

Review

Status and Public Health Significance of *Mycobacterium bovis* in Ethiopia

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Article information

Received: March 1st, 2023; Revised: April 7th, 2023; Accepted: May 11th, 2023; Published: May 18th, 2023

Cite this article

Getahun TK. Status and public health significance of *Mycobacterium bovis* in Ethiopia. *Vet Med Open J.* 2023; 8(1): 18-28. doi: [10.17140/VMOJ-8-173](https://doi.org/10.17140/VMOJ-8-173)

ABSTRACT

Infectious diseases, accounting for 30-50% of the total annual losses, remain a major impediment to Ethiopia's livestock economy. Cattle, other domesticated animals, and some free-range or captive wildlife species are all susceptible to the infectious disease known as bovine tuberculosis, which is caused by the *Mycobacterium bovis* (*M. bovis*). It is typically distinguished by the development of tubercle-like nodular granulomas. The bovine tuberculosis-diseased animal loses 10-25% of their productive efficiency; direct losses due to the infection become evident by a decrease in 10-18% milk and a 15% reduction in meat production. During the final stages of tuberculosis, severe emaciation and acute respiratory distress might happen. It affects animal production, but it also significantly affects public health. Human infection due to *M. bovis* is thought to be mainly through the drinking of contaminated or unpasteurized raw milk and undercooked meat. It is estimated that *M. bovis* causes 10-15% of human cases of tuberculosis in countries. This indicated that tuberculosis in both humans and animals is endemic in Ethiopia. Reporting the status of tuberculosis in animals and humans is so dynamic.

Keywords

Bovine tuberculosis; *Mycobacterium bovis*; Human tuberculosis; Public health.

INTRODUCTION

The livestock industry contributes significantly to the world economy, especially in underdeveloped nations since livestock provides manure for crops, a source of energy, food, and raw materials. So, it should come as no surprise that the livestock industry has grown to be a significant source of income for the great majority of rural residents and a target for agribusiness in the dairy, meat, hide, and skin, as well as numerous other goods in the processed foods sector.¹ Ethiopia is predominantly an agricultural nation in Eastern Africa. Animal production is practiced in all ecological zones of the country.² Ethiopia is one of the countries that possess a huge number of livestock populations in the African continent estimated to be 70 million cattle out of which, about 98.95% of the total cattle in the country are local breeds while the remaining are hybrid and exotic breeds that accounted for about 0.94 and 0.11%, respectively, 30.7 million sheep and 30.2 million goats were found in the country. About 16.5% of the country's gross domestic product (GDP) and 35.6% of the agricultural GDP are contributed by the livestock industry. It also contributes 15% of export earnings and 30% of agricultural employment.³

However, the productivity still remains marginal mainly

due to malnutrition, prevalent disease, the poor genetic potential of local breed, management problems, and inefficiency of livestock development services with respect to credit, extension, marketing, and infrastructure.³ The direct consequence of prevalent diseases is loss of production and productivity, a hindrance to accessing the international animal and animal products market, reduction in the quality of the skin and hide, death of the animals, loss of weight, and poor fertility performance. Among prevalent livestock diseases, *Bovine tuberculosis* (BTB) is constantly deteriorating the health and productivity of domestic and wild animals.⁴

Infectious diseases, accounting for 30-50% of the total annual losses, remain a major impediment to Ethiopia's livestock economy. Cattle, other domesticated animals, and some free or captive wildlife species are all susceptible to the infectious disease known as BTB, which is caused by *M. bovis*. It is typically distinguished by the development of tubercle-like nodular granulomas. Although BTB is sometimes described as a chronic, disabling illness, it can occasionally take a more progressive course. Any body tissue can be affected, but the lymph nodes (especially those in the head and thorax), lungs, intestines, liver, spleen, pleura, and peritoneum are where lesions are most frequently seen.⁵

Despite having identical 16s ribonucleic acid (RNA) sequences and almost 99.9% identity in their genome sequences, other members of the *Mycobacterium tuberculosis* Complex (MTC) that were previously thought to be *M. bovis* have been acknowledged as distinct species. These include *M. caprae*⁶ (in some countries considered to be a primary pathogen of goats) and *M. pinnipedii*,⁷ a pathogen of fur seals and sea lions. These two new species are known to be zoonotic. *M. caprae* has been recognized as a frequent cause of BTB in central Europe.⁸ The illness brought on by *M. caprae* is thought not to differ significantly from that brought on by *M. bovis*, and the same tests can be used to diagnose it. Clinical evidence of tuberculosis in cattle is uncommon in nations with tuberculosis eradication programs because the intradermal tuberculin test enables presumptive identification and culling of infected animals before symptoms manifest. Nonetheless, tuberculosis-related clinical symptoms were frequently seen before the national tuberculosis eradication programs.⁷

Bovine tuberculosis is caused by *Mycobacterium bovis* (*M. bovis*), a member of the MTC, which also includes *M. tuberculosis*, *M. africanum*, *M. canetti* and *M. microti*. BTB is a worldwide animal health issue that continues to pose a significant threat to public health in countries where people live in close proximity to their cattle and milk is not pasteurized. The association between the prevalence of *M. bovis* infection in humans and in local cattle populations emphasizes the disease's potential threat to humans. BTB also has a significant socioeconomic impact, as it has the potential to disrupt international trade in animals and animal products.⁹

Bovine tuberculosis is a serious infectious disease of Ethiopian cattle that have been found in almost every part of the country. Except for routine abattoir inspections, which involve whole or partial condemnation of infected carcasses for the purpose of protecting consumers' health, no discernible control programs are in place. Even so, routine meat inspection procedures with qualified professionals are only carried out in a few municipal and export abattoirs across the country. It is estimated that more than half of all slaughtered animals are illegally processed in backyard systems without proper inspection, posing a significant health risk to consumers. This is exacerbated further by the low sensitivity of routine meat inspection in detecting carcasses with tuberculosis lesions, allowing infected meat to be approved for human consumption.⁵

Although cattle are thought to be the true hosts of *M. bovis*, the disease has been reported in a variety of domesticated and wild animals. Animals isolated include buffaloes, bison, sheep, goats, equines, camels, pigs, wild boars, deer, antelopes, dogs, cats, foxes, mink, badgers, ferrets, rats, primates, South American camelids, kudus, elands, tapirs, elks, elephants, sitatungas, oryxes, adaxes.¹⁰

In many cases, the infection is chronic, and symptoms may be absent, even in advanced cases involving multiple organs. When present, clinical signs vary; lung involvement may be manifested by a cough, which can be induced by changes in temperature or manual pressure on the trachea. Dyspnoea and other signs of

low-grade pneumonia are also evidence of lung involvement. In advanced cases, lymph nodes are often greatly enlarged and may obstruct air passages, the alimentary tract, or blood vessels. Head and neck lymph nodes may become visibly affected, rupturing and draining. In some cases, involvement of the digestive tract manifests as intermittent diarrhoea and constipation.¹⁰ During the final stages of tuberculosis, extreme emaciation and acute respiratory distress are possible. Lesions involving the female genitalia may occur. Male genitalia are seldom involved. At necropsy, tubercles are most frequently seen in bronchial, mediastinal, retropharyngeal and portal lymph nodes and may be the only tissue affected. In addition, the lung, liver, spleen and the surfaces of body cavities are commonly affected. Palpation frequently reveals early nodular pulmonary lesions. The lesions are usually non-odoriferous. Other anatomical sites that may be infected should be investigated. A tuberculous granuloma is typically yellowish in appearance and caseous, caseo-calcareous, or calcified in consistency. Its appearance can occasionally be purulent. Cervids and camelids may have a more purulent appearance. Some non-tuberculous granulomas may be indistinguishable from tuberculous granulomas under the microscope.¹¹

Bovine tuberculosis diseased animal loses 10 to 25% of their productive efficiency; direct losses due to the infection become evident by decrease in 10 to 18% milk and 15% reduction in meat production. Apart from effects on animal production, it has also a significant public health importance.¹² In developing countries like Ethiopia, the socio economic situation and low standard living area for both animals and humans are more contributing in BTB transmission between human to human and human to cattle or *vice versa*.¹³ Human infection due to *M. bovis* is thought to be mainly through drinking of contaminated or unpasteurized raw milk and under cooked meat. The high prevalence of BTB in cattle due to the close contact of cattle and humans, the habit of raw milk and meat consumption, and the increasing prevalence of HIV may all increase the potential for transmission of *M. bovis* and other Mycobacteria between cattle and humans.¹⁴

Bovine tuberculosis is an endemic disease of cattle in Ethiopia, with a reported prevalence of 3.5-5.2% in abattoir (mostly zebu) and 3.5-50% in crossbred farms.⁵ Despite the fact that Ethiopia has a high prevalence of BTB, there is little known about the genotypic characteristics of *M. bovis* strains infecting animals as well as in human communities. In addition to assisting in the design of a more targeted control measure, the availability of such information would aid in the study of the organism's phylogenetic characteristics, which would provide new insight into the natural history of BTB.¹⁵ Despite the large livestock population, no information on BTB is available. Despite the fact that BTB is a public health threat that also causes economic losses, research and control of animal tuberculosis in Ethiopia has received less attention than human tuberculosis.¹⁶

Therefore, the objectives of this review were:

- To highlight the status of BTB in Ethiopia.
- To access its public health importance in Ethiopia.

BOVINE TUBERCULOSIS

Etiology

Bovine tuberculosis is an infectious illness caused by the zoonotic organism *M. bovis* that affects cattle, other domesticated animals, and certain free or captive wildlife species. *M. bovis* is indeed a slow-growing (16-20-hour generation time) aerobic bacterium that causes tuberculosis in livestock (known as bovine TB). It is related to *M. tuberculosis*, the bacterium that causes tuberculosis in humans. *M. bovis* can cross the species barrier and end up causing tuberculosis in humans and other mammals. It is usually distinguished by the formation of nodular granulomas known as tubercles. Although commonly defined as a chronic debilitating disease, BTB can occasionally take a more progressive course.¹⁷

Taxonomy

The genus *Mycobacterium* is classified under the order Actinomycetales and family Mycobacteriaceae.¹⁸ The genus includes a number of species, some being pathogenic to human and animals, some are opportunistic while others are essentially saprophytic. The seven members of the MTC include *M. tuberculosis*, *M. bovis*, *M. bovis* BCG, *M. africanum*, *M. microti*, *M. tuberculosis* sbsp. *canetti* and *M. bovis* sbsp. *caprae*. Four members of this group are responsible for human tuberculosis i.e. *M. bovis*, *M. tuberculosis*, *M. africanum* and *M. canetti*.¹⁹ Genetically, members of the MTBC are remarkably similar, sharing exactly the same 16 ribosomal RNA (rRNA) sequence and at least 99.9% identity at the nucleotide level.²⁰

Physical and Biochemical Characteristics

Morphology and staining: In general mycobacteria are gram positive non-motile, non-spore forming, pleomorphic bacilli or coccobacilli, obligate aerobic, thin rod usually straight or slightly curved having 1-10 µm length and 0.2-0.6 µm width, facultative intracellular microbe and has a generation time of 15-20-hours. They come in single occurrences, pairs, or little bundles. In tissues they appear as rods, which may be straight, curved or in the form of clubs, measuring 1.0-4.0 µm in length and 0.2-0.3 µm in width and occur singly, in pairs or as small bundles. On laboratory media they may appear as cocci or rods measuring 6-8 µm. The formation of characteristic cords are distinguishing features of pathogenic mycobacteria.¹⁸

Unique aspect of mycobacteria from other gram-positive bacteria is that cell wall is made up of four parts. The first component that is comparable to that of other bacterial species is the peptidoglycan layer. Arabinogalactan, a branching macromolecule composed of arabinose and galactose, is found in the second layer.²¹ Mycolic acids, which are lengthy branched chains of fatty acids with varying lengths of 50 and 30 carbon atoms, make up the third layer, which helps to determine the thickness of mycobacterial cell walls. The mycolic acids are responsible for the acid-fast staining reaction of mycobacteria cells as they resist decolorizing with strong acid/alcohol solutions. A major factor in the bacterium's resistance to numerous disinfectants, common laboratory stains, antibiotics, and physical traumas is this waxy coating (my-

colic acid). It presumably also slows down the uptake of nutrients, which slows down the rate at which some species grow.¹⁸

Growth requirement and cultural characteristics: Medium used for the growth of mycobacterium species contain serum, potato and egg. The most commonly used media are Lowenstein-Jensen (LJ) and stone brick's medium. L-J contains egg, glycerol, asparagines, mineral salt and malachite green. In culture medium *M. bovis* grows more slowly than *M. tuberculosis*, which needs more than 8-weeks to appear on primary culture as well as it needs media free from glycerol, but having pyruvate as a nutrient which enhance the growth. The optimal growth temperature is 37 °C.²¹

Pathogenesis

Infection: *Tubercle bacilli* gain entrance to the animal body through different ways which include: the respiratory, alimentary, genital, cutaneous and congenital routes. After entering to the animal body the bacteria may localize in tissues and associated lymph nodes related to the route of infection. Millitary TB represents the most severe course of the disease with haematogenous spreading as a result of lysis of macrophages that release bacteria in to the blood from the primary foci and secondary seeding to various tissues.²²

Virulence: Mycobacteria are intracellular organisms and their ability to survive and multiply within the macrophages appears to be related to virulence. *M. bovis* eludes the bacteriocidal activities of macrophages by escaping from fused phagolysosomes into non-fused vacuoles in the cytoplasm. In addition to these survival mechanisms, an important aspect of pathogenicity of mycobacteria is their ability to subvert the protective immune response. A characteristic feature of virulent strains of mycobacteria is that they form cords when they grow in a liquid culture media whereas the virulent strains develop as clumps.²³

Gross lesion: A primary lesion or focus of infection is established following the interaction of the host and the agent at the site of entry within 8-weeks of bacterial entrance. The Mycobacterium is then taken by the alveolar macrophages to the circulation and establishes in the lymph nodes. Cellular responses attempting to control the disease results in the accumulation of large number of phagocytes and lead to the formation of a macroscopic lesion referred as tubercle. These granulomas are frequently encapsulated, typically yellowish, and either caseous or calcified. Instead of looking like conventional tubercles, the lesion in some species tends to resemble abscesses. Cattle lymph nodes, particularly those in the head and thorax, frequently have tubercles. Most of those lesions found in lymph nodes are associated with the respiratory system. It is also common in the lungs, spleen, liver and the surfaces of body cavities. In disseminated case, lesions are sometimes found on the female genitalia, but are rare on the male genitalia. In countries with good control programs, infected cattle typically have few lesions at necropsy. However, small lesions can often be discovered in the lungs of these animals if the tissues are sectioned.²⁴

Immunity against Mycobacterial Infection

Both humoral and cell-mediated immune responses can be in-

duced to Mycobacterial infection, but the cell-mediated immunity is generally accepted to have the most significant role in protection. The macrophages have a central role in processing and subsequent presenting of Mycobacterial antigens to antigen specific T-lymphocytes.²⁵

Epidemiology of *Mycobacterium bovis* Infections

The disease affects cattle all across the world, although some nations have been able to 'screen and cull' their cow stocks in order to lessen or prevent the disease's spread. Nearly all of Europe and several Caribbean nations, including Cuba, are *M. bovis*-free. The disease BTB is widespread in many developing nations, particularly those in Africa. One of the broadest host ranges of any pathogens, *M. bovis* also has one of the most complicated epidemiological patterns, involving interactions between infections in humans, domestic animals, and wild animals. Unfortunately, there is a dearth of research on the epidemiology of this organism and the epidemiological needs for its management, particularly in underdeveloped countries.²⁶

Infection source and mode of transmission: Cattles are the main reservoir of *M. bovis*, which can transmit the infection to many mammalian species including human. Organisms leave the host in respiratory discharges, faeces, milk, urine, semen and genital discharges. These body excretions may contaminate grazing pasture, drinking water, feed, water and feed troughs or fomites, which may act as source of infection to other animals.²⁷

Inhalation of *M. bovis* bacilli is the most common route of infection with only a small number of mycobacteria required to cause an infection and spread of the infection can happen between animals when that are confined together in the same air space, such as during housing over the winter period. A secondary source of infection is the ingestion of contaminated milk or contaminated pasture and water, though environmental contamination is not believed to be a significant source of infection for bovine TB. Infection of the reproductive system can lead to genital transmission of the bacilli but this is a particularly rare event as is congenital infection.²⁸

Risk factors associated with animal population: The probability of infection with *M. bovis* is influenced by factors, which are linked to environment, host and the pathogen itself.²⁹

Host range: *M. bovis* primarily infects cattle, although it can also infect other domesticated and wild species. Known maintenance hosts include brush tailed opossums (and possibly ferrets), badgers, bison and elk, and kudu and African buffalo.²⁹ Species reported to be spillover hosts include sheep, goats,³⁰ horses, pigs, dogs, cats, ferrets, camels, llamas, many species of wild ruminants including deer and elk; elephants, rhinoceroses, foxes, coyotes, mink, primates, opossums, otters, seals, sea lions, hares, raccoons, bears, warthogs, large cats (including lions, tigers, leopards, cheetahs and lynx) and several species of rodents.³¹ Most mammals may be susceptible, but little is known about bird susceptibility to *M. bovis*, despite the fact that birds are generally thought to be resistant.¹⁸ Zebu (*Bos indicus*) type cattle are thought to be much more resistant to tuberculosis than European cattle, and the effects on these cattle are much less severe but under intensive feedlot conditions a morbidity rate of 60% and a depression of weight gain

can be experienced in tuberculous Zebu cattle.²⁹

Agent: *M. bovis*, the causative organism is moderately resistant to heat, desiccation, and many disinfectants. It is readily destroyed by direct sunlight unless it is in a moist environment. In warm, moist, protected positions, it may remain viable for weeks and months.³²

Environment: Housing with high stocking intensity and a large number of animals on a farm predisposes to the disease, so that the disease is more common and serious where these forms of husbandry are practiced. The closer the animals are in contact the greater is the chance that the disease will be transmitted. In spite of the low overall incidence in countries where cattle are at pasture all the year round, individual herds with 60-70% morbidity may be encountered.³³

Risk Factors Associated with Human Population

Close physical contact: Close physical contact between humans and potentially infected animals is present in some communities, especially in developing region. For example, in many African countries cattle are an integral part of human social life; they represent wealth and are at the center of many events and, therefore gatherings. In addition, with 65% of Africa, 70% of Asian, and 26% of Latin America and Caribbean population working in agriculture, a significant proportion of the population of these regions may be at risk for BTB.³⁴

The increase in the demand for milk and meat: The demand for milk was increasing at estimated rate of 2.5% per year over the period of 1970-1988 in sub-Saharan Africa.³⁵ This rise demand for milk consumption will be met by increasing number of productive animals and intensifying animal production. For developing countries as a group, evidence suggests that income elasticities for animal proteins are relatively high: 0.8-1.7%. This means that a 10% increase in total income will lead to an 8-17% increase in demand for animal proteins. The income elasticities of meat and milk in tropical Africa have been estimated at 0.98 and 0.82, respectively, compared to 0.22 for cereals. Hence, if real income increases in Africa, demand for animal products will rise faster than for cereals. This rise demand for meat and milk consumption will be met by increasing number of productive animals and intensifying animal production.²⁹

Feeding habit: Consumption of raw or soured milk is mainly practiced in some parts of the world. Approximately 90% of the total volume of milk produced in sub-Saharan Africa is consumed fresh or soured and only a very small proportion follows official marketing channels. It is known that consumption of milk contaminated by *M. bovis* is regarded as the principal mode of TB transmission from animals to humans.³⁶

HIV/AIDS infection: In many developing countries, TB is the most frequent opportunistic disease associated with human immunodeficiency virus (HIV) infection. HIV Seroprevalence rates greater than 60% have been found in TB patients in various African countries.³⁷ Persons infected with both pathogens have annual risk of progression to active TB of 5 to 15% depending on their level of immune suppression; approximately 10% of non-HIV infected persons newly infected with TB become ill at some time during

their life. In the remaining 90% effective host defense prevent progression from infection to disease.³⁸

Absence of control mechanism: BTB can be controlled/eliminated from a country or region by implementing the test and slaughter policy. However, because of financial constraints, scarcity of trained man power, lack of political will, as well as the under estimation of the importance of BTB by national governments and donor agencies, control measures are not applied or are applied inadequately in most developing countries.³⁹

Distribution

Although BTB was once found worldwide, control programs in many countries have eliminated or nearly eliminated the disease from domesticated animals. Nations currently classified as tuberculosis free include Australia, Iceland, Denmark, Sweden, Norway, Finland, Austria, Switzerland, Luxembourg, Latvia, Slovakia, Lithuania, Estonia, the Czech Republic, Canada, Singapore, Jamaica, Barbados and Israel.⁴⁰ Studies in Ethiopia revealed a higher prevalence of BTB in cattle kept indoors compared to free grazing animals and a higher susceptibility to *M. bovis* infection of exotic *Holstein Bos Taurus* cattle compared to local Zebu cattle.⁴¹

Diagnosis

Diagnosis of BTB infection in live animals is usually based on delayed hypersensitivity reactions. A presumptive diagnosis of TB in cattle is often made on history, clinical findings, tuberculin skin tests and/or necropsy findings. In-vitro lymphocyte assays, including an interferon gamma assay and enzyme linked immunosorbent assays have been developed for the detection of the disease in cattle.⁴² Necropsy, histopathology, mycobacteriological culture procedures, and other molecular approaches for isolate identification are some of the diagnostic techniques used. The gold standard diagnostic technique for mycobacterial infections is still mycobacterial culture.⁴³

Clinical examination: Tuberculosis is usually a chronic debilitating disease, but it can occasionally be acute and rapidly progressive. Early infections are often asymptomatic. In late stages, common symptoms include progressive emaciation, fluctuating fever, weakness and in-appetence. Animals with pulmonary involvement usually have a moist cough that is worse in the morning, during cold weather, exercise and may have dyspnea or tachypnea. In terminal stage, animals may become extremely emaciated and develop active respiratory distress.⁴⁴

Tuberculin skin test: Tuberculin skin tests are the international standard for ante-mortem diagnosis of BTB in cattle herds and individual animals.⁴⁴ It involves the intra-dermal injection of bovine tuberculin purified protein derivative (PPD) and the subsequent detection of swelling (delayed hypersensitivity) at the site of injection 72-hours later. It is a simple, low-cost method for assessing cell-mediated responses to a variety of antigens, and it is the “gold standard” for diagnosing new or asymptomatic MTC infection. This may be performed using bovine tuberculin (single intradermal test (SIT)) alone or as a comparative test using avian

and bovine tuberculin (single intradermal comparative cervical test (SICCT)).⁴⁵

In spite of its wide use, intradermal tuberculin reactions present some important limitations, related to their sensitivity and specificity. Tuberculin skin test lacks sensitivity and can be confounded by exposure to non-tuberculosis Mycobacteria and cannot be repeated for 60-days due to desensitization. Delayed hypersensitivity may not develop for 3-6-weeks after infection, so tuberculin testing during this time period may result in a false-negative result. In addition, tuberculin test may be unresponsive in chronically infected animals with severe pathology.⁴³

Post-mortem examination: Post-mortem examinations should be supported by a histological examination of samples stained with haematoxylin and eosin.⁴³ Generally, *M. bovis* lesions in cattle are described as having a perimeter of lymphocytes, neutrophils, and epitheloid cells, and a centre of caseous necrosis with some calcification. Some of epitheloid cells may fused together and form multinucleated giant cells. An outer border fibrous of connective tissue is usually present, giving the lesion a focal appearance and providing encapsulation to some extent, which may limit the spread of infection. Since the lesions are not conclusive, it is necessary to demonstrate the etiologic agent using Ziehl-Neelsen stain.⁴³

Tuberculosis is characterized by the formation of granuloma (tubercle), which is an organized pathological structure that consists of differentiated macrophages with a characteristic morphology, T-lymphocytes, some B-lymphocytes, dendritic cells, neutrophils, fibroblasts and extracellular matrix components.⁴⁶ The complex, dynamic interactions within granuloma lesions reflect a composite of macrophage and helper T-cell function, cytokine production and Mycobacterial activity that in-turn influence the morphological appearance of the granuloma. Lesion necrosis, liquefaction, mineralization and regression represent some of the outcomes of these interactions that dictate lesion size and appearance and ultimately the presentation of disease in the host.⁴⁷

Bacteriology: *M. bovis* can be demonstrated microscopically on direct smears from clinical samples and on prepared tissue materials.¹⁸ The acid fastness of *M. bovis* is normally demonstrated with the classic Ziehl-Nielsen stain, but a fluorescent acid-fast stain may also be used. Immunoperoxidase techniques may also give satisfactory results.²⁹

The presumptive diagnosis of tuberculosis can be made if the tissue has characteristic histological lesions (caseous necrosis, mineralization, epitheloid cells, multinucleated giant cells and macrophages). As lesions are often paucibacillary, the presence of acid-fast organisms in histological sections may not be detected, although *M. bovis* can be isolated.⁴⁸

Differential staining: Final confirmatory diagnosis of BTB depends on isolation and identification of the bacteria, but preliminary examination of stained smears from lesions, sputum, milk, urine, pleural and peritoneal fluids, uterine discharges and feces is very important. In the smear, the organism appears red rods against a blue background (Ziehl-Nielsen staining), while in the fluorochrome proce-

dures, the acid fast organisms appear as fluorescent rods, yellow to orange.⁴⁹

Culture media: Cultures of Mycobacteria require only 10 to 100 organisms to detect MTC. Cultures increase the sensitivity for diagnosis of MTC and allow specification, drug-susceptibility testing and, if needed, genotyping for epidemiologic purposes.⁵⁰ Mycobacteria grow on protein enriched artificial media. There are three different kinds of culture medium: liquid media (Middlebrook 7H12), agar-based media (Middlebrook 7H10 and 7H11), and egg-based media (L-J). The most frequently used solid media is the L-J containing eggs, phosphate buffer and magnesium salts; and asparagines. The bacteriological culture differentiation of Mycobacteria is based on growth rate, temperature of growth and production of pigments in light and darkness and colony characteristics. The surface mycosides (glycolipids and peptidoglycolipids) determine the colony characteristics and serologic specificities.¹⁸ With regard to the colony characteristics, on solid media the colony of *M. bovis* is smooth, white, flat, and when touched breaking up easily. *M. bovis* can also be identified based on specific biochemical and metabolic properties, since it requires pyruvate as a growth supplement and it is negative for niacin accumulation and nitrate reduction and is generally resistant to pyrazinamide.⁵¹

In the laboratory, *M. bovis* is microaerophilic, i.e. it grows preferentially at a reduced oxygen tension. The temperature and hydrogen ion concentration ranges, in which the bacillus is able to multiply, are relatively narrow. All the MTBC members are slow growers. Therefore, the inoculated media may have to be incubated at 37 °C upto 8-12-weeks.¹⁸ To process specimens for culture, the tissue is first homogenized using a mortar and pestle, stomacher or blender, followed by decontamination with either detergent (such as 0.375-0.75% hexadecylpyridiniumchloride (HPC)), an alkali (2-4% sodium hydroxide) or an acid (5% oxalic acid). The alkali or acid mixture is shaken for 10-15-minutes at room temperature and then neutralized.²⁹

Immunological/serological diagnostic methods: Apart from the traditional intradermal tuberculin test, a variety of blood tests have been employed. The gamma-interferon assay and the lymphocyte proliferation assay measure cellular immunity, while the enzyme-linked immunosorbent assay (ELISA) measures humoral immunity.⁴⁸

Gamma interferon assay/Bovigam: Serological assays are an important tool for determining *M. bovis* exposure. Among these tests, the gamma-interferon assay is frequently used in conjunction with tuberculin skin testing as a confirmatory test after a positive tuberculin skin test result. The release of lymphokine gamma interferon (IFN-) in a whole-blood culture system is measured in this test. The assay is based on IFN- release from sensitized lymphocytes after a 16-24-hour incubation period with a specific antigen (PPD-tuberculin). The test compares IFN- production in response to stimulation with avian and bovine PPD.⁵²

The IFN- diagnostic test is a whole blood assay that is performed quickly. A sandwich-ELISA employing particular monoclonal antibodies is used to detect plasma gamma-interferon. This test,

in its current form, will not be widely accepted as a direct replacement for skin testing, but it might be used as an accessory test. Benefits of this test include accelerated elimination of tuberculosis from infected herds and the possibility of the test to be performed as soon as 10 days after the application of a tuberculin skin test. Other application of the IFN- γ test is that confirmation of the immunological status of skin test reactors and the investigation of fraudulent intervention into the skin test.⁵³

Enzyme-linked immunosorbent assay: There have been numerous unsuccessful attempts to develop clinically useful sero-diagnostic tests for tuberculosis. The ELISA appears to be the most appropriate of the antibody detection assays and can be used to supplement, rather than replace, testing based on cellular immunity. The ELISA has the benefit of being simple, but its sensitivity is restricted due to the late and irregular development of the humoral immune response in cattle over the course of the illness. When complex antigens such as tuberculin or *M. bovis* culture filtrates are utilised, specificity in cattle is equally weak. Nevertheless, comparing antibody levels to PPD-B and PPD-A has been demonstrated to be effective in enhancing ELISA specificity.⁴³

Molecular Techniques

Multiplex PCR: By Mycobacterial genus typing, multiplex polymerase chain reaction (PCR) distinguishes MTC from *M. avium*, *M. intracellulare*, and other Mycobacterial species.³⁰ As a source of deoxyribonucleic acid (DNA) template, heat-killed acid-fast bacilli (AFB) positive sample DNA was used. The PCR targets the Mycobacterium sequence within the 16S rRNA gene (*G1*, *G2*) sequences, within the hypervariable region of 16S rRNA known to be specific to *M. intracellulare* and *M. avium* (MYCAV-R), and the *MTB70* gene specific for MTC (TB-A, TB-1B). In this method, it is possible to differentiate all members belonging to the genus Mycobacterium and further characterize the groups belonging to the MTC and *M. avium* complex. On the gel electrophoresis result, all members of the genus Mycobacterium produce a band of 1030 bp, members of the Mycobacterium avium complex produce a band of 850 bp (*M. avium* subspecies *intracellulare*) and a band of 180 bp (*M. avium* subspecies *avium* and *M. avium* subspecies *paratuberculosis*), and members of the *M. tuberculosis*.³⁰

Region of difference deletion typing: Region of difference (RD) deletion typing is a PCR-based typing method that makes use of the MTBC chromosomal regions of difference deletion loci. The regions of difference represents the loss of genetic material that arise due to errors in DNA replication, movement of mobile genetic elements, mycobacteriophage-mediated transduction, or recombination between adjacent homologous DNA fragments with loss of the intervening sequence.⁵¹ Some of these long sequence polymorphisms (LSPs) were discovered to be limited to a single MTC strain or subspecies, whereas others appeared to be distributed differently throughout MTC groups. Several PCR primer pairs specific to the loci were used which include; 16S rRNA, Rv0577, Rv1510 (RD4), Rv1970 (RD7), Rv3877/8 (RD1), Rv3120 (RD12), Rv2073 (RD9), Rv1257 (RD13), IS1561 (Mid3) and TbD1.⁵⁴

Spoligotyping: Spoligotyping is based on the variability of spacer sequences interspersed with repeat sequences in the polymorphic chromosomal direct repeat (DR) locus. This locus has a number of 36-bp long direct repeats that are highly preserved (DR). Strains differ in the number of DRs and the presence or absence of certain spacers, with *M. bovis* lacking spacers 39-43 in the spoligotype system. Spoligotyping is thus effective not only for differentiating *M. bovis* strains but also for distinguishing them from *M. tuberculosis*, a difference that is sometimes difficult to achieve using traditional bacteriological techniques.⁵⁵

Mycobacterial Interspersed Repetitive Units-Variable Numbers of Tandem Repeats

This is a PCR-based approach for analysing numerous independent loci with variable numbers of tandem repeats (VNTR) of diverse groups of interspersed genetic elements known as Mycobacterial interspersed repetitive units (MIRU). In its original format, the PCR primers are each run in separate reactions and the sizes of the products are analyzed by gel electrophoresis. The most extensively utilised version of MIRU-VNTR analysis now covers 12 tandem repeat loci. A set of 24 MIRU-VNTR loci was standardized to increase the discrimination power.⁵⁶

Zoonotic Importance of Bovine Tuberculosis

Human tuberculosis of animal origin is an important public health concern in developing countries. Due to poor experimental controls, *M. bovis* was initially believed not to be a disease of human but this was later proved to be inaccurate. The consumption of unpasteurized milk from infected cattle is the primary route of *M. bovis* infection in human and is associated with non-pulmonary TB, particularly in children.⁴⁹ Cervical lymphadenitis and lupus vulgaris (chronic skin TB) are the most common presentations of a non-pulmonary *M. bovis* infection. Pulmonary TB due to *M. bovis* is clinically, pathologically and radiologically identical to one caused by *M. tuberculosis* but it is uncommon and usually associated with animal handlers and abattoir workers.⁵⁷

Human TB caused by *M. bovis* is unusual in countries in the developed world, due to the implementation of eradication programs for domesticated animals, accounting for less than 1% of TB infection.⁵⁸ In the developing world, *M. bovis* is responsible for 5-10% of human TB cases but this varies between countries. Limited laboratory facilities, in most developing countries, means that bacteriological diagnosis of a TB infection tends to be carried out by acid fast bacillus smear examination only, so under diagnosis of *M. bovis* infection may be occurring.⁵⁹

Because the World Health Organization (WHO)-recommended TB treatment regimen is effective against human *M. bovis* infections, one could argue that diagnosis is not required for case management and therapy. The current increasing incidence of tuberculosis in humans, particularly in immunocompromised individuals, has rekindled interest in *M. bovis*'s zoonotic importance, particularly in developing countries. Because of the serious consequences of *M. bovis* infection on animal and human health, strict control measures

must be implemented to reduce the disease's risk in human and animal populations. The institution of proper food hygiene practices and stronger inter sectoral collaboration between the medical and veterinary professions is vital to the control of the disease.³⁸

In contrast, spread from animals to humans in developing countries remains a very real danger, mostly from infected milk. This seems to be a danger, which is being entirely ignored. The animal and public health consequences of *M. bovis* are grave. Disease surveillance programs in animals and humans should be considered a priority, especially in areas where risk factors are present. Other recommendations made by the WHO³⁷ in its memorandum on zoonotic tuberculosis include: Personnel training at all levels of control programmes, as well as the urgent need for more research on illness diagnosis and control, immunology, epidemiology, and socioeconomic factors. International cooperation in all aspects of zoonotic tuberculosis remains essential in the fight against this disease (Table 1).³⁵

Prevention and Control

The effective control and eradication of BTB from herds and/or farms of cattle depend on identifying and isolating potential sources of infection from the herds, through test and slaughter strategy. However, there are also various modifications of eradication and control programs adopted in different countries. In developed countries BTB has nearly been eradicated or drastically reduced in farm animals to low-levels by control and eradication programs. In Ethiopia these measures, however, cannot be adopted in practice due to various reasons such as: lack of knowledge on the actual prevalence of the disease, the prevailing technical and financial limitations, lack of veterinary infrastructures, cultural and/or traditional beliefs and geographical barriers, though certain control measures are in place like identification of animals, improvement of management and hygienic practices; legislation, insurance, sound testing and meat inspection, establishment of areas and/or farms free of BTB should have to be applied.³⁹

CONCLUSION

M. bovis has been known to be the major cause of BTB in cattle. Cattle are the main reservoir of *M. bovis*, which can transmit the infection to many mammalian species including man. Organisms leave the host in respiratory discharges, faeces, milk, urine, semen and genital discharges. These body excretions may contaminate grazing pasture, drinking water, feed, water and feed troughs or fomites, which may act as source of infection to other animals. Inhalation of *M. bovis* bacilli is the most common route of infection with only a small number of mycobacteria required to cause an infection and spread of the infection can happen between animals when that are confined together in the same air space, such as during housing over the winter period. With respect to molecular epidemiology of BTB in livestock of Ethiopia, isolation and molecular characterization of the causative agent of BTB has been carried out in the last decade mainly in cattle and a number of isolates has been reported from different regions of the country. Animal and human health is inextricably interwoven and food animals, especially cattle serve as a

Table 1. Molecular Epidemiology of Bovine Tuberculosis in Ethiopia

Title of the Study	Molecular Techniques Used	Authors
Molecular typing of Mycobacterium bovis isolated from tuberculosis lesions of cattle in north eastern Ethiopia	RD4; PCR; Spoligotyping	Ameni et al ⁶⁰
Prevalence of tuberculosis in pigs slaughtered at two abattoirs in Ethiopia and molecular characterization of Mycobacterium tuberculosis isolated from tuberculous like lesions in pigs.	RD10; mPCR; Spoligotyping	Arega et al ⁶¹
Molecular characterization of Mycobacterium bovis isolates from Ethiopian cattle	Deletion typing; Accuprobe gene probe method; PCR; Spoligotyping	Biffa et al ⁶²
Mycobacteria and zoonoses among pastoralists and their livestock in South-East Ethiopia	RD4; RD9 deletion typing; Genus typing; 16S rDNA sequencing; Spoligotyping of human isolates	Gumi et al ⁶³
Tuberculosis in Goats and Sheep in Afar Pastoral Region of Ethiopia and Isolation of Mycobacterium tuberculosis from Goat	Mycobacterium genus typing; Spoligotyping	Mamo et al ³⁰
Epidemiology and Molecular Characterization of Causative Agents of Bovine Tuberculosis in Ruminants	mPCR	Ashenafi et al ⁶⁴
Prevalence study on bovine tuberculosis and molecular characterization of its causative agents in cattle slaughtered at Addis Ababa municipal abattoir, Central Ethiopia	PCR; RD4 deletion typing; Spoligotyping	Mekibeb et al ⁶⁵
Epidemiology of bovine tuberculosis in Butajira southern Ethiopia: A cross sectional abattoir based study	mPCR	Nemomsa et al ⁴¹
Epidemiology of mycobacterial infections in cattle in two districts of Western Tigray Zone, northern Ethiopia	mPCR	Romha et al ⁵⁸
A prevalence study of bovine tuberculosis by using abattoir meat inspection and tuberculin skin testing data, histopathological and IS6110 PCR examination of tissues with tuberculous lesions in cattle in Ethiopia	mPCR system	Shitaye et al ⁶⁶
Conventional and Molecular Epidemiology of Bovine Tuberculosis in Dairy Farms in Addis Ababa City, the Capital of Ethiopia.	RD; m-PCR; spoligotyping; VNTR analysis	Tsegaye et al ⁶⁷
Molecular Epidemiology of Mycobacterium Tuberculosis Complex at Nekemte Municipality Abattoir, Western Ethiopia	RD deletion Typing; m-PCR	Woyessa et al ⁶⁸
Gross and Molecular Characterization of Mycobacterium tuberculosis Complex in Mekelle Town Municipal Abattoir, Northern Ethiopia	mPCR; spoligotyping	Zeru et al ⁶⁹
Cultural and molecular detection of zoonotic tuberculosis and its public health impacts in selected districts of Tigray region, Ethiopia	m-PCR; Deletion typing; Spoligotyping	Zeweld ⁷⁰
Strain Diversity of Mycobacterium tuberculosis Isolates from Pulmonary Tuberculosis Patients in Afar Pastoral Region of Ethiopia	m-PCR; Deletion typing; Spoligotyping	Belay et al ⁷¹
Identification and Characterization of Mycobacterium Tuberculosis Isolates from Cattle Owners in North Western and North Eastern Parts of Rural Ethiopia	Deletion typing; Spoligotyping; SNPs typing	Mengistu et al ⁷²

reservoir of diseases of public health importance. The food safety of animal origin food with regard to infection by *M. bovis* is worth giving consideration.

SUGGESTION

I suggest all the concerned government and non-government organization body must give attention for tuberculosis and awareness must be created among all individuals.

AUTHORS CONTRIBUTION

Temesgen Kassa conceptualize the idea, design and write up the manuscript while Fikadu Wodajo and Fikadu Gutema edit and review the manuscript.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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