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Chapter

# Diseases Caused by Bacteria in Cattle: Tuberculosis

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## Abstract

Tuberculosis is an infectious, chronic or acute, localized or disseminated granulomatous disease that affects all animal species, caused by members of the genus mycobacteria. In cattle, the disease is caused by obligatory pathogenic and opportunistic species of mycobacteria and is transmitted between animals mainly through inhalation. It is a major public health concern and humans are infected chiefly through consumption of raw animal products. The disease is characterized by progressive emaciation, which may be terminally fatal. Pathological lesions comprising of be caseous or calcified granulomas are found mainly in the respiratory tract but animals infected through ingestion develop lesions in the lymph nodes of the head and the mesentery. Lesions may disseminate to involve other internal organs and tissues. Histologically, lesions manifest typical granulomas with a necrotic center surrounded by inflammatory cells and a fibrous capsule. Diagnosis is based on history, clinical signs, antemortem tests, and postmortem examination. Culture, isolation, and identification of the organism are confirmatory tests. The disease is a listed under the OIE Terrestrial Animal Health Code and the main method of control is testing and slaughter of affected animals. The importance of the disease is the zoonosis, loss in productivity in affected animals, and the cost of control.

**Keywords:** bacterial diseases, cattle, mycobacteria

## 1. Introduction

Tuberculosis in cattle is of serious public health as well as economic concern worldwide but more so in developing world. The disease is a zoonosis, transmitted from animals to humans mainly through the consumption of raw animal products especially milk. Human infections are therefore prevalent in communities with poor food hygiene and unsanitary cultural practices [1]. The resultant disease manifestation is largely similar to the human-type tuberculosis, with socioeconomic costs of stigma, reduced productivity, mortality, and cost of treatment. Rigorous control and eradication programs have drastically reduced transmission to humans in the developed world but in the developing world, it remains a serious threat to human health. Animal to animal transmission is mainly through the inhalation of infective respiratory aerosols. Production systems that involve close contact between animals promote transmission. The disease is listed under World Animal Health Organization (OIE) and therefore a restriction to trade in animals and animal products. Other costs include reduced animal productivity and the cost of control.

Since its identification in 1898, *Mycobacterium bovis* (later split into two subspecies: *M. bovis* subsp. *bovis* and *M. bovis* subsp. *caprae*) has been considered as the etiological agent of tuberculosis but later, other members of the *Mycobacterium tuberculosis* complex (MTBC) were found to cause similar infections [2–4]. More recently, species of mycobacteria hitherto regarded as saprophytic and nonpathogenic, referred to as nontuberculous mycobacteria (NTM) and more recently, mycobacteria other than tuberculosis (MOTTs) have been identified as causative agents [4, 5]. Apart from the requirement for isolation and identification of the causative for confirmatory diagnosis, these species have complicated interpretation of in vivo diagnostic tests, such as the tuberculin test, due to the expected cross-reactive immune responses [6, 7]. Some of these MOTTs have also been found to cause a variety of infections in humans and should therefore be considered potentially as zoonotic, and infestations in cattle and other animals as important and the MTBC. Many other species of animals are also susceptible to *M. bovis*. These include wildlife species, which constitute reservoirs of infection for domestic animals [8, 9]. Transmission to domestic animals and humans is therefore potentially an outcome of human-wildlife conflict. With such a variety of mycobacteria species, now associated with tuberculosis in cattle, perhaps the etiological term mycobacteriosis, rather than the pathological term tuberculosis, should be more applicable. This chapter will explore the etiology, epidemiology, pathogenesis, pathology, diagnosis, public health importance, and control of tuberculosis in cattle. It is expected that the chapter will be found useful by veterinary students, tutors, animal health service providers, and researchers.

## 2. Definition

Tuberculosis is an infectious, chronic or acute, localized or disseminated granulomatous disease that affects mammals, fish, and birds, caused by members of the genus *Mycobacterium*. In cattle, the disease is caused by obligatory pathogenic and opportunistic species of mycobacteria. Animals affected by the disseminated infection progressively emaciate and finally succumb to the infection. The importance of the disease is its zoonosis and the economic losses it causes.

## 3. History

Tuberculosis affects warm- and cold-blooded animals and it is estimated that it has been around for more than 3 million years [10]. The disease in cattle was first observed by the Spaniard farmer, Lucius Junius Moderatus Columella in Northern Italy in the year 14 AD [11]. In 1881, Robert Koch discovered *Mycobacterium tuberculosis* (tubercle bacillus) as the cause of tuberculosis in humans and in 1882, established the connection between human and animal tuberculosis through the observation that consumption of contaminated cow's milk led to infection. In 1898, Theobald Smith identified *M. bovis* as a different species from *M. tuberculosis*. The first compulsory milk pasteurization law was enacted in UK in 1908 following the research that linked consumption of raw milk to extrapulmonary tuberculosis; two French scientists Albert Calmette and Camille Guerin developed the Bacillus Calmette-Guerin (BCG) vaccine for immunizing humans against tuberculosis, by attenuating *M. bovis* through subculture. The vaccine was first used in 1921 [12]. In 1890, Robert Koch extracted tuberculin from the tubercle

bacilli. The extract was initially tried as a vaccine, but later shown to have diagnostic potential to detect infected animals. The tuberculin skin test for animals was thereafter developed [10]. The development of the skin test for humans was then carried out by Von Pirquet and Mantoux in 1907–1908 [13]. In the developed world, bovine TB eradication programs involving herd testing and culling of reactors and pasteurization of milk has largely eliminated the spread of bovine TB. The disease, however, remains a serious public health problem in many developing countries [14].

## 4. Etiology

### 4.1 Classification of mycobacteria

The genus *Mycobacterium* is the only genus in the family *Mycobacteraceae* in the order *Actinomycetales*, which includes other mycolic acid-containing genera, namely *Nocadia*, *Rhodococcus*, *Gordonia*, and *Tsukamurlla*. Currently, the genus comprises of over 150 species and 13 subspecies [15, 16]. Within the genus, classification is based on several factors including growth rate and pathogenicity. Based on pathogenicity, it can be classified into two groups: tuberculous and nontuberculous mycobacteria, the latter also referred to as mycobacteria other than tuberculosis (MOTTs). A refined classification on this basis groups the genus into obligatory pathogens, potentially pathogenic (opportunistic) and saprophytic or ubiquitous microorganisms [17]. Obligatory pathogens belong to *Mycobacterium tuberculosis* complex (MTBC) group that comprise *Mycobacterium bovis* subsp. *bovis*, *M. bovis* subsp. *caprae*, *M. tuberculosis*, *M. africanum*, *M. bovis* BCG, *M. canetti*, *M. microtti*, *M. pinnipedii*, and *M. leprae* [18]. All MTBC species have identical 16S rRNA sequences and a 99.9% similarity at nucleotide level, and may even be considered subspecies, but differ significantly in their host range [19]. The potentially pathogenic mycobacteria, represented by the *Mycobacterium avium* complex (MAC), consists of closely related species and subspecies, which include, among others, *M. avium* subsp. *avium*, *M. avium* subsp. *paratuberculosis*, and *M. avium* subsp. *hominissuis*. The potentially pathogenic species are found in the environment as well as in the susceptible hosts and cause disease mainly in hosts with compromised immunity [20]. Saprophytic mycobacteria are the largest group found in the environment. Some, such as *M. kansasii*, *M. asiaticum*, *M. interjectum*, *M. szulgai*, *M. fortuitum*, *M. celatum*, *M. ulcerans*, *M. smegmatis*, and *M. septicum* have been associated with diseases in humans and animals [21].

On the basis of growth rate, the genus is classified into slow and rapidly growing species, with rapid-growers being those that produce grossly visible colonies in less than 7 days and slow-growers taking over 7 days. Slow-growing species are more commonly associated with pathogenicity than the fast-growing group [22].

In cattle and other ruminants, tuberculosis is caused mainly by the obligate pathogen *Mycobacterium bovis* subsp. *bovis* but infections by *Mycobacterium bovis* subsp. *caprae*, *Mycobacterium tuberculosis* and *Mycobacterium africanum* also occur [2–4]. In Central Europe, *M. bovis* subsp. *caprae* is the major cause of tuberculosis in cattle [23, 24]. The disease caused by *M. bovis* subsp. *bovis* and *M. bovis* subsp. *caprae* is commonly referred to as bovine or zoonotic tuberculosis. Although not as widely as zoonotic tuberculosis, MOTTs infections have been reported in cattle exhibiting granulomatous lesions identical to those caused by the MTBC complex [6, 15, 16].

## 4.2 Cellular morphology and staining

Mycobacteria are nonmotile, noncapsulating, and nonspore forming rods measuring 0.2–0.6 µm by 1.0–10 µm with a slender, straight or slightly curved shape. The cell wall of mycobacteria contains a hydrophobic lipid layer, which includes mycolic acids, phosphatidylinositol, mannosides, phthiocerol dimycocerosates, isoprenoid lipids, glycerophospholipids, lipoarabinomannan, and trehalose mycolates and lipoglycans, which give the organism some unique characteristics:

1. Growth requires complex organic media, containing long-chain free fatty acids necessary for the synthesis of the lipid layer.

2. The hydrophobic lipid layer causes poor penetration of nutrients, hence the slow growth of the organism and the long incubation period of disease.

3. The poor penetration of chemical agents makes the organism difficult to stain by ordinary procedures. Once stained, they resist decolorization even by weak mineral acids such as 3% hydrochloric acid in ethanol, hence the name acid-fast bacilli (AFB). Mycobacteria have a cell wall structure characteristic of Gram-positive bacteria but they cannot be stained by this method although they may stain weakly Gram-positive.

4. The organism is highly resistant to disinfectants and most antibiotics, occasioning lengthy treatment of infection.

5. The presence of fatty acids in the cell wall causes cells to aggregate in a pattern referred to as "cording," observed in stained smears and in broth cultures, due to resemblance to strands of rope cords, and in solid media, growth resembles that of fungi (*myco* = Greek = means "fungus").

6. The lipid layer plays a role in resistance to the host's immune system [25–27]. Paradoxically, the biosynthesis site of some of the lipid components is also the site of action of anti-TB drugs [26].

## 4.3 Cultural characteristics

*Mycobacteria* are obligate aerobes and require complex organic media for growth. Solid media such as the egg-based Lowenstein-Jensen, (L-J), Middlebrook 7H10, and Middlebrook 7H11 or liquid media such as Modified Middlebrook 7H9 broth are used. Like other MTBC members, *M. bovis* is a slow grower. On solid media, colonies are detectable 3–6 and up to 12 weeks of incubation at 37°C weeks depending on the concentration of inoculum [28]. Colonies are small, raised, rounded, off-white (bluff) in color, wrinkled surface, and with irregular margins [29]. Addition of pyruvate is reported to stimulate growth of *M. bovis* and glycerol, which favors growth of *M. tuberculosis,* is said to inhibit it [28, 30]. Other findings however, indicate that *M. bovis* can grow satisfactorily in media containing either substance [5]. Members of MTBC group, including *M. bovis,* are inhibited by paranitrobenzoic acid (PNB), a criteria used to differentiate the group from MOTTs [31, 32]. Growth in liquid media is faster since the organism is surrounded by the media and access to nutrients is more efficient. Growth appears as clumps or "cords." Addition of egg yolk to the growth medium enhances growth, due to the presence of

phospholipids that are required for growth but synthetic phospholipids such as polyoxyethylene sorbate compounds (Tweens) can also be used, which also lower the tendency of the mycobacteria to aggregate, giving a diffuse homogenous turbidity [33].

### 4.4 Biochemical properties

*M. bovis* exhibits strain variation in biochemical characteristics, which can be summarized as follows [28, 34, 35]:

| Test | Reaction | |
| --- | --- | --- |
| | *M. bovis* | *M. tuberculosis* |
| Sensitivity to thiophen-2-carboxylic acid hydrazide (TCH) | + | — |
| Sensitivity isonicotinic acid hydrazide (INH) | + | + |
| Niacin production | — | + |
| Nitrate reduction | — | + |
| Pyrazinamidase test | +* | — |
| Nicotinamidase test | +* | — |
| Amidase test | — | + |
| Urease production | + | Variable |
| Growth under microaerophilic environment | + | — |

*Mycobacterium bovis *subsp.* caprae *is negative.*

The main limitation of biochemical tests is that sufficient amounts of bacterial cells as well and several weeks of incubation are required. The other limitation is that unknown species of mycobacteria cannot be identified. The availability of more rapid methods such as molecular methods has therefore diminished the use of biochemical tests.

### 4.5 Environmental, chemical, and drug resistance

In general, mycobacteria are inactivated by prolonged exposure to heat, direct sunlight, and dry conditions. They are killed by temperatures of 65°C and above for at least 30 minutes and UV light but are resistant to freezing for prolonged periods. Under ordinary temperatures, *M. bovis* can persist in slurry and soil for at least 6 months and can survive for long periods in buildings and transport vehicles under dark, cold, and moist conditions [29, 36, 37]. The high lipid and wax content makes mycobacteria less susceptible to many chemical agents and disinfectants. Chemicals such as quaternary ammonium compounds, hexachlorophene, and chlorhexidine have bacteriostatic effect while formaldehyde vapor, chlorine compounds, 70% ethanol, hydrogen peroxide alkaline glutaraldehyde, and 5% phenol have bactericidal effect. Although treatment of infected animals is not normally practiced, *M. bovis* is resistant to most antibiotics but sensitive to the drugs used in treatment of *M. tuberculosis* (rifampin, isoniazid, streptomycin (STR), and ethambutol). *M. bovis* subsp. *bovis* is resistant to pyrazinamide (PZA), a first-line TB treatment drug in humans [38]. This characteristic is relevant in the management of infection in humans and also useful in differentiating *M. bovis* from *M. tuberculosis*. Multi-drug resistant strains of *M. bovis* have been reported in many countries [39].

## 5. Epidemiology of bovine tuberculosis

### 5.1 Host range

Mycobacteria have one of the widest host range and affects mammal, birds, fish, reptiles, and amphibians. Cattle and related ruminants such as buffalo and bison are regarded as the main hosts of *M. bovis* subspecies. Other mammalian hosts include sheep, goats, camels, horses, llamas, pigs, dogs, cats, humans, and nonhuman primates [40]. Many wild animals, including elephants, rhinoceroses, coyotes, mink, otters, seals, sea lions, hares, bears, warthogs, large cats ferrets, and rodents are affected. Known maintenance hosts include possums and ferrets in New Zealand; badgers, raccoons, and foxes in Europe; bison and elk in Canada; and kudu and African buffalo in Africa and white-tailed deer in the USA [9].

### 5.2 Geographical distribution and prevalence

Zoonotic tuberculosis caused by *M. bovis* has a worldwide distribution. It was reported by 78 of the 181 OIE reporting countries in 2017, distributed in every region of the world [41]. This figure is likely to be much lower due to under-reporting, occasioned by inadequate surveillance. Globally, the prevalence has been estimated at 0.8% [42]. Using reports of zoonotic tuberculosis in humans as indication, the highest prevalence is found in African region followed by South East Asia, Western pacific, Eastern Mediterranean, Europe, and lastly Americas [43]. The disease has been largely controlled in developed world through systematic test and slaughter of infected animals, meat inspection surveillance in abattoirs, and milk pasteurization but complete eradication has been hindered by the existence of reservoirs of the agent in wildlife species [44]. In many developing countries, the disease remains largely neglected [45]. MOTTs have mainly been isolated coincidentally from animal lesions while searching for *M. bovis*. Isolation of MOOTs from cattle carcasses range approximately between 7 and 70% of total isolates [4, 5].

### 5.3 Transmission and risk factors

Infected animals shed *Mycobacterium* via respiratory aerosols, milk, saliva, feces, urine, and discharging lesions. The main route of infection in cattle is mainly through the inhalation of infective aerosols. This is supported by high frequency of tuberculous lesions found in the respiratory tract and associated lymph nodes [46]. Transmission is facilitated by close contact between animals and therefore the production system plays an important role. Intensive livestock farming, referred to as zero-grazing, promotes close contact between animals. In extensive production, such as practiced by nomadic pastoralists in arid and semiarid regions of Africa, close contact between animals occur in, night shelters, watering points, vaccination centers, marketing yards, and at dipping tanks while in intensive production close contact occurs during milking and in watering and feeding troughs [36]. Ingestion of contaminated feed and water is generally considered to be a secondary, less important route of transmission but in countries where untreated manure is commonly used as a fertilizer in farms, such manure can become a source of infection to animals through pasture and vegetation contamination [36, 37]. The oral route is also particularly important in calves nursing from infected cows.

Other rare routes of infection include cutaneous, genital during coitus, congenital through placental or umbilical infection, and transmission through udder infections [47]. Contact between domestic and wild animals through pasture contamination is a risk factor. Domestic species reported to be reservoirs and spill-over

hosts include sheep and goats. The low prevalence of tuberculosis in these species in the African region, however, indicates that they may not be significant in transmission of disease to cattle [48, 49].

Human to animal transmission through aerosols is well documented and patients with pulmonary tuberculosis pose danger to animals [50]. Humans with urogenital tuberculosis represent a source of infection for animals through contamination of pastures with urine. In Ethiopia, the traditional practice of spiting chewed tobacco into mouths of livestock as anti-parasitic treatment is a potential source of infection with *M. tuberculosis* [3].

Male animals were more significantly affected by than female animals while *Bos indicus* (zebu) have been found to be more resistant than *Bos Taurus* (Exotic breeds). At the herd level, herd size increases infection due to increased exposure and introduction of new animals into a herd is a risk factor [51].

The primary source of infection by MOTTs is presumably the environment [16], and although, the specific source of individual infections may not be easily identified, and the route of infection may be deduced from the localization of granulomas.

## 6. Pathogenesis

Animals exposed by ingestion of contaminated feed or water often develop primary foci in lymph nodes associated with the intestinal tract, while aerosol exposure leads to the involvement of the lungs and associated lymph nodes. In case of respiratory infection, the mucociliary clearance in the upper respiratory passages may prevent infection in some exposed animals [52]. In the bronchi, the organism penetrates the mucosa and are trapped and phagocytosed in the bronchial and mediastinal lymph nodes. In the lungs, the bacterial are phagocytosed by alveolar macrophages. In case of oral infection, the organism presumably penetrates the bucal or intestinal mucosa and, via the lymphatics, reaches the phagocytes in the draining lymph node. The phagocytosis causes a localized inflammatory reaction and recruitment of mononuclear cells from neighboring blood vessels. The cellular response results in the accumulation of large number of phagocytes leading to the formation of the granuloma or the tubercle that characterizes the disease [36, 47]. The granuloma consists of infected macrophages surrounded by epithelioid cells, granulocytes, lymphocytes, and later, multinucleated giant cells [53].

Mycobacteria are facultative intracellular pathogens, and survive and multiply within the hosts' phagocyte. The ability of the organism to survive intracellular within macrophages involves interfering with the development of the phagosome into a degradative vesicle. It is thought that the organism prevents the phagosome from maturing and fusing with lysosomes to form the phagolysosome. The mycolic acids of the organism are thought to play a role in blocking this phagosome maturation [54, 55]. Some components of the lipid layer, such trehalose dimycolate, may cause death of macrophages by direct cytotoxicity [52]. The *Mycobacterium* survival and multiplication within the phagosomes eventually destroys the macrophage. When entering into the death phase, infected macrophages release mycobacterial antigens, which are engulfed by uninfected dendritic cells, processed and subsequently presented, via major histocompatibility complex class I, to CD8+ T cells. The cellular hypersensitivity that develops, contributes to cell death and tissue destruction resulting in caseous necrosis. In some instances, liquefaction and cavity formation occur as a result of enzymatic action on proteins and lipids, and the organism multiplies uncontrolled in these cavities. Rapture of the cavities into the bronchi allows aerosol spread of the bacilli. Dissemination by bacteria-containing macrophage may occur through vascular and lymphatic channels to form lesions

in many organs, as in acute miliary TB, which is rapidly fatal [47, 53]. Innate non-specific and specific cell-mediated immunities are the main host defense mechanisms. The innate resistance may clear the initial infection and prevent mycobacteria to proliferate. Specific resistance is mediated by T-lymphocytes. They destroy infected macrophages or activate them to destroy extracellular bacilli through soluble mediators such as gamma interferon [56]. Where the host has been able to contain spread of infection, lesions consistency progress from caseous, fibro-caseous, fibro-calcified to calcified and are surrounded by a fibrous capsule. Calcified granulomas generally indicate a successful suppression of the infection by the immune response and the lesions may regress completely [53]. During pathological processes, mycobacteria are present in tuberculous tissue and in various body fluids, secretions and excretions such as milk, blood, sputum, bronchoalveolar lavages, cerebrospinal fluid, and semen [36].

## 7. Pathology

Pathology of tuberculosis is characterized by the formation of granulomatous lesions mainly in the respiratory and alimentary tracts and associated lymph nodes.
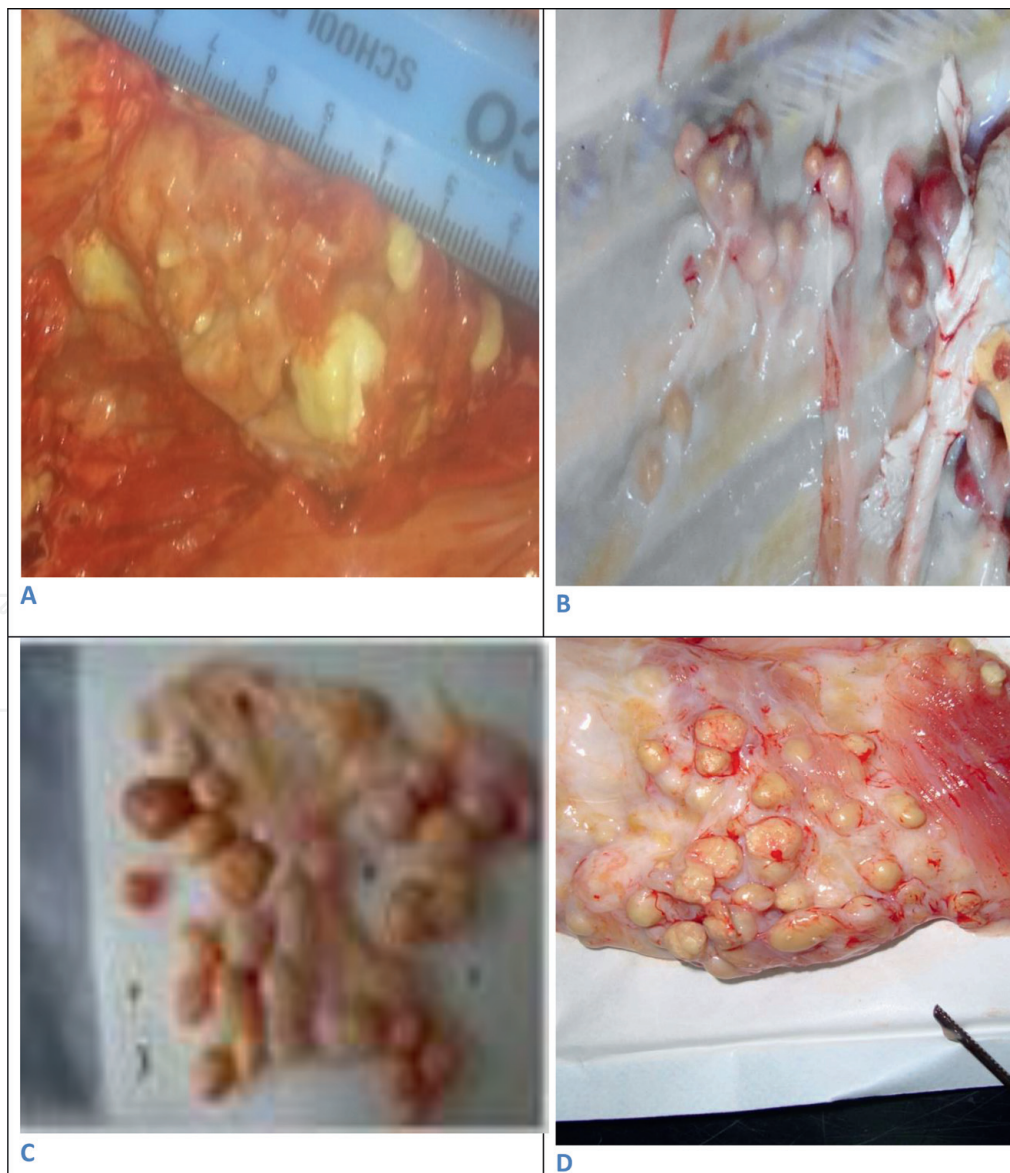


**Figure 1.**
*Multiple tuberculosis lesions observed in lungs (A), pleura (B), mesentery (C) and diaphragm (D) in cattle during postmortem meat inspection.*
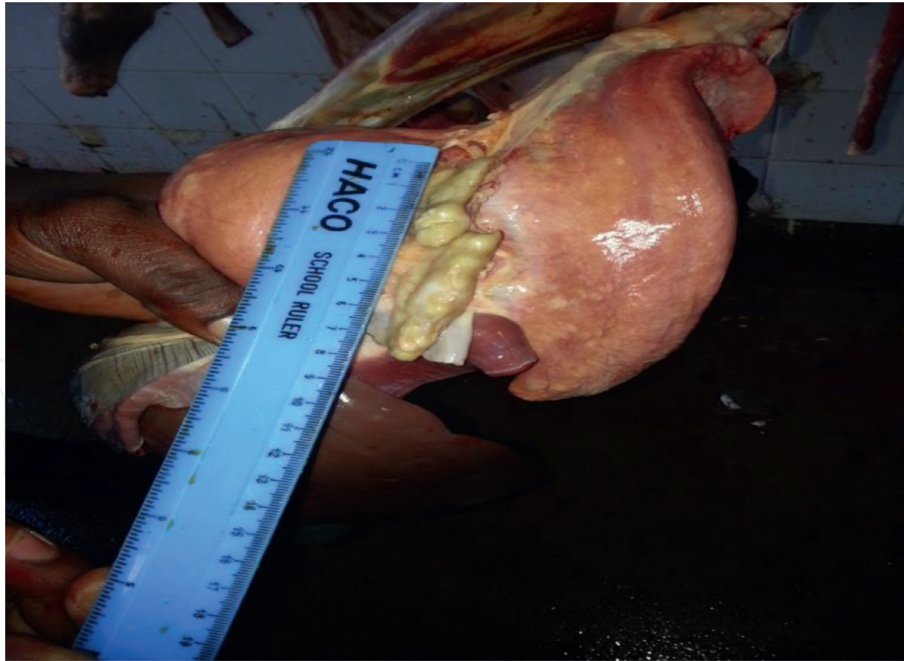
**Figure 2.**
*Tuberculous lesions in the mediastinal lymph nodes of a goat meat carcass. ©2018. JKN Kuria.*

The lesions may be localized to few organs or tissues or disseminated to multiple sites. In the respiratory system, lesions are observed in bronchial lymph nodes, in lungs (**Figure 1A**) and in mediastinal lymph nodes (**Figure 2**). The alimentary system lesions involve the retropharyngeal, parotid, sub-maxillary, and mesenteric lymph nodes (**Figure 1C**), as well as the liver and portal lymph nodes, the spleen and other internal organs such as the kidneys [28]. Lesions may also be found on surfaces of body cavities such as the pleura (**Figure 1B**), diaphragm (**Figure 1D**), and perito-neum. In most cases, lesions are confined to the lymph nodes of the head region and respiratory tract [47]. The size, color, and consistency of the lesions vary widely according to the stage of infection. Lesion sizes are microscopic or large enough to involve the greater part of or the whole organ or tissue. The consistency ranges from caseopurulent, fibro-caseous fibro-calcified to calcified, but may also be thin-walled purulent cavities [4, 5, 53]. Histopathological features of a granuloma show a central area of caseous necrosis with or without calcification, surrounded by macrophages, lymphocytes, plasma cells, neutrophils, epithelioid cells, and Langhan's giant cells and enclosed partially or completely by a fibrous capsule [53].

## 8. Clinical signs

The signs of tuberculosis in cattle usually vary depending on the organ systems affected. In the early stages, clinical signs are not visible and many animals with tuberculosis are clinically normal. The signs have a gradual onset characterized by progressive weakness, debility, and mild fluctuating fever. Advanced lung involve-ment is characterized by dyspnea, chronic moist cough, more marked in the morn-ing and during cold weather, and reduced exercise tolerance [14]. Swollen lymph nodes of the head may be observed and involvement of internal lymph nodes may result in obstruction signs of the system or organ affected. There may be diarrhea or constipation due gastrointestinal tract involvement. Mammary tuberculosis has been found in varying proportions of animals, from 1 to 2%, up to 5.4% and is characterized by persistent mastitis and hypertrophy [40]. Infertility or abortion may result from tuberculous metritis, accompanied by chronic purulent vaginal

discharge. Affected animals generally remain bright and alert and maintain a good appetite despite weakness and sluggishness [57]. Acute or subacute death may result from military tuberculosis, caused by rapid widespread dissemination, from primary or secondary lesions through the hematogenous route.

## 9. Public health importance

Zoonotic tuberculosis in cattle is a public health concern worldwide. The prevalence is estimated at 0.5–1% in developed countries and 10–15% in developing countries [58]. In developing world, high levels of human immunodeficiency virus (HIV) and poverty, especially in Sub-Saharan countries, are contributing factors. Consumption of raw or undercooked products, and especially milk, from infected cattle is the main cause of nonpulmonary tuberculosis [59]. *M. bovis* is excreted in milk of about 1–2% of infected cattle in large numbers such that a single infected cow can contaminate bulk milk by 100 cows to cause infection in susceptible humans [60]. Social-cultural factors, for instance, the tradition by pastoral communities to consume raw blood and milk and raw or undercooked meat and meat products, are risk factors [1]. It is estimated that in Africa, 90% of milk is consumed either raw or fermented, thus increasing the risk of transmission [61]. Cervical lymphadenitis is the commonest manifestation of oral infection (**Figure 3**). Inhalation of infected dust particles or aerosols shed by infected cattle is the second important route especially in rural pastoralist communities. Abattoir workers, farmers, milkers, veterinarians, and animal handlers are also exposed to this mode of transmission [58, 62]. Infection in wildlife puts hunters, trappers, and zoo workers at risk. Trans-cutaneous transmission may occur through handling of infected carcasses [47].



**Figure 3.**
*Raptured lesion (arrowed) in the left retropharyngeal lymph node of a tuberculosis patient infected by* M. bovis. *©2018 JKN Kuria.*

## 10. Diagnosis

Tuberculosis in cattle can be diagnosed in live animals and also during postmortem examination of dead or slaughtered carcasses. In live animals, clinical signs, tuberculin skin test, and gamma interferon assay can be used. At postmortem, pathological lesions and acid fast staining are preliminary tests while culture and DNA analysis are confirmatory.

### 10.1 Diagnosis by clinical signs

Clinical diagnosis may be difficult due to the chronic nature of the disease and the wide variety of symptoms, resembling other chronic debilitating conditions. The disease should be suspected on the basis of history coupled with signs of progressive emaciation, in spite of good appetite, fluctuating temperature, chronic, and moist cough dysphagia and noisy breathing. Enlargement of supramammary lymph nodes may be observed. Differential diagnosis includes contagious bovine pleuropneumonia, pasteurellosis *Trueperella pyogenes* pneumonia, bovine lympho-sarcoma, traumatic pericarditis, and fascioliasis [63]. Animals suspected of tuberculosis infection should be thoroughly examined by palpation of all superficial lymph nodes, the udder in females and percussion and auscultation of the pulmonary area.

### 10.2 Tuberculin skin test

Tuberculin skin test is the standard procedure recommended by the World Organization for Animal Health (OIE) for the diagnosis of bovine TB in live animals. This test measures the delayed type hypersensitivity response to tuberculin, *referred* to as purified protein derivative (PPD), injected intradermally. There are two variations of the test. The single intradermal test (SITT), which uses PPD from *M. bovis* only (PPD-B), and the comparative intradermal test (CITT), which uses PPD-B and PPD from *Mycobacteria avium*, (PPD-A). In the SITT, PPD-B is injected intradermally in the neck region. A positive test is indicated by a delayed hypersensitivity reaction (**Figure 4**). The skin thickness at injection site is measured with a pair of calipers before and 72 hours after injection. A relative change greater than 4 mm in skin thickness at the site is considered positive for *M. bovis* infection [28]. The CITT is designed to address the cross-reaction between *M. bovis* and the *M. avium*. PPD-B and PPD-A are injected side by side, around 12 cm apart, and skin swelling is measured after 72 hours. The test result is considered positive, if the relative difference in the increase of skin thickness at the site of PPD-B injection is 4 mm greater than that at the site of PPD-A injection [28]. The sensitivity and specificity of the CITT has been estimated at 81–85 and 80.0, and 99.9%,
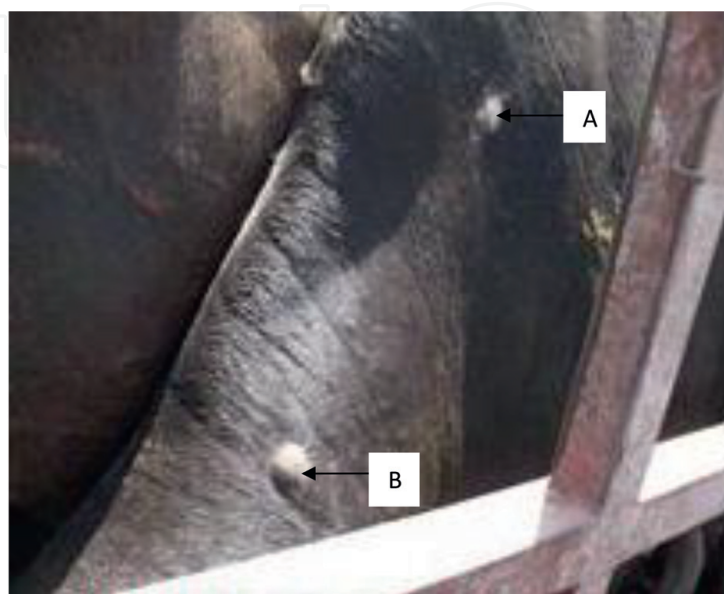


**Figure 4.**
*A comparative intradermal tuberculin test in a cow showing a positive reaction. PPD-A was injected at site A and PPD-B at site B. ©2018. JKN Kuria.*

respectively [58, 64]. The CTT has higher specificity than the SITT since it can distinguish animals infected with nontuberculous mycobacteria, specifically the MAC complex, which include *M. avium s*ubsp. *paratuberculosis*, the causative agent of Johne's disease. Other MOTTs species with ability to cross-react with *M. bovis* have, however, been isolated from tuberculous lesion in cattle and related wild species. The advantage of the CITT over the SITT is therefore limited [5–7].

**10.3 Gamma interferon assays**

The gamma interferon assay (IFN*γ)* is an *in vitro* form of the CITT. It is based on detection of *γ* interferon produced by specific circulating lymphocytes upon stimulation of heparinized whole blood *in vitro* with PPD-B and PPD-A. Detection of IFN*γ* is carried by a sandwich ELISA, using two monoclonal antibodies to bovine gamma-interferon, after incubation of the blood for about 16–24 hours with PPD-B and PPD-A. The IFN*γ* test is reportedly more sensitive than the tuberculin test and can detect infected animals that are negative to the later. The sensitivity and specificity are estimated at 81.8 and 99.1% [65]. It has been observed that more infected cattle can be identified by using both the tuberculin and the IFN*γ* tests and it is recommended that both tests be conducted in parallel [66]. The advantage of the IFN*γ* is that infected animals are detected early and only one visit to the farm is required. It is particularly convenient for animals that are difficult to capture or handle, such as cattle reared in ranches or under nomadic pastoralism, or wildlife, as they need only to be captured once rather than twice. It however requires more technical expertise and facilities and is costly (approximately 10 USD, for consumable materials per test).

**10.4 Postmortem diagnosis**

Detection of tuberculosis using pathological examination involves visual observation, palpation, and incision of organs and tissue to detect lesions. A presumptive diagnosis can be made on the basis of macroscopic granulomatous lesions (**Figures 1** and **2**). Differential diagnosis includes parasitic and mycotic granulomas and abscesses caused by other bacterial pathogens such as *Actinomyces bovis*, *Actinobacillosis,* and *Trueperella pyogenes*, as well as bovine lymphosarcoma [67, 68]. Further, very small lesions may be missed and may only be detected microscopically. Routine postmortem meat inspection has been found to detect approximately only 47% of presumptive lesions [69]. Direct smears of suspected lesions should be stained by the acid fast method and examined for acid-fast bacilli (**Figure 5**).

**10.5 Culture and isolation of mycobacteria**

Culture is considered the "gold standard" for detection of *Mycobacteria* [69]. Samples for culture are first homogenized and decontaminated with sodium hydroxide to inactivate any contaminant bacteria present in the sample, inoculated into solid or liquid media and incubated at 37°C. Solid media include egg-based Lowenstein-Jensen (L-J), Agar-based media such as Middlebrook 7H10, 7H11, and Stonebrink Leslie solid culture media. Solid media is prepared as slants in screw-capped bottles. In Lowenstein-Jensen media, malachite green dye (0.025 g/100 ml) is used as a selective agent. Isolation should target MTBC and MOTTs and it is recommended that each sample is inoculated into three tubes of LJ, one containing glycerol, another pyruvate, and the other PNB. Most mycobacteria are obligatory aerobic but *M. bovis* is microaerophilic. Screw caps should be loosened, to allow in oxygen, and the tubes incubated in a slanting position, to allow bacteria to seed onto
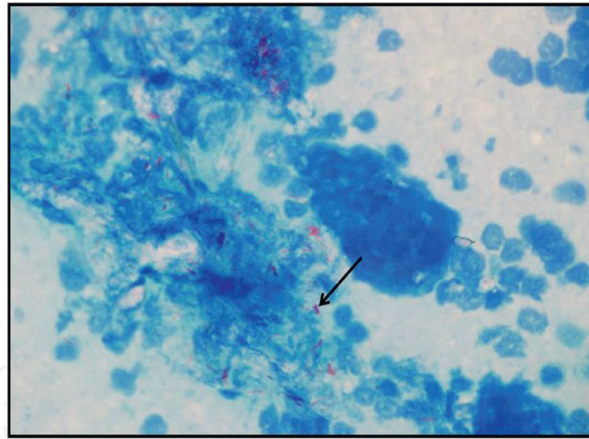
**Figure 5.**
*Direct smear of a tuberculous lymph node lesion from a cow, showing presence of acid-fast bacilli (arrowed).*
*ZN × 1000. ©2018. JKN Kuria.*

the media. Thereafter, the caps are tightened and the tubes incubated vertically for six 12 weeks. Liquid media include BACTEC 460, Mycobacterial Growth Indicator Tube (MGIT), which have enriched Middlebrook 7Ha with antibiotics and growth promoters are added. BACTEC 460 MGIT media is fully automated and can monitor the growth of mycobacteria by the use of oxygen quenching or fluorescent sensor. Mycobacteria may not be recovered in the cultures for a number of reasons:

1. Extended period between sample collection and analysis, leading to death of the organism.

2. Nonviability of the bacilli due to necrosis and calcification of granulomas.

3. Organisms may be inactivated by the decontamination process.

4. Samples may contain microorganisms other than mycobacteria.

Cultures suspected to be mycobacteria are then stained by acid fast method for confirmation. All laboratory procedures must be conducted in a class II biosafety cabinet in a laboratory environment that has been found safe and secure following risk assessment.

### 10.6 Molecular diagnosis

Molecular tools for differentiating *Mycobacterium* species have been developed [70]. Polymerase chain reaction (PCR) technique involves detection of the genetic material that is unique and specific to a species. Convenient commercial kits are available. Genotype *Mycobacterium* (Hain, Nehren, and Germany) are line probe assays available in three different formats: Genotype MTBC differentiate species in MTBC; GenoType *Mycobacterium* common mycobacteria (CM) detects most frequently encountered Mycobacteria species and Genotype *Mycobacterium* additional species (AS) detects less frequently encountered Mycobacteria species. This kit uses reverse hybridization technology on a solid membrane matrix consisting of nitrocellulose strips. The DNA probes are immobilized on parallel lines on the strips. Biotinylated DNA apricon fragments of the 16S-23SrRNA spacer region are incubated with the labeled strips and hybridization detected colorimetrically by addition of an enzyme, Streptavidin-alkaline phosphatase, and a chromogenic substrate. A precipitate is formed on

the membrane, where hybridization takes place. Another line probe assay is INNOLiPa Mycobacteria (Innogenetics, Ghent, Belgium). Line probe assays are convenient in that they can detect many species of mycobacteria simultaneously. The strips can also be conveniently dried and preserved.

AccuProbe (GEN-Probe, San Diego, California, USA), is an in-solution hybridization assay. DNA probes consisting of species-specific, single-stranded DNA oligonucleotides are prepared complementary to ribosomal RNA released from bacterial cultures and labeled with acridinum ester (chemiluminescent). Hybridization is measured by chemiluminescence using a luminometer and expressed as relative light units (RLU). The test can be performed on culture growing from broth or solid media and will detect all members of MTBC but without differentiating the species. However, since no nucleic acid amplification occurs in the assay, identification requires sufficient growth.

Real-time commercial PCR kits are also available for direct detection in clinical specimens and pathological specimens but can also be used for identification of cultures. The current available kits detect MTBC but not individual species.

Restriction fragment length polymorphism (RFLP) or spoligotyping distinguishes between phenotypically different strains of *M. bovis* [71]. It is designed to detect the unique spacers within the direct repeat (DR) locus of the *M. bovis* genome [72] and is a useful epidemiological tool, in that it indicates strains circulating in a population, and therefore the transmission patterns.

DNA tests are more rapid and reliable than the conventional identification methods, but are still limited to the postmortem diagnosis of the infection, in that, tissue samples or isolates are still required. Extraction and detection DNA in nasal swab samples, milk, lymph node aspirates may however be achieved [73].

## 11. Economic impact

Economic losses due to tuberculosis in cattle worldwide are estimated at more than US $3 billion annually [74]. This may be an underestimate since losses in many developing countries have not been examined sufficiently or studied at all. Loss of productivity of infected animals includes reduced milk yields, meat production, and reduced fertility. Among dairy cattle, milk production may decrease between 4 and 18%. Other direct losses include mortalities, infertility, calf mortalities, additional processing for infected animals, and condemnation of carcasses at slaughterhouses. Export market restrictions constitute nontariff barriers to trade. The cost of control involves meat inspection, test and slaughter of positive animals, pasteurization of milk, and compensation schemes to farmers. The public health cost include cost of treatment, mortality, loss of incomes and livelihoods, food insecurity, stigmatization as well as extra working hours for those attending to sick humans [75, 76]. Globally, 147,000 new cases of zoonotic TB in humans were estimated in 2016, resulting in 12,500 deaths. Most of the cases were in the African followed by the South-East Asian region [43].

## 12. Control

Bovine tuberculosis is listed under the OIE Terrestrial Animal Health Code. Control should be aimed at reducing prevalence in animals in order to prevent transmission to humans. The recommended control method in livestock is continuous detection and slaughter of infected animals [28]. Postmortem meat inspection

and pasteurization of milk is an effective method of preventing infected animal products from entering the food chain. Meat inspection can allow trace-back to the herd of origin, which can then be tested and eliminated. Individual testing of cattle and removal of infected and in-contact animals, coupled with animal movement controls reduces prevalence [28]. Testing and slaughter may, however, not be tenable in poor countries because of insufficient financial resources, pastoral production method that is characterized by uncontrolled movement of animals, weak veterinary institutions and political instability [28]. Further, in developing countries especially in Africa, cattle are raised together with sheep and goats, which act as reservoirs and are not targets for test and slaughter.

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