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Review Article

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Review on Bovine Tuberculosis and its status in Ethiopia

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Abstract

Bovine tuberculosis (bTB) is highly prevalent diseases in intensive and semi-intensive dairy farms in Ethiopia. Bovine Tuberculosis is a contagious, bacterial disease of both animals and humans. It is a chronic infectious disease caused by *M. bovis*, characterized by progressive development of granulomas in tissues and organs. This disease is a significant zoonosis that spread to humans, typically by the inhalation of aerosols or the ingestion of un-pasteurized milk. BTB has been widely distributed throughout the world and it has been a cause for great economic loss in animal production. In developed countries, there is strong strategic control and eradication programs to reduce distribution of tuberculosis in cattle, and human disease is now rare; however Bovine tuberculosis is still common in less developed countries like Ethiopia, and severe economic losses can occur from livestock deaths and trade restrictions. Ethiopia is one of the African countries where tuberculosis is wide spread in both humans and cattle mainly due to culture of drinking unpasteurized raw milk. So, different strategic control measures should be implemented in to decrease the prevalence and zoonotic importance of bovine tuberculosis in Ethiopia.

Keywords: Bovine; Ethiopia; *M. bovis;* Tuberculosis; zoonosis

Introduction

Ethiopia has large potential for dairy development due to its large livestock population, which accounts 59.5 million cattle, 30.70 million sheep, and 30.20 million goat populations (CSA, 2017) with dairy cattle having potential production of 3.8 billion liters of milk per year (FAO and NZAGRC, 2017). However there is growing dairy production system in urban and peri-urban areas, diseases like bovine tuberculosis were one of hindering effects of the production system in Ethiopia (MoA, 2015).

Tuberculosis (TB) is a chronic granulomatous disease of humans and animals caused by a group of closely related bacteria termed as *Mycobacterium tuberculosis* (*M. tuberculosis*) complex (MTBC) (Brites and Gagneux, 2017). The main pathogenic species of the MTBC members are *M. tuberculosis*, *M. africanum*, *M. bovis*, *M. caprae*, *M. pinnipedii* and *M. microti* (Forrellad *et al*., 2013). Three most important

types of tuberculosis are bovine TB (bTB) caused by *Mycobacterium bovis*, avian TB caused by *Mycobacterium avium*, and human TB caused by *Mycobacterium tuberculosis*(AFF, 2016). Besides their primary hosts, MTBC species infect other secondary hosts (Smith *et al*., 2006).

Tuberculosis in cattle is caused by *M. bovis* and the disease has become an important infectious disease which spread between species by affecting a wide range of animals and humans (Dwight and Yuan, 1999; OIE, 2010; Tenguria *et al*., 2011). It is widely distributed around the world with significant economic impact on the livestock production sector (Ayele *et al*., 2004; Rodriguez-Campos *et al*., 2014).

The disease is one of the seven most neglected endemic diseases in the world, particularly in developing countries including Ethiopia (WHO, 2006) that can spread to humans through aerosols, by the consumption of unpasteurized milk and dairy products and through meat from infected cows (Olmstead and Rhode, 2004; Ameni, 2010). Even though most of Europe and several Caribbean countries (including Cuba) are virtually free of bovine tuberculosis, the disease is endemic to many developing countries particularly African countries (Abubakar *et al.,* 2011; Spickler, 2019). In developed countries, the occurrence of bovine tuberculosis has declined because of mandatory pasteurization of milk together with tuberculin skin testing of cattle followed by culling/ slaughtering the infected cattle (Palmer *et al*., 2012).As a report of WHO, 143,000 human tuberculosis caused by *Mycobacterium bovis* globally from sixteen countries in 2018 (WHO, 2018).

The disease has also become prevalent in different parts of Ethiopia with varying prevalence (Sibhat *et al*., 2017).Traditional preference of raw milk as compared to pasteurized milk because of its taste, availability and lower price in Ethiopia are one of potential zoonotic risk factor for transmission from cattle-to-human (Gebremedhin *et al*., 2014). Previous study occurred at Southern part of Ethiopia in Wolaita sodo, Hawassa and Shashamane milk shed areas has also indicated 48%, 11% and 48.9%, respectively prevalence at herd level (Abie *et al*., 2019; Gezagegn and Yared,2020; Lemu *et al*., 2020). However, due to changing characteristics of the Mycobacteria and its re-emerging condition in different parts of countries, it is important to assess current disease status in different parts the area.

In Ethiopia, there is custom of raw milk consumption and sharing of shelter human with animal in many areas. There is also free movement of dairy animals for marketing purpose in to different areas without registrations and history of origin. Due to this and other related preconditions probability of bovine tuberculosis disease distribution is high in the area. So it needs routine testing and identification of disease prevalence in different parts of Ethiopia.

Literature review

Etiology

In the year 1882 Robert Koch discovered the tubercle bacillus as the infectious agent of tuberculosis. It was first called *Bacterium tuberculosis* until Lehmann and Neumann suggested to name the agent *Mycobacterium tuberculosis* and to include this species together with the leprosy bacillus into the new genus *Mycobacterium* (Sewpersadh, 2012). This genus was placed in its own family of *Mycobacteriaceae* in the order *Actinomycetales* (Rastogi *et al*., 2001). Today there are four conditions for a bacterium to be included in the genus *Mycobacterium*. These are acid-alcohol fastness, presence of mycolic acids containing 60-90 carbons which can be cleaved by pyrolysis to C22- C26 fatty methyl esters and containing Guanin and Cytosin to 61-71 mol% in the DNA (Shinnick and Good, 1994).

Morphology and Staining

Mycobacteria are non-motile, non-spore forming, pleomorphic bacilli or coccobacilli. In tissues they appear as rods, which may be strait, curved or in the form of clubs, measuring 1.0-4.0 µm in length and 0.2-0.3 µm in width. They occur singly, in pairs or as small bundles. On laboratory media they may appear as cocci or rods measuring 6-8 µm. Spores, flagella and capsules are absent (Dwight and Yuan, 1999; Quinn *et al.,* 1999; Ahmad *et al*., 2014). Mycobacteria tend to be more resistant to chemical agents than other bacteria because of the hydrophobic nature of the cell surface and their clumped growth (Geo*et al*., 2013; Ahmad *et al*., 2014).

Mycobacterium, though cytochemically gram positive, often resist staining with gram stain. Their most noted staining property is their acid fastiness: once stained, they resist discoloration with 3% HCL in ethanol. Acid fastness depends on the integrity of the waxy envelope on the cell wall. Mycobacteria can be stained with fluorescent dyes (auramine-rodamine) (Dwight and Yuan, 1999; Quinn *et al.*, 1999; Geo *et al*., 2013).

Pathogenesis

Infection

The progression of bovine tuberculosis in the body's host is characterized by two stages: the initial infection (primary complex) and a chronic post primary dissemination (Giovana, 2018). The primary complex consists of the lesion at the point of entry and in the local lymph node. A lesion at the point of entry is common when infection is by inhalation. When infection occurs via the alimentary tract, a lesion at the site of entry is unusual, although tonsillar and intestinal ulcers may occur. More commonly, the only observable lesion is in the pharyngeal or mesenteric lymph nodes (Radostits *et al*., 2007).

Tubercle baccili can spread in the host by direct extension, through the lymphatic channels and bloodstream, and via the bronchi and gastrointestinal tract (Geo *et al*., 2013). In previously unexposed animals, local multiplication occurs as macrophages on the scene ingest the organisms. Resistance to phagocytic killing (cell wall chemistry and shunting to endosomal compartments rather than those fuse with lysosomes) allows continued intracellular and extracellular multiplication. An inflammatory response (elicited by mycobacterial cell wall constituents) involving largely histocytes and monocytes develops around the focus of proliferating organisms. Infected host cells and bacteria reach draining lymph nodes, where proliferation and inflammatory response continue (Dwight and Yuan, 1999).

Incubation period

Infection in animals, tuberculosis usually has a slow onset, with clinical signs often taking several months or more to develop. Infections can also remain latent for years and later reactivate and infection in humans systemic signs can develop months to years after exposure in humans, or the infection may remain latent until many years later when waning immunity allows the organisms to reactivate. Tuberculous chancre, a type of skin lesion, typically appears 2-4 weeks after cutaneous exposure (Spickler, 2019). Development and production of lesion also depends on number of mycobacteria in the inoculum and their subsequent multiplication and the type of host (Geo *et al*., 2013). A visible primary focus develops within 8 days of entry being affected by the bacteria. Calcification of the lesions commences about 2 weeks later (Radostitis *et al*., 2007).

Pathology

Infection begins with deposition of tubercle bacilli in the lung or on pharyngeal or intestinal mucous membranes ((Dwight and Yuan, 1999). 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 (Thoen and Bloom, 1995). Tuberculosis is characterized by the formation of granulomas where bacteria have localized. The classic lesion is the tubercle, a yellowish-white or grayish-white granuloma typically enclosed in a capsule of varying thickness. Its interior is usually caseous, caseo-calcareous or calcified in cattle, small ruminants, pigs and a number of other species.

However, tubercles in some species of cervids are often poorly encapsulated, tend to resemble abscesses and may have purulent centers. Tubercles vary in size. Some are small enough to be missed unless the tissue is sectioned; others may form coalescing, confluent lesions that affect most of the organ. In cattle and other species that tend to inhale the organisms, lesions are typically found in the lungs and lymph nodes of the head and thorax. They are more likely to occur in the abdominal organs and lymph nodes (e.g., mesenteric lymph nodes) of animals infected by ingestion. Many other tissues and organs including the skin, bones, joints and CNS can also be affected (Radostitis *et al*., 2007; Spickler, 2019).

Virulence

Mycobacteria are intracellular organisms and their virulence appears to be related to their ability to survive and multiply with in the macrophages (Hermans *et al.*, 1991; Geo *et al*., 2013). *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 (Grange, 1995). The lipid components are implicated in pathogenesis. Mycosides, Phospholipid and sulfolipid apparently protect tubercle bacilli against phagocytic killing. Other lipids can produce granulomas, and, with tuberculo-proteins, stimulate cell mediated responses, a central feature of tuberculosis (Dwight and Yuan, 1999). A characteristic feature of virulent strains of mycobacteria is that they form cords when they grow in a liquid culture media whereas the avirulent strains develop as clumps (Thoen and Bloom, 1995; Ereqat *et al*., 2013).

Epidemiology

Occurrence and distribution

Bovine Tuberculosis (BTB), caused by *Mycobacterium bovis* is a well-known zoonotic disease, which affects cattle worldwide, especially in developing countries, because of deficiencies in preventive and/or control measures, poor sanitation and health care (Smith *et al.*, 2006). A limited number of countries (e.g., Australia, Iceland, Greenland, Singapore, some European nations and Israel) are completely free of *M. bovis;* however, infected livestock herds are also now uncommon in Europe, Canada, the United States, New Zealand and some other locations. Bovine tuberculosis is still common or relatively common in cattle in parts of Africa, Asia, the Middle East and Latin America including Mexico (Spickler, 2019). But some countries have been able to reduce or limit the incidence of the disease through process of 'test and cull' of the cattle stock. Bovine tuberculosis is endemic to many developing countries particularly African countries (Abubakar *et al.,* 2011). It is one of the endemic infectious diseases that have long been recorded in Ethiopia (Hailemariam, 1975; FAO, 2003) and the infection has been detected in cattle in different parts of Ethiopia (Sibhat *et al*., 2017).

Source of infection

Depending on the sites where it has localized, Mycobacterium bovis organism may be found in respiratory secretions, exudates from lesions (e.g., draining lymph nodes, some skin lesions), urine, feces, milk, vaginal secretions and semen (Spickler, 2019). 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 (Alemu, 2015).

Transmission of *M. bovis* can occur between animals, from animals to humans and vice versa and rarely, between humans (Nwanta *et al*., 2010). The common mode of transmission is inhalation. Animal can be infected by inhalation of contaminated air droplet directly from infected animal or by inhalation of contaminated dust. Thus, overcrowding and poor ventilation of the house along with improper management facilitate the transmission. Moreover, the disease can also be transmitted by ingestion of through feed and water contaminated by feces and urine of infected animal. The infected bull may also transmit disease or through artificial insemination with the use of infected semen (Amit *et al*., 2014). In endemic area young animals can be infected by drinking of infected milk. However, since mammary infection occurs late in the course of the disease it is less common route of transmission in countries with advanced control programs. Cutaneous, genital, and congenital infections have been seen but are rare (Radostits *et al*., 2007).

Mode of transmission

Transmission between cattle

Animals can become infected by inhalation, ingestion or direct contact through the mucous membranes or breaks in the skin. Much larger numbers of organisms are generally needed to establish an infection by ingestion than inhalation. The importance of the various transmission routes differs between host species. Cattle are often infected via aerosols during close contact. Ingestion is less important in this species, except in calves that nurse from infected cows. Cutaneous, genital (sexual) and congenital transmission are possible but seem to be uncommon in cattle (Radostitis *et al*., 2007; OIE, 2018; Spickler, 2019).

Diagnosis

Clinical Examination

However tuberculosis is usually a chronic debilitating disease in cattle, it can occasionally be acute and rapidly progressive. In countries with eradication programs, most infected cattle are identified early and symptomatic infections are uncommon (Une and Mori, 2007). In the late stages, common symptoms include progressive emaciation, a low–grade fluctuating fever, weakness and inappetence. Animals with pulmonary involvement usually have a moist cough that is worse in the morning, during cold weather or exercise, and may have dyspnea or tachypnea (Radostits *et al.,* 2007; Une and Mori, 2007). In some animals, the retropharyngeal or other lymph nodes enlarge and may rupture and drain. Greatly enlarged lymph nodes can also obstruct blood vessels, airways, or the digestive tract. If the digestive tract is involved, intermittent diarrhea and constipation may be seen (Une and Mori, 2007). The symptoms of bovine tuberculosis usually take months to develop in cattle and infection can also remain dormant for years and reactivate during periods of stress or in old age. Due to this, it’s difficult to diagnose based only on the clinical signs (Radostits *et al.*, 2007).

Tuberculin Skin Test

Tuberculin skin test is the definitive screening test that is currently used. It is the most commonly used test in practice also an OIE (World Organization of Animal Health) prescribed test for international trade. Tuberculin is a solution of protein material extracted from the cell wall of the Mycobacteria organism. When this protein solution is injected into the skin of an infected animal, the body’s sensitized immune response will cause a localized inflammatory reaction that leads to the typical signs of a positive tuberculin test. The preferential site for conducting this test is the neck area. The largest and most typical reactions to an intradermal tuberculin test occur in the early stages of infection with *M. bovis*. This sensitivity lessens later on during the chronic (advanced) phase of the disease when the animal’s immune system gradually switches from a cell-mediated reaction (responsible for the skin reaction) to a humoral antibody – based reaction. Thus the intradermal test becomes less effective in detecting infected animals and in some cases the sensitivity disappears completely and the animal, although still infected, shows no reaction to the tuberculin test at all which is known as anergy (AFF, 2016; OIE, 2018).

Postmortem Examination

Lesions in cattle are most commonly found in organs rich in reticulo-endothelial tissue, p articularly the lungs and associated lymph nodes.

Lesions are most commonly present in the lower respiratory tract, however the upper respiratory tract and its associated tissues also displays disease in many cases. There is also formation of granulomatous nodules called tubercles which have a center of caseous necrosis with some calcification, whose locations depend largely on the route of infection. In calves, it is usually transmitted by ingestion and lesions involve the mesenteric lymph nodes with possible spread to other organs while in older cattle, infection is usually by the respiratory tract with lesions in the lung and dependent lymph nodes (Aboukhassib *et al*., 2016). Postmortem examinations should be supported by a histological examination of samples stained with haematoxylin and eosin (OIE, 2009) and demonstrating the etiological agent by using Ziehl Neelsen stain (Thoen and Blooom, 1995).

Culture

In diagnosis of mycobacterial infections, culture is still considered the international gold standard. However, due to dysgenic and slow growth characteristics, the identification of *M. bovis* by culture and biochemical methods is cumbersome and time consuming (Aboukhassib *et al*., 2016). Cultures are incubated for a minimum of 8 weeks at 37°C with or without CO2. The media should be in tightly closed tubes to avoid desiccation. Slopes are examined for macroscopic growth at intervals during the incubation period. When growth is visible, smears are prepared and stained by the Ziehl Neelsen technique. Growth of *M. bovis* generally occurs within 3–6 weeks of incubation depending on the media used (Silaigwana *et al.*, 2012). Characteristic growth patterns and colonial morphology can provide a presumptive diagnosis of *M. bovis*; however every isolate needs to be confirmed (Alemu, 2015).

Treatment

Cattle should not be treated at all and as such farm animals with tuberculosis must be slaughtered (culled). This is because the risk of shedding the organisms, hazards to humans and potential for drug resistance make treatment controversial (Nwanta *et al*., 2010). In human tuberculosis, drugs like isoniazid, combination of streptomycin and Para-amino salicylic and other acids are commonly used (Amit *et al*., 2014).

Vaccine against Mycobacterium

Vaccination is nearly always the most cost-effective means to control infectious disease. The BCG vaccine was developed for control of human tuberculosis by Albert Chalmette and Camille Guerin by repeated sub-culturing of the bovine TB bacillus, *Mycobacterium bovis* until it lost virulence for guinea pigs yet protected them from challenge with live virulent TB bacilli. The BCG vaccine was first used in humans in 1921 and since now become the most widely used vaccine for human (Chandran *et al*., 2019).

A long-lasting solution such as the protection of cattle against BTB by vaccination could be an important control strategy in countries where there is the persistence of *M. bovis* infection in wildlife and in developing countries where it is not economical to implement a “test and slaughter” control program. Vaccination of cattle represents an alternative intervention strategy to reduce the impact of BTB on livestock productivity and human health in developing countries (Pokam *et al*., 2019). The highest level of efficacy was achieved when calves were vaccinated at <1 month of age, followed by a revaccination boost at 12-24 months to prevent waning immunity (Marais *et al*., 2018). However, Vaccination with the attenuated strain of the M. bovis pathogen, BCG, is not used to control bovine tuberculosis in cattle at present, due to its variable efficacy and because it interferes with the PPD test (Chandran *et al*., 2019).

Control and Prevention

The prevention and control of zoonotic tuberculosis needs a cross-sectorial and multidisciplinary approach, linking animal, human, and environmental health. The One Health approach is increasingly being endorsed by many prominent organizations to comprehensively address the challenges at the animal–human interface (Olea-Popelka *et al*., 2016). But there is low understanding on prevention of the true burden of bovine tuberculosis disease in human beings are low systematic surveillance for M. bovis as a cause of tuberculosis in people in all low-income and high tuberculosis burden countries where bovine tuberculosis is endemic, and the inability of laboratory procedures most commonly used to diagnose human tuberculosis to identify and differentiate M. bovis from M tuberculosis with the result that all cases can be assumed to be caused by M tuberculosis. Hence, the available data for zoonotic tuberculosis do not accurately represent the true incidence of this disease (Perez-Lago *et al*., 2014; WHO, 2015). There is only little surveillance is done particularly in developing countries on the epidemiology of this organism and the epidemiological requirements for its control (Ali, 2006).

Economic Importance

The Food and Agriculture Organization has prioritized bovine tuberculosis as an important infectious disease that should be controlled at the animal–human interface through national and regional efforts. However, bovine tuberculosis continues to cause important economic losses due to the reduced production of affected animals and the elimination of affected (or all) parts of animal carcasses at slaughter. This economic loss has an important effect on livelihoods, particularly in poor and marginalized communities because bovine tuberculosis negatively affects the economy of farmers (and countries) by losses due to livestock deaths, losses in productivity due to chronic disease, and restrictions for trading animals both at the local and international level (Olea-Popelka *et al*., 2016). Apart from actual deaths, infected animals lose 10-25% of their productive efficiency (Radostits *et al.*, 2007).
There is also extra expenses arise linked to surveillance and regular testing of cattle, removal of infected animals and other in-contact animals in the same herd, and movement control on infected herds (Radun, 2014). However, in Ethiopia, the economic impacts of bTB on cattle productivity and related points as well as bTB control programs have not yet been well documented or studied (Shitaye *et al.*, 2007).

Conclusion and Recommendation

In almost every country of the world, bovine tuberculosis is prevalent and causes great loss in dairy farm productivity. The disease has an importance public health issue due to its zoonotic importance. Human tuberculosis due to *M. bovis* has become very rare in countries with pasteurized milk and bovine tuberculosis eradication programs. However, this disease continues to be reported from developing countries where bovine tuberculosis disease is poorly controlled. The incidence is higher in farmers, abattoir workers and others who work with cattle. In developed countries, mandatory pasteurization of milk combined with tuberculin testing and culling (slaughter) of infected cattle resulted in dramatic decline in the incidence of human TB due to *M. bovis*. In contrast, spread from animals to humans in developing countries remains a very real danger, mostly from infected milk. In rural areas of Ethiopia most people drink raw milk and do have extremely close relation with cattle (such as sharing shelter) that intensifies the transmission and spread of BTB.

Therefore, based on the above concluding remarks the following recommendations are forwarded:

- Create awareness among the people about the public health significance of the disease.
- Government should be enforcing strict rule on test and slaughter policy and compensate affected farmers to elimination of the disease at source.
- Movement control with respect to trade and export from endemic areas into disease free area.
- Should avoid drinking of unpasteurized raw milk.
- Avoid living in humid, ill-ventilated, overcrowded place/house to prevent the zoonosis.

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