected with *M. bovis*, while 6 of the Selalle cattle harbored *M. tuberculosis*, isolated from mesenteric and retropharyngeal lymph nodes. Notably, a traditional practice in Selalle, chewing tobacco and administering the juice orally to cattle, was suspected to facilitate *M. tuberculosis* transmission from humans. Spoligotyping revealed the cattle isolates belonged to SIT149, a type previously found in humans in the same region, highlighting possible human-to-cattle transmission.	Ameni 2011 [21]
2011	Ibadan, Southwestern Nigeria	Post-mortem examination Microscopy Culture Spoligotyping MLVA	–	1	Tissue samples	Between 2005 and 2007, 74 MTBC isolates were obtained—24 from humans and 50 from animals (cattle, goats, and pigs). *M. bovis* was isolated from two human cases, indicating possible zoonotic transmission. Conversely, *M. tuberculosis* was found in a bovine, a pig, and a goat, suggesting reverse zoonosis. Bovine *M. tuberculosis* isolate had the SpolDB4 type 53 from the T1 family.	Jenkins 2011 [40]
2011	2 cattle herds from Northeast and 4 cattle herds from Northwest China	TST Culture ELISA Multiplex PCR for species differentiation Standard biochemical assay MIRU VNTR Spoligotyping	1067	29	Throat swabs and serum samples	During 2007–2008, out of 1067 TST-positive cattle, 43 MTBC strains (14 *M. bovis* and 29 *M. tuberculosis*) and 68 NTM strains were isolated from throat swabs.	Du 2011 [41]
2011	Three Cattle farms, Spain	TST Post-mortem examination Culture Spoligotyping MIRU VNTR	–	3	Tissue samples	Between 2007 and 2009, across the three farms in Spain, identical spoligotype and MIRU-VNTR patterns between human and cattle isolates confirmed possible human-to-cattle transmission, including the introduction of an MDR SIT2537 strain from an infected farm worker in one farm, while the SIT1564 and SIT58 profiles in the other farms similarly matched strains circulating in their respective human contacts.	Romero 2011 [26]
2011	Achefer, Bahirdar Zuria, and Adet districts, Western Gojam, Ethiopia	CIDTT Culture Microscopy biochemical tests	72	2	Milk samples	Milk samples collected from 51 TB patient households and 21 non-TB households were analyzed, and *M. tuberculosis* was detected in two samples and *M. bovis* in 5 samples, both from TB-affected households. In contrast, no *M. tuberculosis*	Fetene 2011 [19]

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Table 1 (*continued*)

Year	Sampling location	Screening test	Total no. of animal specimens screened	*M. tuberculosis* positive	Sample type: Tissue/ milk/blood/nasal swab	Comments	References
						was found in milk from non-TB households.	
2012	Cattle farm, Croatia	CIDTT Post-mortem examination Culture Genotype MTBC assay Biochemical analysis MIRU VNTR typing	–	1	Tissue samples	During routine surveillance involving TST, a six-month-old heifer in Croatia tested positive and was slaughtered. Though no visible lesions were found, *M. tuberculosis* was isolated from bronchial lymph nodes. MIRU-VNTR typing matched the isolate with two human TB cases, including the heifer's owner, and an unrelated human case, marking a likely human-to-cattle transmission.	Špičić 2012 [42]
2013	Central Ethiopia	CIDTT Post-mortem examination Culture Microscopy Multiplex PCR Spoligotyping	2033	2	Milk and tissue samples	In an Ethiopian study involving 2033 cattle and 287 households, TB prevalence was significantly higher in cattle owned by TB-positive farmers. All 141 human isolates were *M. tuberculosis*, while among 16 cattle isolates, 3 were *M. bovis* and 2 were *M. tuberculosis*. The spoligotype patterns of the two *M. tuberculosis* isolates were SIT149 and SIT53, are common among humans in the area, although they did not match the specific strains of the farmers.	Ameni 2013 [20]
2014	Cattle farm, Ghana, West Africa	CIDTT Post-mortem examination Microscopy Biochemical assay Culture Spoligotyping 16S rRNA sequencing Single Nucleotide Polymorphism analysis	685	2	Tissue samples	Among 685 cattle screened in two Ghanaian farms, 17 (2.48 %) tested positive for bovine TB by CITT. Of these, 6 yielded AFB cultures, including 2 *M. tuberculosis*, 1 *M. africanum*, and 3 NTM. Spoligotyping analysis classified two as *M. tuberculosis* sublineage Latin American Mediterranean (LAM) and Ghana, respectively.	Asante 2014 [43]
2014	Dairy farm, Uttar Pradesh, North India	Post-mortem examination Smear microscopy Histopathology Culture Multiplex PCR and sequencing (Differential diagnosis of *M. tuberculosis* and *M. bovis* based on the deletion of the mce-3 operon in *M. bovis*)	30	8	Tissue samples	*M. tuberculosis* was detected in 8 of 30 bovine lung tissue samples that died of suspected tuberculosis infection, using multiplex PCR and sequencing, indicating possible human-to-cattle transmission.	Mittal 2014 [44]
2015	Teaching and Research Hospital, Madras Veterinary College, Chennai, University Research Farm (Kattupakkam), Slaughter Houses (Perambur, Chennai), and also from individual small-holder farmers in the districts of Dharmapuri and Tanjore, India	Post-mortem examination Microscopy Culture MTBC Genus-specific PCR Multiplex PCR	304	4	Raw milk samples and pre-scapular lymph node biopsy samples	A study of 181 bovine milk and 123 lymph node samples found *M. tuberculosis* in 4 samples via PCR, with only one milk sample culture-positive case, indicating possible reverse zoonosis from humans to cattle.	BhanuRekha 2015 [45]
2015	Çukurova region Slaughterhouse, Turkey	Microscopy Culture Biochemical analysis Spoligotyping	95	13	Tissue samples	In a study of 95 cattle with granulomatous lesions, 13 isolates belonging to the T1 family (SIT53) were identified as *M. tuberculosis* using Spoligotyping.	Tuzcu 2015 [46]

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Table 1 (*continued*)

Year	Sampling location	Screening test	Total no. of animal specimens screened	*M. tuberculosis* positive	Sample type: Tissue/ milk/blood/nasal swab	Comments	References
2016	Three official Abattoirs in Menofia governorate, Egypt	Culture MTBC Genus-specific PCR based on IS*6110* insertion sequence Detection of resistance using GenoType MTBDRplus	68	8	Tissue samples	Among 34 cattle and 34 buffalo examined, 5 (14.7 %) and 3 (8.8 %) tested positive for *M. tuberculosis*, with two cattle strains showing drug resistance to rifampicin and isoniazid. Of 90 abattoir workers, butchers, and meat inspectors from the same abattoirs, tested using blood samples for IgG antibodies, 5.6 % were seropositive for *M. tuberculosis* −6.1 % in butchers and 8.3 % in abattoir workers.	Abdel-Moein 2016 [47]
2016	Cattle farm, Nigeria	SICCT Post-mortem examination Microscopy Culture SD Bioline assay to differentiate MTBC from NTM Hain GenoType MTBC assays	350	1	Tissue samples	During 2010, in a national bovine TB survey, a 2-year-old heifer with strong reactivity to the tuberculin test, along with two other positive cows, was slaughtered for bacteriological investigation. Although no visible TB lesions were present, cultures from lymph nodes yielded AFB. A farm attendant who showed TB symptoms also tested positive. Both the heifer and human isolates were identified as *M. tuberculosis*.	Ibrahim 2016 [22]
2017	Abattoir and postmortem samples, India	Microscopy Culture Biochemical analysis MTBC Genus-specific PCR based on IS*6110* insertion Spoligotyping Multiplex PCR	148	43	Milk and tissue samples	Out of 148 cattle samples from emaciated cattle suspected of tuberculosis, and also from the caseous nodular lesions from abattoir and postmortem samples, 67 cultures yielded 51 MTBC isolates—43 (84.3 %) were *M. tuberculosis* and 8 (15.6 %) *M. bovis*. Spoligotyping of 31 *M. tuberculosis* isolates revealed that 28 belonged to the MANU1 strain. The study highlights significant *M. tuberculosis* infection in cattle, likely due to human spillover in endemic areas.	Sweetline 2017 [48]
2017	Two epidemiologically unrelated farms, Eastern Cape, South Africa	CIDTT Post-mortem examination Microscopy Culture VNTR MTBC genus-specific PCR PCR using primers targeting 3 regions of difference (RD4, RD9, and RD12)	28	2	Tissue samples	This first report of *M. tuberculosis* infection in cattle in South Africa identified strains from two unrelated farms using RD-PCR and VNTR typing. Although no human strain matches were found, the study highlights the need for One Health-based surveillance	Hlokwe 2017 [49]
2018	Farms and Abattoirs, Telangana, Maharashtra, and Gujarat, India	SIT Microscopy Culture nested PCR kit based on the IS6110 MTC-specific nucleotide sequence Spoligotyping	841	7	Bovine Milk, Nasal swabs, and post-mortem tissue samples	From 2010 to 2015, Mycobacterial infection was screened from 841 bovine samples—825 from cattle and 16 from buffaloes—in Telangana, Maharashtra, and Gujarat. Seven *M. tuberculosis* isolates were confirmed by IS*6110*-based PCR. Spoligotyping identified human-associated lineages including EAI3_IND, EAI5, CAS1_DELHI, U, and T1, indicating possible human-to-animal transmission.	Mukherjee 2018 [50]

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Table 1 (*continued*)

Year	Sampling location	Screening test	Total no. of animal specimens screened	*M. tuberculosis* positive	Sample type: Tissue/ milk/blood/nasal swab	Comments	References
2018	Gusau abattoir, Zamfara State, northwest of Nigeria	Microscopy Culture RD deletion typing	226	1	Tissue samples	This study investigated tubercle bacilli in 226 slaughtered cattle with TB-like lesions in Zamfara State, Nigeria. Ziehl-Neelsen staining confirmed AFB in 16.4 % (37/ 226) of cases, and MTBC was detected in 91.9 % (34/37) of these. Molecular typing identified 30 *M. bovis*, 3 *M. caprae*, and 1 *M. tuberculosis* isolates.	Ahmad 2018 [51]
2019	Ebonyi State, South-Eastern Nigeria	Culture RD deletion typing Spoligotyping MIRU-VNTR	144	2	Milk samples	This study investigated zoonotic TB transmission between pastoralists and their cattle. Among 149 human sputum and 144 cattle milk samples, 54 MTC isolates were identified (*M. tuberculosis*: 42 humans, 2 cattle; *M. bovis*: 1 human; *M. africanum*: 9 humans). Spoligotyping revealed dominant Uganda I/ SIT46 strains (59.2 %), followed by LAM/SIT61 (16.3 %) and T/SIT53 (2.0 %). MIRU-VNTR typing showed identical patterns in Uganda I/SIT46 strains from one cow and nine pastoralists, suggesting potential cross-species transmission.	Adesokan 2019 [8]
2019	Ebonyi and Sokoto States, Nigeria	Post-mortem examination Culture RD deletion typing Spoligotyping	1905	2	Milk and tissue samples	This study genotyped 64 MTBC isolates from livestock workers (n = 47) and cattle (n = 17; milk = 2, lesion = 15) across three Nigerian states. Among these, 42 were *M. tuberculosis*, 13 *M. bovis*, and 9 *M. africanum*. Notably, *M. tuberculosis* was identified in 2 cattle samples (1 from milk, 1 from a lesion), with the milk isolate belonging to the Uganda I/SIT46 family, a rare lineage.	Adesokan 2019 [16]
2019	Farm Chennai, India	Post-mortem examination Microscopy Culture DST Spoligotyping WGS	167	4	Tissue samples	This cross-sectional study in Chennai, India, screened 271 livestock handlers and 167 cattle for tuberculosis, using WGS to assess isolate relatedness. *M. tuberculosis* was isolated from 6 handlers and 4 cattle across two farms; *M. bovis* was not detected. All isolates belonged to Lineage 1. Three isolate pairs (two human-human, one cattle-cattle) showed close genetic similarity (<5 SNPs). One *M. tuberculosis* isolate from cattle was multidrug-resistant, while no drug resistance was detected among the human isolates.	Palaniyandi 2019 [9]
2019	Environment of pastoralists and villagers in the Iringa district, adjacent to the Ruaha National Park in Tanzania	FastDNA spin kit – DNA extraction from cattle Faecal and Boma samples Quantitative PCR targeting RD4 and RD9 regions	764	54	Cattle faeces Cattle boma	Between the 2012 dry season and 2014 wet season, *M. tuberculosis* was detected in 7.1 % (28/394) and 7.0 % (26/ 370) of cattle faeces, respectively, with no significant seasonal difference. One cow was positive for both *M. tuberculosis* and *M. bovis*. In	Emma 2019 [17]

(*continued on next page*)

Table 1 (*continued*)

Year	Sampling location	Screening test	Total no. of animal specimens screened	*M. tuberculosis* positive	Sample type: Tissue/ milk/blood/nasal swab	Comments	References
						cattle boma soil, only 1.3 % (1/ 75) tested positive for *M. tuberculosis*, linked to a household with infected cattle.	
2021	Three state dairy farms (Adaberga, Bishoftu, and Holeta), Ethiopia	SICCT Post-mortem examination Culture Pathology Scoring Spoligotyping	720	2	Tissue samples	Out of 720 dairy cattle screened using the SICCT test, 108 suspicious tissues were cultured, yielding 14 *M. bovis* (SB0912) and 2 *M. tuberculosis* (SIT54) isolates, confirming both species in bovine TB lesions. SIT54 is one of the frequent *M. tuberculosis* spoligotypes isolated from humans in central Ethiopia, further supporting the likelihood of human-to-cattle transmission in this setting.	Ambaw 2021 [52]
2021	Dairy in New Mexico, US	TST Post-mortem examination Culture Spoligotyping WGS	–	1	Tissue sample	In early 2018, a 1-day-old heifer calf was transported from a dairy in New Mexico to Texas, where post-import TB testing is required. At 4 months of age, the calf tested positive for TB. *M. tuberculosis* (sub lineage 4.2.2) was cultured from its retropharyngeal lymph node despite no visible lesions. The isolate matched a rare spoligotype (007000024000000) also found in three human TB cases in the region, indicating probable human-to-cattle transmission; however, the exact source could not be confirmed because WGS data from the human cases were unavailable for comparison.	Lombard 2021 [53]
2022	Abattoir of Tanta, centre of the Nile delta, Egypt	Microscopy Histopathology Culture RT-PCR targeting all members of MTBC MPB70-targeting PCR and sequencing	750	1	Tissue samples	Post-mortem examination of 750 (569 cows and 181 buffalo) tissue samples in Tanta abattoir identified nine *M. bovis* strains and, interestingly, one *M. tuberculosis* MDR strain was isolated from a buffalo.	Borham 2022 [12]
2022	Abattoir, Rwanda	Microscopy Culture PCR targeting MPB 70 antigen, 16s rRNA sequence RD-based PCR GeneXpert/MTB/RIF assay	300	1	Tissue samples, including tonsils	A cross-sectional study of 300 cattle from six abattoirs identified *M. bovis* in 4 cases and rifampicin-resistant *M. tuberculosis* in 1 case, with NTM detected in 36 samples.	Ntivuguruzwa 2022 [27]
2022	Maiduguri Abattoir, Borno State, Nigeria	Post-mortem meat inspection for TB-like lesions Culture Microscopy SD-BiolineTB Ag MPT64 GenoType MTBC assay	1003	2	Tissue samples	Out of 1003 cattle slaughtered in the Maiduguri abattoir, 51 showed TB-like lesions. Genotype MTBC identified 44 (86.3 %) as *M. bovis*, 3 (5.9 %) as *M. tuberculosis*, and 2 (3.9 %) as *M. africanum*, with no significant difference between sexes.	Bello 2022 [54]
2024	Slaughterhouse, Lahore -Pakistan	Post-mortem inspection of carcasses Microscopy Culture PCR for MTBC differentiation WGS	3581	8	Tissue samples	Between Nov 2021–Mar 2022, this slaughterhouse study identified 10 *M. orygis* and 8 *M. tuberculosis* sensu stricto isolates from 3581 slaughtered animals (441 cattle and 3140 buffalo), while no *M. bovis* was detected in any of the examined specimens	Maqsood 2024 [55]

(*continued on next page*)

Table 1 (*continued*)

Year	Sampling location	Screening test	Total no. of animal specimens screened	*M. tuberculosis* positive	Sample type: Tissue/ milk/blood/nasal swab	Comments	References
2024	Slaughterhouse in Chennai, India	Microscopy Culture Histopathology PCR targeting MPT64 antigen DST WGS	567	3	Tissue samples	Over 3 years, 567 samples were collected from 500 animals, including various lymph nodes and organs. WGS of 16 MTBC-positive samples identified 13 *M.* orygis and 3 *M. tuberculosis.* One of the *M. tuberculosis* isolates had a mixed infection with *M. tuberculosis* Lineage 1 and *M. orygis;* the remaining Two *M. tuberculosis* isolates showed pure growth of *M. tuberculosis* Lineage 2, both of which were pre-extensively (pre-XDR) drug-resistant.	Ramanujam 2024 [11]
2024	Central Ethiopian slaughterhouse	Post-mortem examination Microscopy Culture Multiplex RT- PCR detecting speciation of MTBC	7640	1	Tissue samples	Out of 7640 slaughtered cattle examined, 173 animals had tuberculosis-like lesions (TBLs), from which 388 tissue samples were collected. Of these, 94 TBLs were culture and multiplex RT-PCR-positive. All were identified as infected with *M. bovis*. Among them, two animals had mixed infections—one zebu cattle with both *M. bovis* and *M. caprae*, and one crossbred cow with *M. bovis* and *M. tuberculosis*.	Fromsa 2024 [56]
2025	Slaughterhouse, Wolaita, Ethiopia	Microscopy Culture Histopathology Loopamp™ LAMP MTBC kit targets in *gyrB* and IS*6110* loci of MTBC PCR targeting the RD4 and RD9 loci of the MTBC WGS	2251	1	Tissue samples	This study investigated bTB in 2251 cattle from four abattoirs in the Wolaita zone, identifying tuberculous lesions in 122 animals. Of 180 tissue samples processed, 18 were culture-positive. WGS confirmed *M. bovis* in four cases and *M. tuberculosis* lineage 4 in one, suggesting possible human-to-animal transmission.	Zaba 2025 [18]

Abbreviations: SIT- Single intradermal tuberculin test; AFB- Acid fast bacilli; RFLP- Restriction fragment length polymorphism; NTM- Non-tuberculosis Mycobacteria; RD deletion- Region of difference deletion; MIRU-VNTR- Mycobacterial interspersed repetitive unit variable number tandem repeats; IFN-γ- Interferon- γ; PCR- Polymerase chain reaction; TST- Tuberculin skin test; CIDTT- Comparative intradermal tuberculin test; SICCT- Single intradermal cervical comparative tuberculin; MLVA- Multi-locus Variable-number tandem repeat; RT-PCR- Real time PCR; N-PCR- Nested PCR; ELISA- Enzyme- Linked Immunosorbent Assay; DST- Drug sensitivity test; RIF- rifampicin; WGS- Whole-genome sequencing; EDTA- Ethylene diamine tetra-acetic acid.

M. tuberculosis $H_{37}Rv$ or *M. bovis* AF2122/97, and the results were evaluated 17 weeks after infection. Both strains triggered strong IFN-γ and tuberculin responses; however, only cattle infected with *M. bovis* developed visible pathology, while *M. tuberculosis* persisted at low levels without causing lesions. These results confirm the reduced virulence of *M. tuberculosis* in cattle and emphasize the importance of host immune status and bacterial genotype in determining infection outcomes, laying the groundwork for exploring host preference and virulence mechanisms in *M. bovis* [24]. Building on earlier research, another study examined whether an *M. tuberculosis* isolate (BTB1558) from an Ethiopian bull would provoke similar immune and pathological responses in cattle as the $H_{37}Rv$ reference strain. Both *M. tuberculosis* strains remained significantly attenuated compared to *M. bovis* AF2122/97, causing minimal pathology and granulomas that largely halted at early stages, despite persisting in host tissues for over 10 weeks. Peripheral blood immune responses were generally similar across infection groups, but RNA-Seq revealed distinct gene expression profiles in *M. bovis*-infected cattle, indicating accelerated disease progression [25]. Interestingly, Palaniyandi et al. (2019) found that natural *M. tuberculosis* infection in cattle caused by a lineage 1 strain could lead to active pathology, including visible lesions in the lymph nodes, lungs, spleen, and udder. This contrasts with experimental models and may be attributed to several factors: only certain *M. tuberculosis* strains have been tested in experimental models, mainly lineage 4 strains like $H_{37}Rv$ and BTB1558, which may affect observed pathogenicity in cattle; experimental infections are time-limited, whereas livestock may live longer, allowing disease progression; natural infections often involve repeated exposure and diverse entry routes; there may be species-specific susceptibility differences and the age of cattle varies, as experimental animals are typically six months old. Additionally, variations in host immune response or pathogen virulence, as well as co-infections with other pathogens, may further influence susceptibility and enable productive infections [9,26]. Collectively, these findings highlight the complexity of *M. tuberculosis* infection in cattle and the necessity of considering both experimental and natural contexts when evaluating virulence and disease outcomes.

However, the presence of *M. tuberculosis* in cattle, particularly drug-resistant strains, is a significant concern as it underscores the issue of reverse zoonosis and suggests the potential for livestock to become secondary reservoirs of drug-resistant tuberculosis. Notably, studies

from Chennai, India, have documented drug-resistant *M. tuberculosis* infections in cattle. A 2019 study conducted on a farm used whole genome sequencing (WGS) to identify Lineage 1 isolates in both cattle and their handlers, with one bovine isolate confirmed as multidrug-resistant (MDR) [9]. In a 2024 study at a slaughterhouse, three *M. tuberculosis* isolates were found—two Lineage 2 isolates from tissue samples were pre-extensively drug-resistant (pre-XDR), and one was a mixed infection involving Lineage 1 and *M. orygis* [11]. Similar results have been reported from other areas: a 2022 post-mortem study at a slaughterhouse in Tanta, Egypt, discovered an MDR *M. tuberculosis* lineage 2 strain in buffalo tissue, and a 2022 abattoir-based study in Rwanda identified a rifampicin-resistant *M. tuberculosis* strain in cattle tissue [12,27]. These findings collectively highlight the urgent emergence of drug-resistant *M. tuberculosis* in livestock populations in both farm and slaughterhouse environments, posing a significant public health risk within the One Health framework.

3. Diagnostics: methods and challenges

3.1. Sampling strategies

Effectively identifying *M. tuberculosis* and differentiating it from *M. bovis* and other MTBC species in bovine samples remains a significant challenge, particularly in regions with limited resources. The types of samples collected from cattle suspected of having TB can vary by location, with non-invasive methods often involving the collection of milk, faeces, nasal swabs, and environmental samples from cattle sheds [17,33]. Although intermittent shedding has been demonstrated primarily for *M. bovis* and has not been characterized in natural *M. tuberculosis* infections in cattle, the low bacillary load typical of bovine *M. tuberculosis* cases means that non-invasive samples may still fail to detect the pathogen consistently, limiting both diagnostic sensitivity and species confirmation [57,58]. Moreover, Several studies in Table 1 relied on such non-invasive samples, which may not reliably capture low-level or sporadic shedding, possibly leading to under-detection and confirmation of *M. tuberculosis* in cattle.

When animals react positively to blood-based immunological tests and subsequently die, are culled, or are slaughtered due to TB suspicion, post-mortem tissue samples can then be collected to facilitate confirmatory diagnosis. Blood supports field-level screening using the Single Intradermal Tuberculin Test, Comparative Cervical Tuberculin Test, and Interferon-Gamma Release Assays, which indicate MTBC exposure but cannot differentiate *M. tuberculosis* from other MTBC species [38]. Slaughterhouse investigations offer a passive surveillance method for bTB detection, as all lung lobes and major lymph nodes that drain the respiratory and gastrointestinal tracts, including the retropharyngeal, mandibular, mediastinal, hepatic, bronchial, and mesenteric lymph nodes, can be systematically examined using lesion-scoring protocols [20,59]. However, this approach is limited as only animals entering the slaughter chain are evaluated, clinical histories are often unavailable, traceback to the farm of origin is usually not possible, and infected animals may sometimes lack visible gross lesions at post-mortem, complicating detection [56]. Accordingly, studies relying solely on slaughterhouse tissues in Table 1 may have under-detected early infection or no lesion samples, influencing diagnostic yield and the strength of the conclusions drawn.

3.2. Histopathological, microscopic, and biochemical approaches

Histopathology of bovine tissue sections using haematoxylin–eosin (H&E) staining can identify granulomatous lesions with epithelioid macrophages, Langhans giant cells, and caseous necrosis, but these features are not specific to MTBC; similar pathology can occur with NTM and other actinomycetes, limiting its utility for species-level differentiation [60]. Microscopy-based detection of acid-fast bacilli also lacks discriminatory power. Ziehl–Neelsen staining may fail to detect *M. tuberculosis* in cattle due to low bacillary load, as demonstrated by Ramanujam et al. (2024), where several culture-confirmed *M. tuberculosis* isolates were smear-negative [11]. Auramine O staining with fluorescence microscopy, as described by Jean Bosco Ntivuguruzwa et al. (2022), has also been applied to bovine specimens [27]. Culture on LJ or Middlebrook media enables isolation of MTBC from milk, swabs, or tissues, as shown by Mukherjee (2018) and Adesokan et al. (2019), yet culture characteristics alone cannot differentiate *M. tuberculosis* from other MTBC species [16,50].

Earlier studies also relied on biochemical profiling, such as niacin accumulation, nitrate reduction, pyrazinamide susceptibility, and aerobic growth in the Lebek test, as well as resistance to thiophene-2-carboxylic acid hydrazide (TCH), along with additional assessments such as catalase activity, aryl sulfatase activity, and Tween hydrolysis to support *M. tuberculosis* identification from other MTBC species such as *M. bovis* and *M. africanum*. While the majority of these reactions aligned with the expected *M. tuberculosis* profile, variations were noted in certain tests, and the limited discriminatory power of these assays across MTBC species substantially reduces their reliability and increases the risk of species misclassification [32,61].

3.3. Molecular diagnostics

3.3.1. Conventional PCR-based approach

PCR assays targeting IS*1081* and IS*6110* have been widely used as initial confirmatory tests for MTBC detection in bovine samples. IS*1081* is conserved across all MTBC members, whereas IS*6110* shows variable copy numbers—typically higher in *M. tuberculosis*, although strains with a single copy or none have also been reported [62]. Early molecular studies used single-gene targets such as *hupB*, which contains a characteristic 27-bp deletion in *M. bovis* relative to *M. tuberculosis*, and assays targeting deletions within the mce3 operon, which are specifically absent in *M. bovis*, to distinguish these two species in bovine isolates. While useful at the time, these assays were limited because they only distinguished *M. tuberculosis* from *M. bovis* and could not resolve other MTBC members [31,44]. This is particularly relevant for bovine investigations today, as additional species within the complex, such as *M. orygis*, which has been reported in parts of South Asia, would not be detected using these earlier PCR methods [63].

Regions of difference (RD) are stable, vertically inherited polymorphic markers that form the basis for phylogenetic classification and species identification within the MTBC [2], as illustrated in (Fig. 4). Conventional or multiplex PCR studies on bovine-derived samples have typically targeted RD1, RD4, RD9, and RD12 to discriminate mainly between *M. tuberculosis* and *M. bovis*, limiting resolution for other MTBC members [37,49]. More recently, as described by Fromsa et al. (2024), multiplex PCR methods combine conserved IS targets (IS*6110*/IS*1081*) for broad MTBC detection with RD- and SNP-based markers—such as RD4, yrbE3A, RD7, *lepA*, and *rskA*—allowing simultaneous differentiation of *M. tuberculosis*, *M. bovis*, *M. orygis*, and *M. caprae*. Nevertheless, these approaches may fail to detect rare or emerging variants, restricting comprehensive species-level resolution [6].

3.3.2. Genotyping approaches for Human–Cattle transmission analysis

Genotyping tools have been central to investigating potential transmission of MTBC strains between humans and cattle. Earlier studies relied on IS*6110* RFLP, spoligotyping, and MIRU-VNTR, which enabled preliminary comparison of lineage patterns between human and bovine isolates and provided the first clues of possible cross-species transmission. With the advent of WGS, higher-resolution insights have become possible, allowing more definitive assessment of transmission links, species identification, and drug-resistance profiles [9,26,32].

3.3.2.1. IS6110 RFLP. IS*6110* RFLP played a crucial role in strain-level fingerprinting by analyzing the numbers and position of IS*6110*

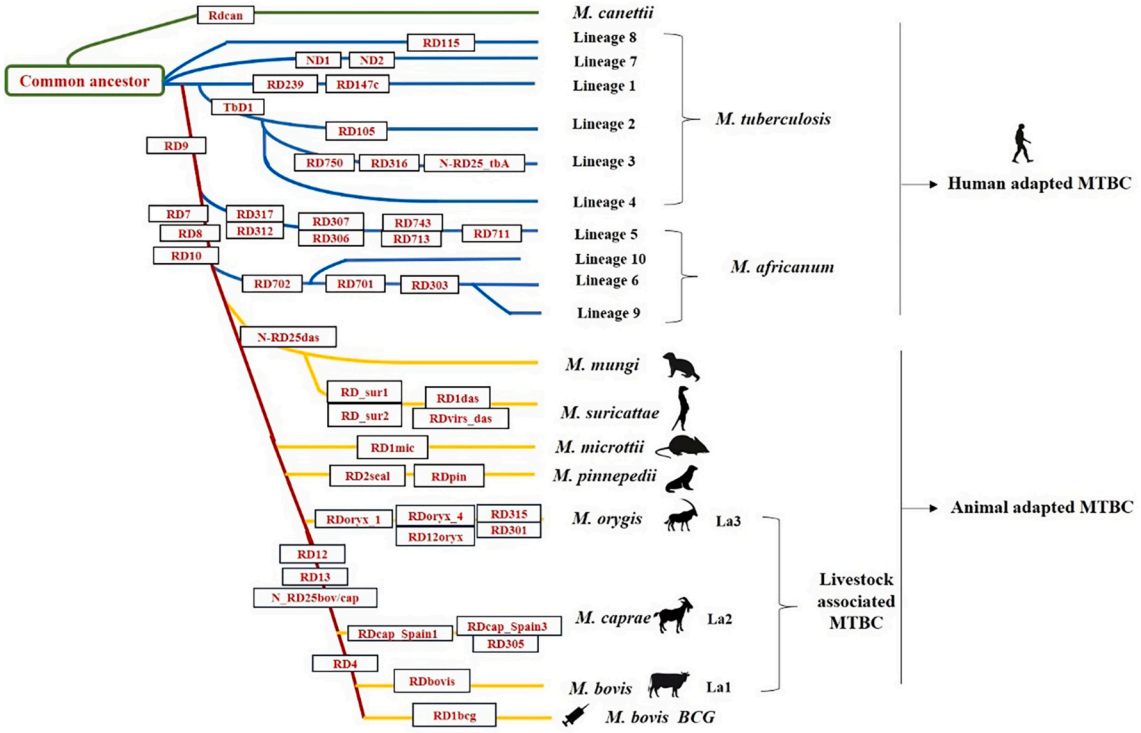

Fig. 4. Evolutionary scheme of *Mycobacterium tuberculosis* complex species based on Region of difference.
This schematic illustration depicts the evolutionary relationships within the MTBC, reconstructed from Region of Difference (RD) deletion patterns previously reported by Bespiatykh et al. (2021) following the advent of whole-genome sequencing (WGS). Each box represents a specific RD deletion event, and the branching structure shows how successive deletions have given rise to distinct MTBC human-adapted lineages (including *M. tuberculosis* Lineages 1–4, 7, and 8, and *M. africanum* Lineages 5, 6, 9, and 10) and animal-adapted subspecies (including *M. bovis*, *M. caprae*, *M. orygis*, *M. microti*, *M. mungi*, *M. suricattae*, and *M. pinnipedii*).

elements within the genome of *M. tuberculosis*. A notable instance of human-to-cattle transmission was documented in a Cattle farm in, Slovenian capital, Ljubljana, when a routine check found that a two-year-old cow was infected with *M. tuberculosis*. The IS*6110* RFLP analysis revealed an exact pattern match between the bovine isolate and a strain from a farm worker who had previously undergone TB treatment, marking the first molecular proof of direct transmission from humans to cattle. Nevertheless, the method's limited resolution and the necessity for high-copy-number IS*6110* elements restricted its broader use, leading to a growing preference for Spoligotyping [32,64].

3.3.2.2. Spoligotyping. Spoligotyping, which relies on detecting the presence or absence of spacer sequences in the Direct Repeat region of *M. tuberculosis*, facilitates the assignment of lineages and clustering [65]. For instance, a 2013 study conducted in central Ethiopia, involving 2033 cattle from 287 households, found a higher prevalence of TB in animals owned by farmers who tested positive for TB. Two strains of *M. tuberculosis*, identified as SIT149 and SIT53, were isolated from five cattle and are common among humans in the area, although they did not match the specific strains of the farmers, indicating possible environmental exposure through contaminated feed, sputum, or urine [20]. Similarly, a study conducted in an abattoir in India, which examined 148 animals that were either emaciated or had lesions, discovered that 28 out of 31 *M. tuberculosis* isolates were associated with the MANU1 lineage, which is prevalent among humans in Mumbai and southern Andhra Pradesh. It was suggested that human spillover was the probable source, as most of the animals had been transported from Andhra Pradesh [32].

3.3.2.3. MIRU VNTR. MIRU-VNTR typing, which examines variable-number tandem-repeat loci throughout the *M. tuberculosis* genome, provides enhanced resolution within lineages and has been utilized to explore epidemiological connections between bovine and human isolates. In Croatia, a six-month-old heifer that tested positive for TST was slaughtered; despite the absence of visible lesions, *M. tuberculosis* was isolated from its bronchial lymph nodes. MIRU-VNTR matched this isolate with two human TB cases, including the heifer's owner, suggesting probable human-to-cattle transmission [42]. Similarly, in Ebonyi State, Nigeria, MIRU-VNTR analysis of 44 *M. tuberculosis* isolates (42 human, 2 cattle) revealed identical Uganda I/SIT46 patterns in one cow and nine pastoralists, supporting potential cross-species transmission [8].

While both Spoligotyping and MIRU VNTR tools are informative for shared lineage detection, their limited discriminatory power and susceptibility to homoplasy remained insufficient for fine-scale transmission mapping, prompting the adoption of WGS [66,67].

3.3.2.4. WGS. WGS now offers the most detailed resolution for genotyping the MTBC. This method facilitates the use of SNP comparisons to evaluate genetic distances and deduce transmission pathways. Furthermore, it allows for the classification of lineages, species identification, and the detection of virulence and drug-resistance markers. In a farm-based investigation, WGS was utilized to examine 10 *M. tuberculosis* lineage 1 isolates from cattle and their handlers, revealing three pairs of isolates with fewer than five SNP differences. Among these, two highly related cattle isolates suggest possible cattle-to-cattle transmission; however, independent infection from a human handler not captured in the study remains plausible [9]. Similarly, lineage 2 *M. tuberculosis* isolates from a Chennai slaughterhouse clustered within seven SNPs, suggesting either human-to-cattle spillover or localized transmission among cattle. These findings emphasize the importance of integrating genomic and field data to interpret cross-species transmission accurately [11].

Drug-resistance detection in cattle has similarly evolved. Earlier

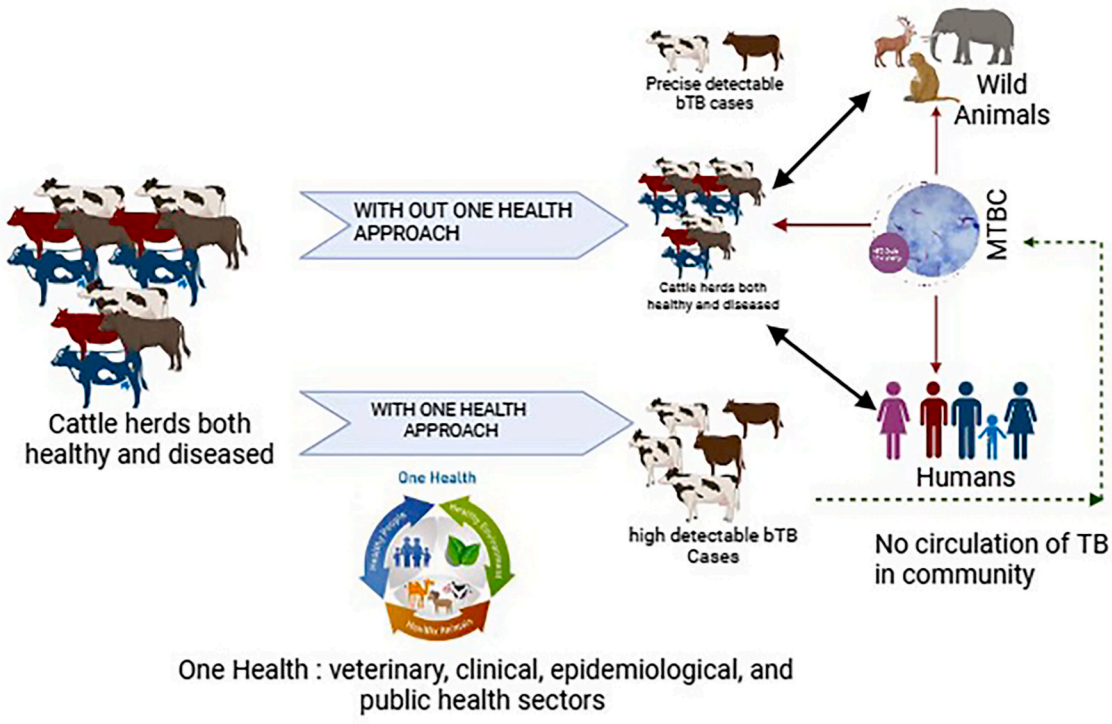

Fig. 5. Importance of the One Health approach to control and prevent TB in cattle
This schematic illustrates how the integration of veterinary, clinical, epidemiological, and public health sectors enhances the detection and control of bovine tuberculosis (bTB). Without a One Health approach, TB transmission between cattle, wildlife, and humans remains largely undetected, allowing persistent circulation of MTBC across species. In contrast, implementing a coordinated One Health strategy enables earlier identification of infected cattle, improved surveillance at the livestock–wildlife–human interface, and more effective containment of MTBC transmission.

approaches, such as GeneXpert/MTB/RIF, were limited to rifampicin resistance, as exemplified by a Rwanda abattoir study reporting a rifampicin-resistant *M. tuberculosis* isolate from cattle tissue [27]. With WGS, comprehensive genotypic DST is now possible. Using TB-Profiler, two lineage 2 *M. tuberculosis* isolates were classified as pre-XDR, with predicted resistance to streptomycin, isoniazid, rifampicin, pyrazinamide, levofloxacin, and moxifloxacin. Although these isolates were expected to be ethambutol-susceptible, two *embB* mutations of uncertain significance were detected, and phenotypic DST confirmed all predicted resistances except ethambutol [11]. In a Chennai farm-based study, WGS identified mutations in *rpoB* and *katG* that conferred rifampicin and isoniazid resistance, consistent with the phenotypic results. Unlike GeneXpert, which identifies only rifampicin resistance, WGS enables full resistance prediction across first- and second-line drugs, detects additional mutations of uncertain significance, and corroborates phenotypic DST [9].

However, interpretation of transmission dynamics remains limited by region-specific constraints, LMICs, where the high cost of WGS prevents its routine implementation. As a result, many surveillance programs continue to rely on lower-resolution tools such as spoligotyping and MIRU-VNTR. Strengthening capacity through centralized sequencing hubs and the use of low-coverage or targeted sequencing could help reduce costs and make higher-resolution genomic analysis feasible, particularly in high TB-burden regions.

4. Towards effective surveillance and control of *M. tuberculosis* infection in cattle

While bovine TB control programs have traditionally focused on *M. bovis*, evidence shows that *M. tuberculosis* can infect cattle in regions with a high human TB burden, particularly where humans and livestock interact closely, highlighting gaps in surveillance systems not designed to detect human-adapted MTBC strains. The detection of MDR and pre-XDR *M. tuberculosis* in cattle emphasizes the critical need for enhanced surveillance and collaborative control efforts at the human-animal boundary.

4.1. Diagnostic preparedness and species-level identification

In the past, the use of phenotypic and biochemical methods masked the variety of MTBC lineages found in cattle, postponing the identification of infections from strains that have adapted to humans [15]. The advent of molecular techniques such as RD-based PCR, spoligotyping, MIRU-VNTR, and notably WGS has revealed that cattle can carry human-associated *M. tuberculosis* lineages, which are sometimes closely linked to strains found in local human populations [9,18]. By integrating species-level identification into routine monitoring, particularly in regions with high infection rates, and by standardizing molecular diagnostics across different laboratories, we can enhance the comparability of research, promote the early detection of drug-resistant strains, and improve the understanding of epidemiological connections.

4.2. Abattoir surveillance as a critical detection platform

Several cases discussed in this review were discovered solely through post-mortem examinations, highlighting the ability of slaughterhouse surveillance to identify infections that might be overlooked by live-animal testing. However, its effectiveness is hindered by issues such as incomplete traceability, inconsistent record-keeping, and variations in tissue sampling. Despite these obstacles, abattoirs play a crucial role in detecting human-to-cattle spillover events and should be systematically integrated into national TB surveillance programs, particularly in areas where routine herd-level testing is not conducted [59].

4.3. Contextual control strategies for reverse zoonosis

As *M. tuberculosis* has not been proven to facilitate direct transmission between cattle, addressing reverse zoonosis necessitates a strategy centered on humans rather than conventional methods for eradicating bovine TB. Essential measures include prompt diagnosis, effective treatment, and TB monitoring among farmers, livestock handlers, and abattoir workers, who are the main sources of cattle exposure. Enhancing hygiene in animal shelters, improving ventilation, and adopting occupational safety practices can further mitigate transmission risks. In herds where human TB exposure is confirmed, targeted surveillance and, when possible, test-and-segregate strategies offer viable alternatives to culling, especially in areas where culling poses cultural or economic difficulties [13]. In the nations listed in Table 1, which are mainly located in Africa and South Asia, the identification and management of MTBC infections in cattle are largely dependent on passive surveillance at abattoirs, opportunistic diagnostic testing, and the effectiveness of human TB control programs, rather than on organized livestock-based eradication systems. These areas often lack national cattle identification systems, compensation mechanisms, regular intradermal testing, and movement control measures, making standardized bTB eradication programs impractical [20,68,69]. This situation is markedly different from the comprehensive test-and-slaughter, traceability, and wildlife management strategies used in high-income countries like Australia and New Zealand, where *M. bovis* has been successfully eradicated [70].

4.4. Drug-resistant M. tuberculosis in livestock environments

While cattle are typically seen as epidemiologically dead-end hosts, the presence of drug-resistant *M. tuberculosis* in livestock environments presents a possible occupational risk for veterinarians, slaughterhouse workers, and animal handlers [60]. Although direct transmission from cattle to humans has not been recorded, the lack of comprehensive genomic research leaves this risk ambiguous. These observations underscore the urgent need to incorporate antimicrobial resistance(AMR) monitoring and routine drug susceptibility testing in bovine samples, especially in regions with a high incidence of resistant human TB. Consequently, adopting a One Health strategy that combines human, animal, and environmental health is crucial for the early identification, coordinated monitoring, and effective management of MTBC transmission at the human–cattle interface [3], as depicted in (Fig. 5).

5. Conclusion

Cattle can host *M. tuberculosis* strains that have adapted to humans, illustrating the significant connection between human and animal health. Molecular evidence indicates that MDR and pre-XDR variants are capable of infecting cattle, yet there is no documented evidence of continuous cattle-to-cattle transmission or transmission back to humans, highlighting crucial knowledge gaps and intervention opportunities. These findings stress the necessity of integrating surveillance with species-specific MTBC identification, comprehensive genomic AMR monitoring, and coordinated One Health strategies. By linking human, veterinary, and environmental health efforts, early detection of reverse-zoonotic events is possible, reducing occupational and public health risks. Thus, effective control of *M. tuberculosis* in cattle depends on strong human TB management and proactive, cross-sector interventions, which collectively support both animal and human health and advance global TB elimination goals.

CRediT authorship contribution statement

Vaishnavi Vivekanandan: Writing – original draft, Visualization, Methodology, Formal analysis, Data curation. **Arun K:** Writing – original draft, Methodology, Formal analysis. **Sindhu Hasini Doredla:** Methodology, Formal analysis, Data curation. **Harini Ramanujam:** Methodology, Investigation, Data curation. **Ranjani Singaraj:** Methodology, Formal analysis, Data curation. **Kannan Palaniyandi:** Writing – review & editing, Writing – original draft, Visualization, Resources, Funding acquisition, Conceptualization.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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