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Battling Bovine Tuberculosis: Modern Approaches to Diagnosis and Control

Mahendra Pal[1,*], Motuma Regassa[2], Mulugeta Gizachewu[3], Kumasa Shifara[4], Ravindra Zende[5], Aishwarya Nair[5], Giovanni Sgroi[6]

[1]Narayan Consultancy on Veterinary Public Health and Microbiology, Bharuch, Gujarat, India
[2]Toke Kutaye Woreda Agricultural Office, Guder, West Shewa Zone, Ambo, Oromia, Ethiopia
[3]School of Veterinary Medicine, Ambo University Guder Mamo Mezemir Campus Veterinary Teaching Clinic, Ambo, Ethiopia
[4]Toke Kutaye Woreda Agricultural Office, Guder, West Shewa Zone, Ambo, Oromia, Ethiopia
[5]Department of Veterinary Public Health, Mumbai Veterinary College, Parel, Mumbai, Maharashtra, India
[6]Animal Health Department, Experimental Zooprophylactic Institute of Southern Italy, 2 Via della Salute, 80055 Portici (Naples), Italy
*Corresponding author: palmahendra2@gmail.com

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Abstract Bacterial zoonoses have serious implications on human and animal health, and are significant causes of morbidity as well as mortality in developing and developed nations of the world. Bovine tuberculosis (BTB) caused by *Mycobacterium bovis* is a chronic highly infectious bacterial disease, which affects both people and animals. This disease primarily affects animals, though it can also occasionally harm humans. It is extensively distributed around the world. BTB poses a threat to human health because of its potential for zoonotic diseases, impacts the ecology through wildlife transmission, and can have an effect on both the domestic and global economies. Its primary economic significance stems from its potential to directly impact animal reproduction, milk and meat output, and other related processes. Several methods, including the single intradermal test, comparative intradermal test, short thermal test and Stormont test, are typically used to diagnose tuberculosis in cattle. Methods that enable direct identification of the Mycobacterium tuberculosis Complex are blood-based diagnostic technique such as gamma interferon assays, enzyme-Linked immunosorbent assays, lymphocyte proliferation assay, Culture of *Mycobacterium*, and molecular tests like polymerase chain reactions. Variable number tandem, restriction fragment length polymorphism and spoligotyping methods utilized for simultaneous strain-level mycobacterium species detection and typing are called repeat typing. A conclusive diagnosis requires the identification of *M. bovis* using biochemical, molecular, and cultural methods. The workers in slaughterhouses, veterinary clinics, and agriculture can prevent occupational infections by protecting themselves from airborne pathogens. The management of humans and animals' environments, together with immunization, is the key component for disease control and prevention.

Keywords: *Bovine tuberculosis, Control, Diagnosis, Mycobacterium bovis, Prevention, Public health, Zoonotic implications*

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1. Introduction

With an estimated 56 million cattle, 29 million sheep, 29 million goats, 57 million poultry/chickens, 13.33 million equines and 7 million bee colonies, Ethiopia is one of the countries with the greatest livestock populations on the African continent [1]. The total annual milk production is estimated at 7.1 billion litres in 2022 and only a fraction of the recommended per capita annual milk consumption of 200 litres according to the World Health Organization (WHO) [2]. However, diseases like bovine tuberculosis hinder the growing dairy production system in urban and peri-urban areas in Ethiopia. The *Mycobacterium tuberculosis (M. tuberculosis)* complex is a group of closely related bacteria that cause tuberculosis (TB), a chronic granulomatous illness that affects both humans and animals.

Among the members of the MTBC, *M. tuberculosis*, *M. africanum*, *M. bovis*, *M. caprae*, *M. pinnipedii*, and *M. microti* are the principal pathogenic species. Bovine tuberculosis (BTB), which is caused by *Mycobacterium bovis*, *Avian tuberculosis* (avian TB) and tuberculosis (human) caused by *Mycobacterium tuberculosis* are the three most common forms of tuberculosis and should be taken seriously. Additionally, to their primary hosts, MTBC species can infect other secondary hosts [3]. 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 [4].

It has a major economic influence on the cattle production industry and is widely disseminated throughout the world [5]. TB was one of the seven most neglected endemic diseases in the world. It can infect humans through aerosols, unpasteurized milk and dairy products, and meat from infected cows. It is most common in developing nations like Ethiopia. Although bovine tuberculosis is almost completely eradicated in much of Europe and a few Caribbean nations, including Cuba, the illness is still widespread in many developing nations, especially in Africa. There are an estimated 140 000 cases of zoonotic tuberculosis each year that result in approximately 11,400 deaths globally [6].

Due to the mandated pasteurization of milk and tuberculin skin testing of cattle, followed by the culling or slaughter of diseased cattle, the incidence of bovine tuberculosis has decreased in developed countries. *Mycobacterium bovis* caused 143,000 cases of tuberculosis in humans worldwide in sixteen countries in 2018 [7]. The disease has also spread to several regions of Ethiopia, with differing prevalence rate [8]. Ethiopians' traditional preference for raw milk over pasteurized milk due to its taste, availability, and lower cost [9] acts a potential zoonotic risk factor for transmission from cattle to humans. Therefore, the objective of this communication is to present a comprehensive review on the current diagnostic techniques and control strategies of bovine tuberculosis.

2. Bovine Tuberculosis

2.1. 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. This genus was placed in its own family of *Mycobacteriaceae* in the order *Actinomycetales* [10]. 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 mil% in the DNA.

2.2. Morphology and Staining

Mycobacteria are pleomorphic bacilli or coccobacilli that are non-motile and do not generate spores. Within the tissues, they manifest as rods that range in length from 1.0 to 4.0 μm and in diameter from 0.2 to 0.3 μm. These rods can be straight, bent, or shaped like clubs. They can be found alone, in couples, or in little bundles. They can show up as 6–8μm long rods or cocci on lab media. There are no capsules, spores, or flagella present [11]. Even though Mycobacterium is gram positive cytochemically, it does not stain well with gram stain owing to their acid fastness, such as the ability to withstand discoloration with 3% HCL in ethanol is their most well-known staining characteristic. The integrity of the cell wall's waxy coating determines acid fastness. Fluorescent dyes (auramine-rodamine) can be used to stain mycobacteria [11].

2.3. Pathogenesis

2.3.1. Infection

There are two stages in the development of bovine tuberculosis in the host body: the primary complex, which is the original infection, and the chronic post-primary spread. When an infection occurs through inhalation, a lesion at the point of entry is typical. Although tonsillar and intestinal ulcers may develop, a lesion at the site of entry is uncommon when infection happens through the alimentary canal. Most frequently, the mesenteric or pharyngeal lymph nodes have the only visible lesion [12]. About 52.5% of the lesions were observed in the lung and associated lymph nodes, contributing to a higher percentage of lesions than the head and gastrointestinal area as presented in Figure 1 below [13].

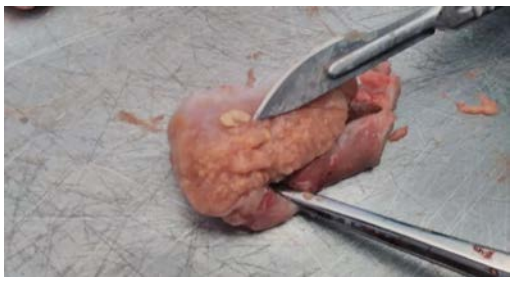

Figure 1. Tuberculous lesions in the mediastinal lymph node of cattle (Source: 13)

2.4. Incubation Period

Animal tuberculosis infections typically have a slow onset, requiring many months or longer manifesting clinically. Additionally, infections may lie dormant for years before resurfacing in humans. Human exposure can cause systemic symptoms to appear months or years later, or the infection may stay dormant for many years until the organisms are able to reactivate due to declining immunity. Skin lesions such as tuberculous chancre usually develop 2-4 weeks following cutaneous contact. Lesion development and production are also influenced by the type of host, the quantity of mycobacteria in the oculum, and their subsequent proliferation. Within eight days of the wound being contaminated by the bacterium, a discernible primary focus appears. Calculation of the lesions commences about 2 weeks later [12].

2.5. Mode of Transmission

2.5.1. Transmission Between Cattle

Animals can contract an infection through direct contact, ingestion, or inhalation through skin breaks or mucous membranes. The mode of ingestion requires a far higher concentration of organisms as compared to inhalation. Depending on the host species, different transmission channels have varying degrees of significance. Aerosols can spread disease to cattle when they come into close contact. With the exception of calves who nurse from diseased cows, ingestion is less

significant in this species. Although they appear to be rare in cattle, cutaneous, genital (sexual), and congenital transmission are all possible [12,14].

2.5.2. Transmission Between Animals and Humans

Humans and animals can get infected via the same routes of transmission such as ingestion, inhalation, direct contact with mucous membranes and skin breaches [15]. Ingestion is a frequent method of exposure due to consumption of unpasteurized dairy products. Additionally, raw or undercooked meat as well as other animal tissues may contain viable *M. bovis*. Rarely, human cases have been connected to animal bites. Transmission from person to person is conceivable, particularly in cases when the respiratory system is impacted [15]. Families and other intimate relationships are typically involved in these incidents, however at least one analysis raised the possibility of *M. bovis* respiratory transmission during informal community contact. It was also proposed that M. microti could spread from person to person. Rare instances of *M. bovis* and M. orygis transfer from humans to animals have been reported. Although urine-contaminated hay was suspected to be involved in one occurrence involving cattle, the organism was most likely spread through aerosols in most cases. Atypical ulcerative cutaneous tuberculosis reported in a middle-aged person due to *M. bovis* that was confirmed by PCR [16].

3. Diagnostic Methods of Tuberculosis

3.1. Clinical Examination

Although tuberculosis in cattle often progresses slowly over time, it can occasionally be severe and rapidly worsen. Most infected cattle are discovered early in eradication campaigns, and clinical infections are rare. The late stages are characterized by a low-grade fluctuating fever, weakness, gradual emaciation and inappetence. Due to intensive activity or cold climatic conditions, animals with pulmonary involvement typically have a wet cough that gets worse in the morning. They may also exhibit dyspnea or tachypnea [12]. The retropharyngeal or other lymph nodes may swell, burst and drain in certain animals. Profoundly swollen lymph nodes might also block the digestive tract, lungs, or blood vessels. Intermittent diarrhea and constipation may occur if the digestive tract is affected. Bovine tuberculosis symptoms often take months to manifest in cattle and the illness might lie latent for years before coming back when the animals are stressed out or elderly. Thus, diagnosis based on clinical indicators is a challenge [12].

3.2. Postmortem Examination

The organs with high reticulo-endothelial tissue content in cattle, especially the lungs and related lymph nodes are most frequently affected by lesions. Though the upper respiratory system and related tissues exhibit disease, lesions predominantly found in the lower respiratory tract. Additionally, there is the formation of granulomatous nodules known as tubercles at the sites of which are primarily determined by the route of infection. Tubercles have a centre of caseous necrosis with some calcification. While older cattle are typically infected through the respiratory tract, resulting in lung lesions and dependent lymph nodes, calf infections are typically caused by ingestion and entail lesions in the mesenteric lymph nodes with potential organ dissemination [17]. In order to provide support for postmortem exams, materials stained with hematoxylin and eosin should be examined histologically [18] and Ziehl-Neelsen stain should be used to identify the etiological agent [19].

3.3. Pathology

The most common lesions are tubercles, which are often yellow-white or gray-white granulomas that are encased in capsules of varying-thickness and size. The interior of the tubercles is typically caseous, caseo-calcareous, or calcified in pigs, cattle, small ruminants and several other animals. On the other hand, tubercles in certain cervid species are frequently poorly encapsulated, have a purulent center, and resemble abscesses. Some are so small that they are visible only in sectioned tissue, while others can grow into confluent, coalescing lesions that cover most of the organ. The lesions are generally detected in the lungs, head and thoracic lymph nodes in cattle and other animals when mode of entry is via inhalation route. Animals infected via ingestion exhibit lesions in their abdominal organs and lymph nodes (such as the mesenteric lymph nodes). The central nervous system, bones, joints, skin, and many other tissues and organs may also be impacted [12].

3.4. Culture of Mycobacteria

3.4.1. Media for Mycobacteria

Veterinary diagnostic laboratories mostly utilize the egg-based Lowenstein Jensen and Stone brinks media. One may alternatively employ an agar-based medium, such as blood based agars medium or middle brook 7H and 7H based agar medium [4]. The prepared media are placed in screw-capped bottles as solid slants. A typical selective agent is malachite green dye (0.025g/100ml). Glycerol is necessary for the growth of *M. tuberculosis*, *M. avium* and other atypical mycobacteria. Glycerol, on the other hand, inhibits the growth of *M. bovis*, whereas 0.4% sodium pyruvate promotes it. Therefore, it is recommended to inoculate both the glycerol-containing and glycerol-free medium (but still containing sodium pyruvate).

Addition of cycloheximide (400µg/ml), lincomycin (2µg/ml), and nalidixic acid (35µg/ml) can increase the selectivity of the medium. Every new batch of culture media needs to be infected with the stock strains of Mycobacteria in order to guarantee that the medium supports adequate development [20]. The inoculation media may need to be incubated for up to 8 weeks at 37°C, and for the mycobacteria in the tuberculosis group, it is preferable to incubate for 10 to 12 weeks with or without carbon dioxide [4]. When it comes to culture media, Mycobacterium avium and tuberculosis prefer loose caps, although Mycobacterium bovis thrives best in sealed containers [21].

3.4.2. Histology and Acid-fast Staining:

Tissue samples are taken and analyzed for histopathological (microscopic) lesions consistent with *M. bovis* during the necropsy of calves suspected of having BTB infection. Pathologists utilize specialized dye known as acid-fast staining to identify organisms compatible with *M. bovis*, in addition to searching for specific lesions under a microscope [22,23]. Approximately equal amounts of polysaccharide are present in the cell walls of these acid-fast bacteria. The ability of these bacteria to withstand decolorization with acidified organic solvents is mostly due to their high lipid content, which varies between 20 and 40% of the dry cell weight [24]. The bacteria that absorb this stain, such as *M. bovis*, appears as short red or pink rods under the microscope [22]. The development of appropriately stained smears should be part of the preliminary examination process for tissues suspected of being tuberculous. Using scrapings of the sliced tissue surface, a new slide can be used to create the recognizable smear. Since no heat is needed, the Kinyoum adaptation of the Zeihl-Neelsen stain is advised [25]. The staining of mycobacteria is frequently done using the Zeihl-Neelsen technique. The smears are heated in the same way as concentrated carbol fuchsin and then decolored with an alcohol and sulfuric acid solution and counterstained with methylene blue or malachite green. Using a standard light microscope, the stained slides are examined for the presence of acid-fast bacilli, which are red, colloidal, or bacillary cells that range in length from three to six microns and can occur individually or in clusters [24,25].

3.4.3. Culture

Culture remains the international gold standard for mycobacterial infection diagnosis. However, the identification of *M. bovis* by culture and biochemical approaches is laborious and time-consuming because of its dysgenic and slow-growing properties [17]. Cultures are kept at 37°C in an incubator with or without CO_2 for a minimum of 8 weeks. The medium must be kept in securely closed tubes to prevent desiccation. Slopes are periodically checked during the incubation period for macroscopic growth. Smears are made and stained using the Ziehl-Neelsen method once growth is apparent. Depending on the medium, *M. bovis* typically grows 3-6 weeks after incubation.

3.5. Biochemical Tests

The definitive identification of the species of mycobacteria is largely based on biochemical criteria [26].

3.5.1. Niacin Production Test

The commercially available niacin test strips (DIFCO, USA) are easier and safer to use as a convectional test which reveals *M. tuberculosis* is positive and *M. avium* is negative in test [20].

3.5.2. Nitrate Reduction

A loop full of a young *Mycobacterium* culture should be added to a screw-capped tube along with a few drops of sterile distilled water. Unvaccinated tube can be used as a reference point. Pour in two milliliters of the NaNo solution (0.01 M NaNo solution in 0.022 M phosphate buffer, pH 7), followed by shaking and incubating in a water bath at 37°C for two hours. Addition of two drops of 0.2% aqueous solution of sulphanilamide, two drops of a 1:1 dilution of concentrated HCl and two drops of a 0.1% aqueous solution of N-(1-naphthyl) ethylene diamine dihydrochloride to check for any pink to red colour development and contrast with the negative control. Strong red denotes a reduction of nitrate to nitrite. Addition of a pinch of powdered zinc to all negative tubes (converts nitrate to nitrite). The production of a red colour indicates a negative test (nitrates not reduced).

3.5.3. Deamination of Pyrazinamide

The medium is a broth base with 15.0g of agar, 0.2g of pyruvic acid, and 0.1g of pyrazinamide per liter. Transfer 15 milliliter portions into screw-capped tubes. For 15 minutes, autoclave at 121°C until solidified in an erect position. For four days, incubate the agar at 37°C with a heavy suspension of a young culture. A pink band appears in the agar and indicates a favorable response. As negative and positive controls, use an uninoculated tube and an *M. avium* tube, respectively [20].

3.5.4. Urease Test

Preparation of the medium by combining one-part concentrated urea-agar base with nine parts sterile water. Transfer contents into screw-capped tubes (16 x 125 mm) in 4 ml increments. Inoculate each tube with a loopful of a young culture and incubate at 37°C. A positive result is indicated by a color change from amber to pink or red. Discard after three days if no change occurs [27].

3.5.5. Inhibition and Tolerance Test

Reagents such as 5% NaCl and thiophen-2-carbonic acid hydrazide (TCH) 10µg/ml are incorporated into Lowenstein-Jensen media for conducting inhibition and tolerance test [24].

3.6. Immunological Diagnostic Techniques

3.6.1. Tuberculin Skin Test

The typical ante mortem test for tuberculin skin test in cattle and humans is a delayed type hypersensitivity to mycobacterial tuberculo protein. It is a practical and economical way to evaluate cell-mediated reactions to different antigens and defined as the "gold standard" for diagnostic screening to identify and isolate new or asymptomatic MTC infections. In cattle, the response is typically seen 30 to 50 days following infection. The cultures of *M. tuberculosis* or *M. bovis* cultivated on synthetic media are used to make tuberculin. Although it can also be done in the caudal fold of the tail, the tuberculin test is typically conducted between the mid neck [28,29]. The skin of the neck is more sensitive to tuberculin than the skin of the caudal fold. To compensate for this difference, higher doses of tuberculin may be used in the caudal fold of the tail.

The International unit (IU) is used to express the potency of tuberculin, which must be determined by biological procedures based on comparison with standard

tuberculin. Bovine tuberculin is more potent and selective. If the estimated potency of bovine tuberculin assures at least 2000 IU of tuberculin per bovine dose in cattle, it is deemed appropriate in various countries. Cattle with reduced allergy sensitivity require a greater dose of bovine tuberculin, and each injection dose should not include more than 0.2 ml. When the tuberculin test or its purified protein derivative (PPD) is administered by the subcutaneous, conjunctival, or intradermal routes, respectively, cell-mediated hypersensitivity acquired through infection can be systematically demonstrated by fever, conjunctivitis, or dermally by local swelling [28].

3.6.2. Single Intradermal Test

Administration of 0.1 ml of bovine tuberculin PPD intradermal injection into the cervical fold or a skin fold at the base of the tail, followed by the observation of swelling due to delayed hypersensitivity. After injection, the reaction is measured between 48 and 96 hours, ideally between 48 and 72 hours for optimum sensitivity and 96 hours for maximum specificity. A positive reaction is indicated by generalized swelling occurs at the injection site. The primary drawback of the SID test are its lack of specificity and the frequency with which reactor lesions appear and inability to distinguish between reactions resulting from *M. bovis* infection and infections with *M. avium*, *M. tuberculosis* and *M. paratuberculosis* using mammalian tuberculin including vaccination or *Nocardia farcinicus [28]*. The failure of the SID test to identify cases of minimal sensitivity in elderly and recently calved cows, as well as early infection in some cattle in an unresponsive state is known as anergy, which is a condition brought on by immunosuppression or excess antigen, which is itself brought on by non-specific factors like stress and malnourishment are some of the test's other drawbacks [30,31,32].

3.6.3. Comparative Intradermal Test (CID)

Prior to tuberculin injection, the mid neck is shaved at two locations, 10 to 12 CMs apart, and the thickness is measured using a calliper in millimetres. Purified protein derivatives from *M. avium* (PPD-A) and *M. bovis* (PPDB) are intra dermally injected into two different clipping sites on the side of the neck, respectively, to perform the CID test. The injection site varies from place to place in the skin carefully. The thickness of the skin is measured again at the spots after a 72-hour period [33,30]. The result is regarded as negative for BTB when there is a larger change in skin thickness at the PPD-A injection location.

The difference between the two modifications is considered when the skin thickness changes at both locations. The animal exhibiting signs of infection is referred to as a reactor if the increase in skin thickness at the injection site for the bovine (B) is greater than the increase in skin thickness at the injection site for the avian (A) and (B-A), is less than 1 mm, between 1 and 4 mm, or 4 mm and above. The results are categorized as negative, doubtful, or positive for BTB, respectively. Animals reacting to bovine tuberculin as a result of exposure to other *Mycobacterium* are distinguished from those infected with *M. bovis* using the comparison test. This sensitivity is explained by the antigenic cross reactivity among mycobacterial species and related genera [34].

3.6.4. Short Thermal Test

Cattle that have a rectal temperature of no more than 39°C both at the time of injection and for two hours afterward are given a subcutaneous injection of 4 millilitres of intradermal tuberculin in the neck. The animal is considered a positive reactor if its temperature increases above 40°C at 4, 6 and 8 hours after injection. Peak temperatures typically occur between 6 and 8 hours and exceed 41°C [35].

3.6.5. Stormont Test

This test is more sensitive than the cattle short thermal test for tuberculosis and depends on heightened sensitivity at the test site following a single injection. The test is carried out in the neck in a manner akin to the SID test, with an additional injection given at the same location after seven days. A good outcome is observed 24 hours after this second injection if there is a 5 mm or greater increase in thickness. The widespread immunological hypo reactivity associated with parturition is most likely the cause of the decrease of sensitivity [30]. The comparative study of tuberculin tests is enlisted in below Table 1 [30].

Table 1. Comparison of Tuberculin tests

S. No	Tests	Advantage	Disadvantage
1	Single Intradermal	Routine testing	Simple prone to false positive and poor sensitivity
2	Comparative Intradermal Test	When avian TB or Johne's disease is prevalent, more specific than SID	More complex than SID
3	Short Thermal Test	Used in postpartum animal's and in infected animals, high efficiency	Time consuming and risk of Anaphylaxis
4	Stormont Test	Used in postpartum animal's and in advanced case very sensitive and accurate	Three visits required may sensitize animal

(Source: 30)

3.7. Blood Based Diagnostic Techniques

3.7.1. Gamma Interferon Assays

This laboratory test uses circulating lymphocytes to identify particular cell-mediated immune reactions. Using a whole-blood culture technique, the test measures the release of the lymphokine gamma interferon (IFN). The technique relies on sensitized cells releasing IFN-between 16 and 24 hours after being incubated with a particular antigen. The test compares IFN production after stimulation with PPD-A and PPD-B. By using two monoclonal antibodies to bovine gamma-interferon, a sandwich ELISA is used to detect bovine IFN- is carried out with a sandwich ELISA that uses two monoclonal antibodies to bovine gamma-interferon.

It is advised that the assay be set up and the blood samples should be transported to the laboratory as soon as is reasonably possible, but no later than the day following blood collection. The capacity of the IFN-test to identify

infections at an early stage means that using both tests simultaneously can identify more infected animals before they become a source of infection for other animals or contaminate the environment. Improved specificity may be achieved by using recognized mycobacterium antigens like CFP-10 and ESAT-6 [36,37]. It might be possible to distinguish between animals that have received the Bacillus Calmette Guerin (BCG) vaccination and those that have not by using these antigens.

The IFN test has an advantage over the skin test when handling tough or dangerous animals, including agitated cattle or other Bovidae, because the animals only need to be trapped once. The IFN- test is employed in both parallel and serial testing to improve sensitivity and specificity, respectively. The heightened sensitivity of the IFN-assay, the potential for quicker repeat testing, the elimination of the requirement for a second farm visit, and more objective test methods are its benefits. IFN has several drawbacks, including low specificity, high logistical requirements (cultivation must begin within 24 hours of blood sample), a higher risk of nonspecific reaction in young animals due to natural killer (NK) cell activity, and a high cost [18,38].

3.7.2. Lymphocyte Proliferation Assay

This type of *in-vitro* assay compares the reactivity of peripheral blood lymphocytes to tuberculin PPD-B and PPD-A. They can be performed on whole blood or purified lymphocytes from peripheral blood samples. This test endeavours to increase specificity of the assay by removing the response of lymphocytes to “non-specific” or cross-reactive antigens associated with non-pathogenic species of mycobacteria to which the animal may have been exposed. Results are usually analyzed as the value obtained in response to PPD-B minus the value obtained in response to PPD-A. The assay has scientific value, but is not used for routine diagnosis because the test is time-consuming and the logistics and laboratory execution are complicated due to long incubation period and the use of radioactive nucleotides.

As with the IFN- test, the lymphocyte proliferation assay should be performed shortly after blood is collected. Peripheral blood lymphocyte reactivity to tuberculin PPD-B and PPD-A is compared in this kind of *in-vitro* experiment. They can be carried out on purified lymphocytes from peripheral blood samples or on whole blood. This test aims to remove the lymphocyte response to "non-specific" or cross-reactive antigens linked to non-pathogenic species of mycobacteria that the animal may have been exposed to, hence increasing the assay's specificity. Results are calculated as the difference between the values obtained in response to PPD-B and PPD-A. Although the test is time-consuming and involves complex logistics and laboratory execution, including the use of radioactive nucleotides and lengthy incubation periods, it has scientific significance but is not utilized for routine diagnosis. The test is relatively expensive and has not been subjected to inter laboratory comparisons [28] [39].

3.7.3. Enzyme-linked Immunosorbent Assay (ELISA)

ELISA appears to be the most suitable of the antibody-detection tests and can be utilized as a complementary test rather than an alternative, based on cellular immunity. An advantage of ELISA is its simplicity and cheap technique which many studies have shown good results with a high sensitivity and specificity especially for antigen detection in cerebrospinal fluid (CSF). However, other studies have shown that it is not more specific than other serodiagnostic methods [18]. The sensitivity of the test is limited mostly because of the late and irregular development of the humoral immune response in cattle during the course of the disease. Specificity is also poor in cattle when complex antigens such as tuberculin or *M. bovis* culture filtrates are used. However, a comparison of antibody levels to PPD-B and PPD-A has been shown to be useful in increasing specificity in the ELISA.

3.8. Molecular Diagnostic Techniques

3.8.1. Polymerase Chain Reaction (PCR)

PCR has been widely utilised for the detection of *M. tuberculosis* complex in clinical samples (mainly sputum) in human patients and has recently been used for the diagnosis of tuberculosis in animals [18]. Several commercially available kits and various ‘in-house’ methods have been evaluated for the detection of the *M. tuberculosis* complex in fresh and fixed tissues. Various primers have been used. Amplification products have been analyzed by hybridization with probes or by gel electrophoresis. Commercial kits and the in-house methods, in fresh, frozen or boric acid-preserved tissues, have shown variable and less than satisfactory results in inter laboratory comparisons. False-positive and false negative results, particularly in specimens containing low numbers of bacilli, have reduced the reliability.

Variability in results have been attributed to the low copy number of the target sequence per bacillus combined with a low number of bacilli. Variability has also been attributed to decontamination methods, DNA extraction procedures, techniques for the elimination of polymerase enzyme inhibitors, internal and external controls and procedures for the prevention of cross-contamination. Improvement in the reliability of PCR as a practical test for the detection of *M. tuberculosis* complex in fresh clinical specimens will require the development of standardized and robust procedures. Cross contamination is the greatest problem with this type of application and this is why proper controls must be set up with each amplification. However, PCR is now being used on a routine basis in some laboratories to detect *M. tuberculosis* group in paraffin embedded tissues. Although direct PCR can produce a rapid result, it is recommended that culture be used in parallel to confirm a viable *M. bovis* infection [18].

3.8.2. Spoligotyping

Recently, a unique technique for simultaneously detecting and typing mycobacterium tuberculosis complex bacteria has been created. It is known as spacer oligonucleotides typing or spoligotyping. The highly polymorphic direct repeat (DR) locus in the *M. tuberculosis* genome is amplified using the polymerase chain reaction (PCR) as the basis for this technique. The direct repeat sequences in the DR region of *M. bovis* BCG are 36 bp long, and they are separated by non-repetitive DNA spacers that range in length from 35 to 41 bp. One or

more IS6110 elements in the DR-region are present in other MTC strains [40].

Spoligotyping applied to culture is simple, robust and highly reproducible [35]. Results can be obtained from a *M. tuberculosis* culture within one day. Thus, the clinical usefulness of spoligotyping is determined by its rapidity, both in detecting causative bacteria and in providing epidemiologic information on strain identities. It can also be useful for identification of outbreak and can facilitate contact tracing of tuberculosis. PCR based methods are available as diagnostic and confirmatory test for tuberculosis and are expected to detect as low as 1 to 10 organisms [34,40].

Implementation of such a method in a clinic setting would be useful in surveillance of tuberculosis transmission and intervention to prevent further spread of this disease [34]. The specificity and sensitivity of this technique has been found to be 98 and 96%, respectively with the clinical samples [40]. The most prominent advantage of spoligotyping over IS6110 RFLP typing is that, in principle, spoligotyping can be used simultaneously for the detection and typing of MTC bacteria in one assay and requires viable organisms [35].

3.8.3. Restriction Fragment Length Polymorphism

Differentiation of strains of *M. tuberculosis* complex using nucleic acid-based technology is based on strain specific differences and frequencies of certain DNA sequences in chromosomal DNA. This is usually demonstrated by digestion of the genomic DNA with specific restriction enzymes and analysis of the generated patterns after separation of the DNA fragments on agarose gel: restriction fragment length polymorphism (RFLP) [35]. This kind of analysis is technically possible without requirement of a hybridization step and defined probes.

4. Bovine Tuberculosis Treatment, Control and Prevention Methods

Bovine tuberculosis can be controlled through test-and-slaughter or test-and-segregation methods [41]. Antibiotics are used to treat TB in pets and zoo animals. However, one must consider the chance of improving clinical outcomes without a bacterial cure, particularly when treatment was insufficient with frequent relapses (e.g., too brief or using only one medicine). The controversy surrounding treatment, stems from the possibility of organism shedding, human risks (particularly in cases of infection or draining lesions), and the development of drug resistance. Certain nations do not allow treatment. Members of *M. tuberculosis* complex are only treatable with a restricted range of tuberculocidal drugs; they are not responsive to several conventional antibiotics. Animal treatments often involve the concurrent administration of two or more medications for several months, and are based on effective human methods [17].

It is crucial to remember that *M. bovis* is inherently resistant to pyrazinamide, a frequent "first line" treatment for tuberculosis, except for rare cases. Animals with *M. caprae* infections, however, may utilize this medication. The treatment regimen of isoniazid, rifampicin, pyrazinamide and ethambutol is recommended for humans as well as animals diagnosed with TB [42]. In addition to tuberculocidal medications, surgery is occasionally performed to remove tiny masses, cure ocular tuberculosis (e.g., enucleation), or amputate an infected joint.

The true burden of bovine tuberculosis disease in humans, however, is poorly understood. This is due to the lack of systematic surveillance for *M. bovis* as a cause of tuberculosis in people in all high-burden and low-income countries where the disease is endemic, as well as the incapacity of the laboratory procedures most frequently used to diagnose tuberculosis in humans to distinguish between *M. bovis* and *M. tuberculosis*, meaning 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 [43, 7]. There is only little surveillance is done particularly in developing countries on the epidemiology of this organism and the epidemiological requirements for its control. A recent study had reported that zoonotic TB is reemerging as an infectious disease in high-income countries and as a neglected disease in low- and middle-income countries [44]. The 30 high burden countries accounting for 87% of total estimated cases globally, wherein 8 countries (Figure 2) accounts for more than two-thirds of the global total [45].

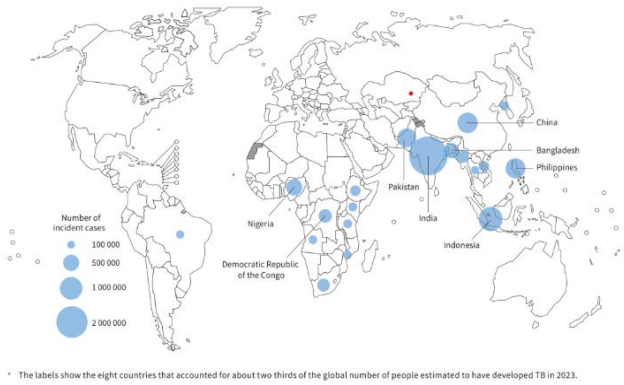

Figure 2. Estimated number of incident TB cases in 2023 [Source: 45]

4.1. Control and Prevention

A cross-sectoral, multidisciplinary strategy that integrates human, animal, and environmental health is required for the prevention and management of zoonotic tuberculosis. Increasingly well-known organizations are endorsing the One Health approach to address the problems at the animal–human interface in a comprehensive manner [17]. But there is little knowledge about how to prevent the true burden of bovine tuberculosis disease in humans. For example, in all high-burden and low-income countries where bovine tuberculosis is endemic, there is a lack of systematic surveillance for *M. bovis* as a cause of tuberculosis in humans, and the most widely used laboratory procedures to diagnose tuberculosis in humans are unable to distinguish between *M. bovis* and *M. tuberculosis*, so all cases can be assumed to be caused by *M. tuberculosis*. Hence, the available data for zoonotic tuberculosis does not accurately represent the true incidence of this disease [7,43]. Surveillance on the epidemiology of this organism

and the requirements for its control is limited, particularly in developing countries.

4.2. 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. Apart from actual deaths, infected animals lose 10-25% of their productive efficiency [12]. Additional expenses also arise due to surveillance and routine testing of cattle, removal of infected animals and other in-contact animals in the same herd, and movement control on infected herds.

4.3. Zoonotic Importance of TB

4.3.1. Treatment and Prevention

In most parts of Ethiopia, animals are kept near dwellings and maintained under very poor management and hygienic status, thus increasing the risk of acquiring infection for animals and humans. Creating awareness among the people to meet the standard hygienic requirement and to improve husbandry practices has been described to have a paramount importance in BTB control. In sub-Saharan Africa, BTB vaccination research in livestock is ongoing but has shown various successes so far [46]. Rifampicin, isoniazid, and pyrazinamide are frequently used first-line treatments for tuberculosis in humans; drug-resistant isolates are treated with alternative (second-line) medications. There have been sporadic reports of *M. bovis* strains that are resistant to many treatments, including at least isoniazid and rifampicin, as well as a small number of strains that are resistant to second-line medications. Antibiotic-resistant strains of *Mycobacterium bovis* are mostly believed to emerge during human tuberculosis treatment rather than animals. Treatment for active tuberculosis often involves the use of two or more medicines at the same time for at least six months.

The standard first line tuberculosis treatment for *M. tuberculosis* can usually be employed for cases caused by *M. caprae*; however, *M. bovis* is intrinsically resistant to pyrazinamide and the recommended length of treatment for this organism is currently 9 months. Compliance can be an issue, and some researchers have been investigating whether shorter treatment periods would also be effective. Surgery might occasionally be considered for some isolated forms of tuberculosis such as cutaneous disease. The risk of contracting zoonotic tuberculosis can be decreased by controlling the organisms in domestic animals. Pasteurization destroys the members of the *M. tuberculosis* complex in dairy products. Thorough cooking is effective in meat and other animal tissues; however, some organisms may survive in undercooked (e.g., rare) meat. Carcasses with lesions should not be eaten [46].

Butchers, veterinarians and animal scientists handling infected animals or their tissues, are an occupational risk for BTB [47]. Hence should use gloves and other Personal Protective Equipment (PPE), including respiratory protection where aerosolization could be an issue. Open wounds should be thoroughly covered. Hunters should also use PPE when in close contact with infected wildlife. The use of high-pressure hoses for cleaning animal facilities has been associated with some infections, probably by aerosolizing bacteria. Some countries administer a BCG vaccine, which contains an attenuated strain of *M. bovis* to infants. It can protect young children from severe tuberculosis, especially disseminated disease and CNS disease; however, its efficacy differs between individuals, the protection wanes early in adolescence, and revaccination does not seem to be helpful. The BCG vaccine is expected to be protective against *M. bovis* as well as *M. tuberculosis*.

4.3.2. Vaccination

Generally, human tuberculosis can be effectively controlled through BCG vaccination within few days of birth and up to six months old and administration of adequate chemotherapy [7]. In Ethiopia, health education is practiced as one of the pivotal means to control TB through increasing awareness of the community about the disease. In pastoral areas where people have the habit of consuming raw milk and meat, public education about hygienic practices like milk pasteurization has been described to be very important. Corner One Health can be defined as any added value in terms of health of humans and animals, financial savings or environmental services achievable by the cooperation of human and veterinary medicine when compared to the two medicines working separately [7].

The human TB vaccine, Bacille Calmette Guerin (BCG), has advantages for use in cattle because the vaccine is inexpensive and commercially produced for human application, and DIVA test prototypes are available. BCG is a live attenuated strain of *M. bovis* developed from a case of bovine tuberculoid mastitis by multiple passages through potato: glycerol culture. Calmette and Guerin first reported in 1911 that BCG induced protection in cattle against experimental challenge with *M. bovis*, and trials were undertaken in several different countries in the early twentieth century to determine the efficacy of the BCG vaccine for cattle. Although studies investigating protection against experimental challenge provided encouraging results, more variable efficacies were reported in field trials [48].

5. Conclusion

Bovine tuberculosis is prevalent in almost every country of the world, and causes great loss in dairy farm productivity. The disease has public health significance due to its zoonotic importance. The disease was reported from developing countries where bovine tuberculosis

disease is poorly controlled. In order to improve the control of disease, complementary tests may be required, particularly in the final stages of eradication programs, when the occurrence of reactive animals to skin tests is higher. An additional obstacle to the diagnosis and treatment of the condition is the existence of anergic animals. Owing to the unique and intricate features of BTB, a growing belief suggests that no single technique can reliably identify all reactive animals at every stage of infection. Furthermore, sub-Saharan Africa presents a diagnostic problem due to a high proportion of HIV infection. The most effective TB diagnostic tools are combinations of conventional, immunological, and molecular procedures. However, there are few molecular techniques available in Ethiopia and other impoverished nations.

Therefore, based on the above conclusions, the following recommendations are suggested:

- Create awareness among the people about the public health significance of the disease.
- Government should enforce strict rule on test and slaughter policy and compensate affected farmers to elimination of the disease at source.
- Detailed research should be done on these diagnostic techniques.
- Government should have to widen the availability and accessibility of these diagnostic techniques as much as possible.

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Authors' Contributions

All authors made a substantial, direct, and intellectual contribution to the work.

Conflict of Interest

The authors declare that they have no conflict of interest.

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