

Burden of *Mycobacterium tuberculosis* in Animals Versus *M. bovis* in Human

Abstract

M. tuberculosis and *M. bovis* are member of Mycobacterium complex (MTBC). *M. tuberculosis*, a primarily pathogen of human tuberculosis (TB) can infect cattle and vice versa *M. bovis* can infect human beings as well as animals with progressive disease. The burden of either of infections is not known in human and animals. *M. tuberculosis* does not cause over disease in cattle, however, *M. bovis* cause TB in human indistinguishable from *M. tuberculosis*. This review provides detailed information about the status of *M. tuberculosis* infection in cattle and the magnitude of cases wherein *M. tuberculosis* was isolated from animals.

Keywords: Tuberculosis; *M. tuberculosis* infection; Cattle; BTB

Review Article

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Introduction

It appears interesting to write on *Mycobacterium tuberculosis*, primarily pathogen of human tuberculosis (TB) and *M. bovis*, primarily pathogen of cattle (BTB), other domestic animals and wild life in context of burden of these two closely related species in human and animals. The common notion in the mind of researchers has been their strong believe that *M. tuberculosis* does not infect cattle vice-versa *M. bovis* seldom infects human. As a result of this notion, laboratories handling human sample only cultivates them on Lowenstein-Jensen (LJ) medium containing glycerol but not onto LJ medium containing 0.5% sodium pyruvate for cultivation of *M. bovis*. This appears one of the strongest reason that neither burden of *M. tuberculosis* in animals nor burden of *M. bovis* is precisely known or reported anywhere in the world except of the fact both of these closely related species have been reported both in human and animals. They are members of *M. tuberculosis* complex (MTBC) which includes human and animal-associated pathogens. Other members of MTBC include *M. caprae* (goats), *M. microti* (voles), *M. pinnipedii* (seals), *M. mungi* (infecting mongoose), *M. orygis* (antelopes) and the chimp bacillus. The phenotypic similarity is less than 65% among the members of MTBC, there is about 99.9% genetic identity observed at deoxyribonucleic acid (DNA) level [1] and 99.95% at the nucleotide level [2]. *M. tuberculosis* may have developed through the domestication of cattle infected with *M. bovis*, some 15 to 20 thousand years ago. *M. tuberculosis* can also infect cattle; however, it is not as virulent as *M. bovis* in cattle [3,4]. *M. tuberculosis* causes less severe disease in cattle than that caused by *M. bovis* [5] and usually causes minor non-progressive infections in domestic cattle [6]. Not much is known about the prevalence of *M. tuberculosis* in its spill over hosts. *M. tuberculosis* infected animals including cattle react positively when subjected to tuberculin skin testing (TST); however, the infection seems to vanish rather quickly and does not normally

lead to a progressive disease [7-9]. Ayele et al. [10] reported human to cattle transmission of *M. tuberculosis* and according to them, the isolation of *M. tuberculosis* from any species other than human, especially from cattle, was interesting and important. The infective dose of *M. bovis* is low with studies demonstrating less than 10 viable bacilli are sufficient to cause infection [11]. Cattle infected with *M. tuberculosis* by tuberculous owners can be sensitized to tuberculin and develop a false-positive reaction for *M. bovis* infection [12].

Several workers have reported *M. tuberculosis* in domestic and wild animals, most frequently living in close contact with humans [13-17]. Among domestic animals, infection with *M. tuberculosis* has most frequently been identified in cattle [7,12,18]. The only reported cases of *M. tuberculosis* in cattle in western Europe were described in Great Britain and dates back to the 1950s [19]. Recent studies have reported isolation of *M. tuberculosis* in cattle with prevalences of 4.7%-30.8% in African and Asian countries [20-22]. Isolation of *M. tuberculosis* from cattle in countries that include India, Nigeria, Ethiopia and China [23,24]. With the advent of molecular techniques like PCR and micro assay [25] there are now increasing reports of occurrence of *M. tuberculosis* in cattle. Supporting data suggesting that humans suffering from active TB are the most probable source of *M. tuberculosis* in cattle have been described in the past, but the first unequivocal evidence of human-to-cattle transmission of *M. tuberculosis* confirmed by IS6110 restriction fragment length polymorphism (RFLP) analysis showed that the isolates from the cattle and farm worker who suffered from pulmonary tuberculosis 1 year prior to this case were the same strains [26]. Except for slight macroscopic enlargement, no gross pathological changes were visible on any lymph node specimen collected from the three slaughtered animals. Histopathological examination of hematoxylin-eosin-stained tissues revealed nonspecific findings. No acid-fast bacilli were detected on Ziehl-Neelsen stained impression smears.

However, after a 28-day incubation at 37°C, a few colonies appeared on a Löwenstein-Jensen slant inoculated with the mixed specimen of mediastinal and portal lymph nodes collected from the 2-year-old cow. The growth characteristic and biochemical tests proved isolate from 2 years cow as *M. tuberculosis*. The authors opined in the areas where bovine TB and human TB coexist, a detailed microbiological investigation of the specimens of slaughtered tuberculin-positive animals should always be performed in order to discriminate between *M. tuberculosis* and *M. bovis* infections [26]. *M. tuberculosis* infections in 3 cattle farms in Spain. The epidemiologic investigation traced humans as the source of infection, with 1 of the strains showing multidrug resistance. No tuberculosis-compatible lesions were observed in the 3 animals. *M. tuberculosis*-infected animals were <9 months of age. The possibility of infection in young animals could be more probable than infection in older cows [27]. Smear microscopy, histopathology and PCR were applied to investigate presence of *M. tuberculosis* complex organisms in 30 bovine lung tissues from an organized dairy farm located in the North India. Differential diagnosis of *M. tuberculosis* and *M. bovis* was made based on the deletion of mce-3 operon in *M. bovis*. Eight of these samples were positive for *M. tuberculosis* by multiplex PCR. Sequencing was performed on two PCR-positive representative samples and on annotation, and BLAST analysis confirmed the presence of gene fragment specific to *M. tuberculosis*. The presence of *M. tuberculosis* in all the positive samples raised the possibility of human-to-cattle transmission and possible adaptation of this organism in bovine tissues [28].

M. bovis accounts for only a small percentage of the reported cases of TB in humans and little information is available on the incidence of *M. bovis* infection in humans. Unlike transmission of *M. bovis* from cattle to humans, the role of human-to-human airborne transmission in the spread of *M. bovis* has been somewhat

controversial. According to LoBue et al. [29] investigations are needed to elucidate the relative importance of *M. bovis* on TB incidence in humans, especially in developing countries. Between 1994 and 1996, three elephants from an exotic animal farm in Illinois died of pulmonary disease due to *M. tuberculosis*. In October 1996, a fourth living elephant was culture-positive for *M. tuberculosis*. 22 handlers at the farm screened for tuberculosis (TB), 11 had positive reactions to intradermal injection with purified protein derivative. One had smear-negative, culture-positive active TB. DNA fingerprint comparison by IS6110 and TBN12 typing showed that the isolates from the four elephants and the handler with active TB were the same strain. This investigation indicated transmission of *M. tuberculosis* between humans and elephants. The necropsy of the elephant that died in 1994 showed caseous necrosis of the lungs and pleural exudates whose culture yielded *M. tuberculosis* [30].

A total of 54 *M. tuberculosis* complex isolates were obtained from different types of specimens from cattle suspected to be suffering from tuberculosis in certain organized cattle farms in north India. Of them, 40 were identified as *M. bovis* and 14 as *M. tuberculosis* on the basis of biochemical tests. Further, the investigation revealed that 28.5 per cent of the milk samples collected were culture positive for *M. tuberculosis*. Moreover, 7.1 per cent of pharyngeal swabs taken from animals were also

positive for *M. tuberculosis* [31]. By using PCR diagnostics [32] showed that among cerebrospinal fluid specimens from TB meningitis patients from India, 17 per cent were positive for *M. bovis* and 2.8 per cent were positive for *M. tuberculosis*. A total of 181 bovine raw milk and 123 pre-scapular lymph node biopsy samples were collected and subjected to acid fast staining, fluorescent staining, isolation and identification. Genus specific PCR to identify the *M. tuberculosis* complex (MTBC) organism, and multiplex PCR (mPCR) were used to differentiate *M. tuberculosis* and *M. bovis*. Among the milk samples tested, only one sample was culture positive for *M. tuberculosis*. Four samples were positive by MTBC-PCR and mPCR; all these four were proved to be *M. tuberculosis*. The authors contended that animals can be infected with human-originated *M. tuberculosis*, which in turn may act as a source of infection in humans, becoming a reverse zoonosis [33].

By PCR, Mycobacterium TB complex was detected in 19 (15.70%) animals out of which 4 (3.30%) animals were positive for *M. bovis* [34]. Lungs, bronchomediastinal and prescapular lymph nodes aseptically at necropsy were processed for bacteriological examination. On gross examination small tuberculous nodules were found in lungs of Cow A and C but no visible growth in other two. Histopathological examination revealed characteristic tuberculous granuloma in cow A and C with non-specific findings in other two cows. After 28-day incubation at 37°C, small moist colonies appeared on L-J slants inoculated with the specimens of lungs of cow A and C. Standard biochemical tests identified the isolate as *M. bovis*. From the mixed specimen of bronchomediastinal and prescapular lymph nodes collected from the cow B the growth was confirmed to be *M. tuberculosis*. However, no growth was found in the lungs of the same animal. Samples from the others animals showed no growth. Among four dead cattle, two cows were of Jersey breed 9 years (cow A) and 14 years of age (cow B), respectively, one Jersey cross 15 years (cow C) and one Holstein-Friesian 14 years (cow D) [35].

A case of transmission of *M. tuberculosis* infection from a man to cattle was reported [36]. *M. tuberculosis* was isolated from the bronchial lymph nodes of a heifer that reacted positively to bovine tuberculin but showed no gross pathological changes at slaughter. The cattle owner died of tuberculosis the same year the heifer was diagnosed with *M. tuberculosis* infection. *M. tuberculosis* strains isolated from the heifer and its owner genotyped by mycobacterial interspersed repetitive units-variable-number tandem repeat (MIRU-VNTR) typing revealed identical MIRU profiles for both isolates. This is the first described case of *M. tuberculosis* infection in cattle and the first case of human-to-animal transmission of *M. tuberculosis* in Croatia. *M. tuberculosis* does not appear to have an indigenous animal host or reservoir and the infected animals most probably represent accidental hosts. In this reported case, a young animal was affected, no TB-characteristic pathological changes were detected and subsequent skin TB testing revealed no inconclusive or positive reactors among the other animals on the farm. The fact that the farm owner was infected with TB strongly suggested the possibility of an anthrozoönotic transmission of *M. tuberculosis* infection.

In India, an investigation of 98 animal tissue samples, yielded 7 *M. tuberculosis* from bovine and 1 *M. tuberculosis* from swine [37]. MIRU-VNTR typing is used widely for genotyping *M. tuberculosis*

and *M. bovis* isolates, it has been employed only twice to characterize *M. tuberculosis* isolates from cattle [20,38]. Chen et al. [20] reported a common MIRU profile revealed for both human and cattle *M. tuberculosis* isolates, suggesting a common source of infection in the cow and an epidemiological link between the cow and human *M. tuberculosis* infections. In a recent investigation of throat swabs from cattle that reacted positively to bovine tuberculin, *M. tuberculosis* strains were isolated in an even larger number than *M. bovis* strains [38]. *M. tuberculosis* strains isolated in cattle mostly belonged to the Beijing and Beijing-like family [20,38]. This can be explained by the fact that China is a highly-burdened TB country (incidence rate 96 per 100,000 population in 2009) [39] where humans and animals still live in close contact. This is the first report of cases of *M. tuberculosis* infection in cattle in South Africa. The VNTR profiles of the *M. tuberculosis* strains identified in the current study will form the basis for creating *M. tuberculosis* [40].

Experimental Infection of *M. tuberculosis* in Cattle

The virulence of *M. tuberculosis* as investigated as early as 1901 by Robert Koch. The experimental infections of cattle where human “tubercle bacilli or the sputum were injected the skin, in others into the peritoneal cavity, in others into the jugular vein. Six animals were fed with tuberculous sputum almost daily for seven or eight months; four repeatedly inhaled great quantities of bacilli.” The results of these infections were that “none of these cattle (there were nineteen of them) showed any symptoms of disease and they gained considerably in weight. From six to eight months after the beginning of the experiments they were killed. In their internal organs not a trace of tuberculosis was found. This early work of Robert Koch on strain phenotypes indicated that *M. tuberculosis* was avirulent in cattle [41].

Cattle were infected by endobronchial instillation with approximately 106 CFU of *M. tuberculosis* H37Rv or *M. bovis* 2122/97 and sacrificed 17 weeks post-infection. IFN-gamma and tuberculin skin tests indicated that both *M. bovis* 2122 and *M. tuberculosis* H37Rv were equally infective and triggered strong cell-mediated immune responses. Postmortem examination revealed that while *M. bovis* 2122/97-infected animals all showed clear pathology indicative of bovine tuberculosis, the *M. tuberculosis*-infected animals showed no pathology. From the postmortem examinations, it was clear that while *M. bovis* had caused extensive gross pathology demonstrated by the presence of visible lesions in the lungs and associated lymph nodes, cattle infected with *M. tuberculosis* showed no pathological signs of disease. Statistically, these difference were highly significant for the numbers of affected lung lobes and lung-associated lymph nodes, as well as the lymph node, lung, and total pathology scores ($P < 0.01$). This apparent attenuation of *M. tuberculosis* in animal hosts was all the more intriguing given that genome studies have shown us that the *M. bovis* genome was merely a reduced version of the *M. tuberculosis* genome; hence, *M. bovis* does not have any “virulence” loci for animals per se that have been lost in *M. tuberculosis*. Instead it appears likely that differential expression of a range of genes between *M. tuberculosis* and *M. bovis* explains their specific host predilections [42].

Villarreal-Ramos et al. [43] experimentally infected cattle using *M. bovis* AF2122/97, *M. tuberculosis* H37Rv, and *M. tuberculosis* BTB1558 (isolated in 2008 during a TB surveillance project in Ethiopian cattle) with a dose of 1×10^4 119 CFU for each strain; inocula endobronchially in 2 ml of 7H9 medium. After ten weeks of infection, the animals infected with *M. tuberculosis* and six weeks after infection with *M. bovis* were killed respectively. The post-mortem examination showed both *M. tuberculosis* strains caused reduced gross and histopathology in cattle compared to *M. bovis*. Using *M. tuberculosis* H37Rv and *M. bovis* AF2122/97 as the extremes in terms of infection outcome, they used RNA-Seq analysis to explore differences in the peripheral response to infection as a route to identify biomarkers of progressive disease in contrast to a more quiescent, latent infection. Their work showed the attenuation of *M. tuberculosis* strains for cattle, and emphasizes the potential of the bovine model as a One Health approach to inform human TB biomarker development and post-exposure vaccine development. According to authors, their work provides further evidence of the distinct host preference of tubercle bacilli as a basis to explore the molecular basis of virulence in *M. bovis* as compared to *M. tuberculosis*, and also offers a model in which to explore the reactivation of latent TB infection.

Therefore, it can be concluded that there is sufficient evidence now that *M. tuberculosis* infects cattle, however, it causes no overt disease in cattle. The evidence of *M. tuberculosis* in cattle emerged from mostly PCR based studies, except few wherein *M. tuberculosis* has been cultured as “gold standard test”. There is lack of information on type of granuloma and detailed histological lesions seen in tissues of cattle infected with *M. tuberculosis*. There is no evidence based research to reveal out the endogenous factors of host responsible for non progression of the disease due to *M. tuberculosis* infection in cattle. The findings of experimental infection of *M. tuberculosis* in cattle showed *M. tuberculosis* to be attenuated for cattle. We opine that cattle are not as susceptible to *M. tuberculosis* infection as much as for *M. bovis*. It leaves enormous scope to study implication of *M. tuberculosis* infection in cattle and its pathology in naturally infected case of *M. tuberculosis*. *M. tuberculosis* and *M. bovis* infecting cattle makes the problem of TB more complex.

Acknowledgement

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Conflict of Interest

None.

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