MATHEWS

Review Article

Mathews Journal of Veterinary Science

Review on the Diagnostic Assay of *Bovine Tuberculosis* in Cattle

Muberik Hussen Gada[1], Meles Yilma[1], Gelan Dule Dahesa[1], Isayas Asefa Kebede[2,*]

[1]*School of Veterinary Medicine, Wolaita Sodo University, Wolaita Sodo, Ethiopia*

[2]*School of Veterinary Medicine, Ambo University, Guder, Ethiopia*

ABSTRACT

Bovine tuberculosis is an endemic disease of cattle in Ethiopia and is classified as a "List B" disease. This category includes all animal diseases that are considered important because of their socio-economic and public health impacts. *Bovine tuberculosis* is an infectious disease caused by *Mycobacterium bovis*, which belongs to *Mycobacterium tuberculosis complex* (MTBC) which infects man and animals. It is a chronic bacterial disease characterized by the progressive development of tubercles in any tissue or organ of the body. The rapid detection and identification of BTB is essential for good management and infection control. Zoonotic tuberculosis is one of the greatest threats to global health. It is a prevalent disease in most developing countries of the globe. The intradermal tuberculin skin test method is the most widely adopted method for the detection of Bovine tuberculosis. However, this method is not sufficiently sensitive or specific. Alternatively, the culture method is highly sensitive but requires 4–8 weeks for the quantifiable detection of bacteria in a selective medium. In addition, detection by this method is limited to regions and facilities with the required equipment for conducting such tests. Currently, there are need for rapid, simplified, and low-cost diagnostic methods for integrated use in many developing countries. Eradication with test-and-slaughter strategies requires access to sufficient financial and human resources to accurately determine the program it's quite challenging in resource-poor settings. The quite open and possible approach in developing regions where *bovine tuberculosis* is prevalent and endemic is abattoir surveillance to inspect macroscopic lesions coupled with rapid diagnostic confirmation as zoonosis control. Therefore, the objective of the current review was to provide an overview of the diagnostic methods used for *Bovine tuberculosis* diagnosis in cattle.

Keywords: *Bovine tuberculosis*, Diagnostic Assay, *Mycobacterium bovis*.

LIST OF ABBREVIATION

BTB: *Bovine tuberculosis*; CID: Comparative Intradermal; DRs: Direct Repeat; MTC: Mycobacterium Tuberculosis Complex; PCR: Polymerase Chain Reaction, PPD: Purified Protein Derivative; RD: Regions of Difference; RFLP: Restriction Fragment Length Polymorphism; rRNA: Ribosomal Ribo Nucleic Acid, SID: Single Intradermal.

Vol No: 08, Issue: 01

Received Date: February 02, 2024

Published Date: March 16, 2024

***Corresponding Author**

Isayas Asefa Kebede

School of Veterinary Medicine, Ambo University, P.O. Box 19, Guder, Ethiopia, Tel: +251-(09)-11-89-49-73

Email: isayasasefa@ambou.edu.et

Citation: Gada MH, et al. (2024). Review on the Diagnostic Assay of *Bovine Tuberculosis* in Cattle. Mathews J Vet Sci. 8(1):37.

INTRODUCTION

Bovine tuberculosis is a highly contagious bacterial zoonosis that is recognized as one of the most serious hazards to humans and animals worldwide, causing mortality, morbidity, and economic losses, particularly in underdeveloped countries [1]. The disease is often recognized by the appearance of granulomatous nodular lesions in the visceral organ parenchyma [2]. *Bovine tuberculosis* is classified as a "List B" disease [3]. The category encompasses all animal diseases deemed significant due to their socioeconomic and public health implications [4].

Bovine tuberculosis is an endemic disease of cattle in Ethiopia, with a prevalence of 3.5–5.2% in abattoirs and 3.5–50% in crossbreed farms [5,6]. Nevertheless, the available information is limited due to inadequate disease surveillance and a lack of better diagnostic facilities [7,8]. This species has non-motility, non-capsularity, non-spore formation, obligatory aerobicity, thin rods that are normally straight or slightly curved, 1-10 mm long and 0.2-0.6 mm wide, facultative intracellular microbes, and generation times of 15-20 hours. Its cell wall is rich in lipids (mycolic acid), which give it the thick waxy covering that offers it acid resistance and hydrophobicity [9]. BTB is hydrophobic and acid-resistant due to the high lipid content of its cell wall. Furthermore, it dramatically boosts bacteria's resistance to various disinfectants, antibiotics, and physical harm [8].

The development and enhancement of diagnostic methods for detecting BTB infection is an important step in establishing a definitive diagnosis. However, the validation of BTB diagnostic tests is limited due to difficulties in collecting samples from animals [10]. Clinical evidence of tuberculosis in cattle is frequently absent until very large lesions have formed. As a result, previous to the introduction of the tuberculin test, its diagnosis in individual animals and an eradication program were not conceivable, and a method such as skin testing sometimes has practical difficulties [11].

Historically, BTB diagnosis relied on phenotypic changes, biochemical tests, animal inoculation, and chromatographic analysis [12,13]. BTB may currently be identified utilizing numerous molecular approaches that have recently been developed. These are a group of polymerase chain reaction (PCR) approaches based on changes in DNA sequences in Mycobacterium tuberculosis complexes in M. bovis [14]. Blood-based techniques for detecting circulating antibodies, such as the gamma interferon assay and enzyme-linked immunosorbent assay, were extensively utilized in BTB diagnosis in cattle laboratory samples [15,13].

Existing diagnostic methods are either insufficient to confirm BTB in live animals, or they take a long time, cost a lot of money, and require specialist labs that are not available in undeveloped countries where the disease is prevalent [16]. Because of the dynamics of M. bovis transmission, the microscopic size of early lesions, and the time it takes for an animal to mount a detectable immune response, no single ante mortem (or postmortem) test for BTB can be expected to detect every infected herd and every infected animal in such herds [17,18]. As a result, a multidisciplinary approach must be employed based on the current status of the disease [19]. Therefore, the objective of this paper is to review on the diagnostic assay of *bovine tuberculosis* in cattle.

BOVINE TUBERCULOSIS

General Description

Bovine tuberculosis is an infectious, chronic, and progressive disease with a global distribution caused primarily by M. bovis, and the presence of the disease in a herd is of critical importance to public health because it poses a significant risk of human infection [20,18]. Although BTB mostly affects cattle, it can be transmitted to humans [21]. Zoonosis is primarily transmitted through the respiratory tract after close or direct contact with sick cattle or by the ingestion of contaminated animal products such as raw meat or unpasteurized milk [22]. Animals are typically infected through inhalation, however, cattle grazing on polluted grass get infected through ingestion. Due to lower productivity, limits on movement, screening fees, and culling of highly productive farm animals, the disease causes significant economic loss [23].

In cattle, BTB clinical signs include chronic cough, decreased appetite, and emaciation [24]. Chronic irritability, weakness, anorexia, influenza-like fever, and local disease can affect the lymph nodes, skin, bones and joints, genitourinary system, definitions, or respiratory system [25]. A BTB-infected animal loses 10 to 25% of its productive efficiency; direct losses related to the infection manifest as a 10% to 18% decline in milk output and a 15% decrease in meat production [26]. The costs of disease control (testing and compensation fees, losses from animal movement and sale limitations, and lower milk and meat output) all result in financial losses [27].

Diagnostic Methods

Clinical diagnostic techniques

Clinical diagnosis of BTB infection in cattle is difficult due to the chronic nature of the disease and the wide range of symptoms depending on the location of the infection [28,29]. Clinical signs of *bovine tuberculosis* are used to provide a preliminary diagnosis [1]. Clinical indications of *bovine tuberculosis* frequently take months to appear in cattle [30]. Infections can also lay latent for years before reactivating

during times of stress or old age. As a result, the number of severe cases of animals with clinical indications may be restricted or nonexistent, particularly in industrialized nations where most are detected by regular examination or discovered at the butcher [29,22].

Most sick cattle are detected early in countries with eradication initiatives, and clinical infections are rare. Early infections are typically asymptomatic [16]. In the late stages, frequent symptoms include growing emaciation, grade-changing fever, and loss of appetite. Animals with pulmonary involvement typically have a cough that worsens in the morning, during cold weather, or activity, and may have dyspnea or tachypnea. Animals in the terminal stage may become exceedingly malnourished and exhibit respiratory distress [31,18].

BTB symptoms in cattle can take months to show, and infections can remain dormant for years before resurfacing under stress or as they age. Despite this, diagnosing BTB solely on clinical signs can be difficult, particularly in developing countries where the prevalence of severe cases of animals with clinical evidence may be low or nonexistent, and the majority are identified through routine testing or discovered at the slaughterhouse [32,18,33].

Immunological diagnostic techniques

A tuberculin skin test (TST): A tuberculin skin test (TST) includes injecting BTB pure protein derivative (PPD) tuberculin intradermally, followed three days later by looking at the injection site for swelling (delayed hypersensitivity) [34]. For decades, the tuberculin skin test (TST) has been used in live animal BTB screening as an effective diagnostic and epidemiological technique [1]. The TST has several documented disadvantages, including difficulties with administration and interpretation of results, the requirement for a second-step visit, a lack of standardization, and unsatisfactory test accuracy [30]. However, these drawbacks exceed the TST's benefits, contributing to its widespread usage for fast screening of BTB in live animals [18].

According to the existing published document, the assay has a wide range of sensitivity and specificity estimated at 77% - 95% and 98% - 99.9%, respectively. The potency and dosage of the tuberculin delivered, the post-infection interval, desensitization, intentional interference, post-partum immunosuppression, and variations in the observer's interpretation can all have an impact on the sensitivity [35].

Single intradermal Test (SIDT): The single intradermal test (SIDT) is performed by injecting 0.1 milliliters of bovine tuberculin PPD intradermally into the cervical fold or the cutaneous fold at the base of the tail and then monitoring the animal for swelling due to delayed hypersensitivity [28]. The reaction is read between 48 and 96 hours following injection, with 48-72 hours being preferred for greatest sensitivity and 96 hours being preferred for maximum specificity. The SIDT's main limitations are its lack of specificity and the frequency with which it encounters visible-lesion reactors (NVLs) [36,22].

THE SIDT's other drawbacks include its inability to identify cases of minimal sensitivity, in old cows and cows that have recently given birth, as well as in early infection, in some cattle that are in an unresponsive state known as anergy, which is developed due to an excess of antigens or immunosuppression, which is in turn brought on by non-specific factors like stress and malnutrition [37]. Recently, a Bayesian approach has been used to estimate the sensitivity and specificity of the SIDT showing sensitivity between 53% (27.3% –81.5%, 95% CI) and specificity between 69.4% (40.1% – 92.2%, 95%CI) depending on the interpretation criteria used [38].

Comparative Intradermal Test (CIDT): The Comparative Intradermal Test (CIDT) is used to distinguish between animals infected with M. bovis and those exposed to another Mycobacterium and hence reacting to bovine tuberculin. This sensitization is caused by antigenic cross-reactivity between mycobacterial species and related taxa [39]. Before tuberculin injection, the injection site should be shaved at two 10-12 cm apart areas on the mid-neck, and the response thickness measured in millimeters with a caliper. During the CID test, Purified Protein Derivatives from Mycobacterium avium (PPD-A) and Purified Protein Derivatives from *Mycobacterium bovis* (PPDB) are injected intradermally at two different clipping areas on the side of the neck [40].

When the change in skin thickness is greater at the PPD-A-Injection site, the result is considered negative for BTB; when it increases at both sites, the difference between the two changes is evaluated. Thus, if the increase in the skin thickness at the injection site for the bovine (PPDB) is greater than the increase in the skin thickness at the injection site at the avian (PPDA) and the measurement substruction (for PPDB - PPDA), is less than 1mm, between 1mm and 4 mm, or a 4 mm and above 4mm, the result is classified as negative, doubtful, or positive for BTB, respectively and the animal with the evidence of infection is termed as reactor [39].

Table 1. Comparison of tuberculin test

Assay categories	Advantage	Disadvantages	Published report on the assay Se/ Sp		
			Se (95% CI)	Sp(95%CI)	Reference
TEST	Low costs, high availability long history of use, and, for a long time the lack of alternative methods to detect Btb	Difficulties in administration and interpretation of results, low degree of standardization, and imperfect test accuracy	77–95%	98–99.9%,	[35]
SIDE	Simple and routine testing	Lack of specificity and the number of visible-lesion reactors (NVLs)	50%	69.4%	[36,38]
CIDT	When avian TB or Johne's disease is prevalent, more specific than SIDT	More complex than SIDT	80.4% - 93%	89.2%-95.2%	[41,42]

Blood-based diagnostic assay

In addition to the conventional intradermal tuberculin test, a variety of blood tests have been used. Laboratory-based assays are often utilized as supplemental tests to boost the identification of infected animals and used as parallel testing to support skin screen tests due to their higher cost and more technical nature [33].

Gamma interferon assay test: Gamma interferon assay test: is also known as 'bovigam' and is based on the detection of IFN- released in response to a specific antigen. The principle of the test is the measurement of IFN release from sensitized lymphocytes in a whole-blood culture system during a 16 to 24-hour period of incubation, usually with PPD [44]. The assay compares IFN- production in response to avian and bovine PPD stimulation [21]. A sandwich ELISA is used to detect bovine IFN-, which uses two monoclonal antibodies to bovine gamma-interferon [45]. ESAT-6 and CFP-10 are two mycobacterium antigens that have been identified as having the potential to enhance specificity [46,11,22].

The IFN- assay has several advantages, including enhanced sensitivity, the ability to perform quicker repeat testing with no need for a second visit to the farm, and more objective test processes. IFN- has several disadvantages, including low specificity, high logistical demands (culture must begin within 24 hours after blood collecting), an increased chance of non-specific response in young animals due to natural killer (NK) cell activity, and a high cost [46,33]. The commercial IFN-g test's sensitivity and specificity were reported to be 95.5% and 87.7%, respectively [47].

Enzyme-linked immunosorbent Assays (ELISA): Techniques for the diagnosis of *Bovine tuberculosis* have been performed in Turkey for the first time by Keskin [48]. The ELISA assay is relatively inexpensive and can be easily automated to process large numbers of samples and appears to be the most suitable of the antibody-detection tests and can be a complement, rather than an alternative, to test based on cellular immunity [1].

An advantage of the ELISA is its simplicity, but both specificity and sensitivity are limited mostly because of the late and irregular development of humoral immune response in cattle during the course of the disease [49]. The ELISA may also be useful for detecting BTB infections in wildlife. For example, a lateral flow-based rapid test (TB statpak) is useful for detecting tuberculosis-diseased animals, particularly some domestic animals, wildlife, and zoo animals, where no cellular immunity tests like the gamma-interferon test are available and where skin testing has been proven unreliable [50]. However, its sensitivity in cattle is relatively low [14]. The sensitivity and specificity rates of ELISA as 86.7% and 90.6%, respectively, for *bovine tuberculosis* [51].

Lymphocyte Proliferation Assay: Lymphocyte Proliferation Assay: This is an in-vitro test that compares the response of peripheral blood lymphocytes to PPDs from Mycobacterium avium (PPD-A) and tuberculin (PPD-B). The test can also be performed with purified lymphocytes or whole blood from peripheral blood samples [52]. The difference between the values obtained in response to PPD-B and PPD-A is frequently utilized to assess results. To improve either the specificity or sensitivity of the diagnosis, the B-A value must be greater than a variable cut-off point [42]. The advantage of the tests is that they attempt to increase the specificity of the assay by removing the response of lymphocytes to 'nonspecific' or cross-reactive antigens associated with non-pathogenic species of mycobacteria to which the animal may have been exposed and have scientific value, but they are not used for routine diagnosis because the test is time-consuming and the logistics and laboratory execution are complicated. The assay necessitates lengthy incubation durations and the use of radioactive nucleotides [53]. The test's weakness is that it is relatively expensive and has not been submitted to inter-laboratory comparisons [54].

Table 2. Comparison of the blood-based assay test

Assay categories	Advantages	Disadvantages	Published report on the assay Se/ Sp		
			Se (95% CI)	Sp (95% CI)	Reference
Gamma interferon assay test	Increased sensitivity and no need for a second visit to the farm	Reduced specificity, high logistical demands, and high cost.	95.5%	87.7%,	[46,55]
Enzyme-linked immunosorbent Assays (ELISA)	Inexpensive and easily automated to process large numbers of samples and its simplicity	Both specificity and sensitivity are limited	86.7%	90.6%	[1,49,51]
Lymphocyte Proliferation Assay	To increase the specificity and scientific value	Relatively expensive and has not been subjected to inter-laboratory comparisons	87.5%	84.6%	[54,56]

Se: Sensitivity, Sp: Specificity

In general, the ELISA may be useful for identifying BTB infections in animals when no cellular immunity assays, such as the gamma-interferon test, are available and skin testing has been demonstrated to be erroneous. For example, a lateral flow-based fast test (TB statpak) is useful in identifying tuberculosis in animals, notably in some domestic animals, wildlife, and zoo animals. Similarly to the IFN test, the lymphocyte proliferation assay should be performed as soon as blood is taken. According to Tewodros and Girja, 2012, the test is highly pricey and has not been the subject of any inter-laboratory comparisons [57].

Post- mortem examination

The post-mortem examination approach is used to detect bovine tuberculosis-like gross lesions on the viscera of slaughtered beef cattle, which is indicative of BTB surveillance [3]. The identification of macroscopic lesions in the lung, as evidence findings, suggests that tuberculosis in cattle is largely a respiratory disease [58]. The digestive system is also a route of infection for bovine tuberculosis, particularly in calves fed colostrum milk from BTB-infected cows or through ingestion of contaminated water or fodder [59]. In this scenario, the main complex is seen in the digestive organs and lymph nodes [60]. Numerous research have been conducted to investigate the prevalence of BTB at the animal and herd levels in various regions of Ethiopia; Numerous studies have examined the prevalence of BTB at the animal and herd level in various regions of Ethiopia; they found that the prevalence was 1.5% (7/476) and 7.4% (7/95) respectively [61].

Routine Postmortem Inspection (RPMI): Visual examination, palpation, and incision of intact organs such as the liver and kidneys, as well as examination, palpation, and incision of tracheobronchial, mediastinal, and prescapular lymph nodes, were all part of the process. When tumors were found in one of these tissues, further lymph nodes, organs, or bodily systems were examined. At the detection of a large lesion, condemnations of complete or afflicted sections of organs or carcasses are a possibility [62,33].

Detailed Postmortem Inspection (DPMI): The inspection of each carcass was undertaken at a glance [63]. The seven lobes of the two lungs, lymph nodes, and organs were also thoroughly examined, and the cut surfaces were examined under bright light sources for the presence of an abscess, cheesy mass, and tubercles [64,65].

Table 3. Ethiopian Abattoir report of BTB gross lesion prevalence (%) on postmortem examination

Study	BTB gross lesion prevalence (%) reported		Se (95% CI)	Sp (95% CI)	Abattoir type (public/ export)
	Routine abattoir inspection (RAI)	Detailed abattoir inspection (DAI)			
[66]	9% (5-13)	25% (12-30)	94% (86-98)	95% (76-99)	Mojo export abattoir
[65]	0.5% (2/384)	4.7% (18/384)	11%	100%	Debre Birhan Municipal Abattoir
[26]	14.3 % (2/14)	85.7 % (12/14)	85.71 %	7.27 %	Woldiya municipal abattoir

Se: Sensitivity, Sp: Specificity, RAI: Routine abattoir inspection, DAI: Detailed abattoir inspection

According to the above Table and based on a thorough meat inspection, the overall lesion prevalence of BTB in cattle slaughtered at the Mojo Export Abattoir, Debre Birhan Municipal Abattoir, and Woldiya Municipal Abattoir during the study period was 25% (12-30), 4.7% (18/384), and 85.7% (12/14) at 95% CI. However, 95% confidence intervals revealed that only 9% (5-13), 0.5% (2/384), and 14.3% (2/14) of cattle exhibited recognized BTB similar lesions.

Histopathological diagnosis

Tissue samples are obtained and evaluated for histopathological (microscopic) lesions compatible with *Mycobacterium bovis* during postmortem examinations of cattle suspected of being infected with BTB [36, 65]. Histopathology examination samples can be obtained from the following sources: Fine needle aspiration of lymph nodes, impacted peripheral lymph nodes, particularly cervical nodes, can be aspirated [16].

The presumptive diagnosis of mycobacteriosis can be made if the tissue has characteristic histological lesions such as caseous necrosis, mineralization, epithelioid cells, multinucleated giant cells, and macrophages make microscopic cell structural landmark called granuloma [67]. Histological analyses are possible, inexpensive, and useful for reaching findings on highly suspect bodies [36]. Despite these advantages, the diagnostic approach is limited by the availability of postmortem samples, and the majority of lesions may be paucibacillary, leading to false-negative results [68,69].

The histopathologic findings were evaluated microscopically and classified as:Positive: If tubercular granuloma displays central necrosis with or without mineralization surrounded by macrophages, and lymphocytes. Inconclusive: Lesion characterized by irregular unencapsulated clusters of epithelioid macrophages but no multinucleated giant cells and necrosis, consistent with an initial stage. Negative: features not consistent with tubercular granuloma [8].

Conventional culture isolation method

The bacteriological culture isolation is the 'gold standard' method to confirm *Mycobacterium bovis*. Isolation of M. bovis from tissue lesions of animals suspected of BTB is routinely performed using egg-based solid bacteriological media with agar [8]. The most popular egg-based solid media is Lowenstein Jensen medium which contains sodium pyruvate (0.4%) enhances its growth of M.bovis. M.bovis grows well on pyruvate-containing media without glycerol, with the characteristic of sparse, small moist-sheen colonies that break up easily and thin growth on glycerol-containing media that is called dysgenic [70,71]. The culture system is very sensitive and specific, and it can be used for drug sensitivity testing and species characterization, but it requires characterization for species identification and takes weeks to grow [8]. Culture is also expensive, time-consuming, and requires a biosafety level 3 facility [72,73].

Acid-fast staining: It is used to detect acid-fast bacilli (AFB) in clinical specimens using Ziehl-Neelsen (ZN) or fluorescence staining, and it is a low-cost method for diagnosing BTB and monitoring treatment progress, particularly in developing countries. However, there are numerous disadvantages, including the difficulty in acquiring the swab sample and the limited sensitivity, particularly in immune-compromised patients with AFB smear positive ranging from 31 to 90 percent [74]. Smear microscopy, culture, and phenotypic identification have been used in the laboratory to diagnose BTB. While acid-fast staining is the quickest, easiest, and cheapest approach available, its low sensitivity (45%-80% of positive cultures) has limited its usefulness in BTB, particularly in geographical areas with lower incidence [3,75].

Molecular Diagnostic Technique

Polymerase chain reaction (PCR): Polymerase chain reaction (PCR) is a potent instrument that may be employed in a range of diagnostic techniques to detect the presence of DNA that is particular and unique to an organism of interest [3,76]. Instead of needing three to six weeks to establish the presence of Mycobacterium using traditional culture methods, PCR may do it in as little as one to three days [15]. Although direct PCR can provide quick findings, parallel cultures are indicated to demonstrate viable M. bovis infection [3,73]. PCR is used to identify M. bovis in tissue obtained at necropsy from animals suspected of being infected with BTB. PCR is only used on samples that demonstrate histological (microscopic) evidence of BTB. The results are normally available within seven days and are labeled as either positive or negative. A positive PCR test indicates that the animal is infected with BTB [5].

PCR techniques are widely used for the diagnosis of BTB and have several advantages; they are quick, applied within a few hours which mean rapid diagnosis and efficient control, overcome the lack of specificity of other traditional tests such as histopathology, and can identify the mycobacteria either from culture or clinical specimens [77]. PCR techniques have several limitations; limited to the PM diagnosis [78], not specific for pathogen identification, being restricted only to members of MBTC or M. avium complexes [79]. The Mycobacterium TB complex can be recognized directly utilizing PCR methods, which can detect fewer than ten germs in a clinical sample. PCR has a sensitivity range of 70 to 90% and a specificity range of 90 to 95% when compared

to culture results. PCR has a sensitivity of more than 95% in smear-positive patients but only 50-60% in smear-negative individuals [80].

Spoligotyping: Spoligotyping, also known as spacer oligonucleotide typing, is a recently developed method for simultaneous detection and typing of *mycobacterium tuberculosis complex* bacteria that is based on polymerase chain reaction (PCR) amplification of a highly polymorphic direct repeat (DR) locus in the M. tuberculosis genome [80,76]. Spoligotyping in culture is a simple, robust, and highly reproducible PCR-based approach that uses little amounts of DNA [81]. The clinical usefulness of spoligotyping is determined by its speed, both in detecting causative bacteria and in providing epidemiologic information on strain identities, which would be useful in BTB transmission surveillance and interventions to prevent disease spread [26].

As diagnostic and confirmatory tests for tuberculosis, PCR-based approaches are available and are predicted to identify as little as 1 to 10 organisms [82,73]. One of the most obvious advantages of Spoligotyping over RFLP typing is that it does not require living organisms and can theoretically be used for both MTC detection and typing in the same experiment [81]. The specificity and sensitivity of this approach are 98 and 96%, respectively [83].

Restriction Fragment Length Polymorphism (RFLP): It is regarded as the gold standard for BTB molecular typing due to its great repeatability and discriminative strength. It may also be used to identify epidemics and make contact tracing for tuberculosis easier [80-86]. Because the combined procedure of probe labeling, DNA fragmentation, electrophoresis, blotting, hybridization, washing, and auto-radiographing requires a significant amount of DNA, this approach is only relevant to mycobacterial cultures and takes 20 to 40 days to obtain. Furthermore, this procedure is technically difficult, labor-intensive, and expensive, and requires the use of advanced analysis tools for result interpretation [70,76].

Table 4. Comparison of molecular diagnostic techniques

Assay categories	Advantages	Disadvantages	Published report on the assay Se/ Sp		
			Se (95% CI)	Sp (95% CI)	Reference
Polymerase chain reaction (PCR)	Allows rapid detection of M. bovis in tissue samples from carcasses of non-bovine animals to confirm or negate BTB infection.	Length of time it takes 6-22 weeks to obtain the result.	70 - 90%	95%	[80,84]
Spoligotyping	Simple, rapid robust, highly reproducible PCR-based method and requires low quantities of DNA.	Limited discriminatory power.	98%	96%,	[81,83]
Restriction Fragment Length Polymorphism (RFLP)	To identify a carrier of a disease-causing mutation in a family.	It requires a large DNA sample, the isolation of which can be a laborious and time-consuming process.	100%	100%	[85,86]

Se: sensitivity, Sp: specificity

CONCLUSION AND RECOMMENDATIONS

Bovine tuberculosis is an infectious, chronic, and progressive disease with a worldwide distribution that mainly infects cattle and is zoonotic to humans. To diagnose BTB, clinical, immunological, blood-based, post-mortem, histopathological, conventional culture, and molecular diagnostic methods must be employed. Despite the wide range of techniques that could be used in the diagnosis of Bovine tuberculosis. However, in most instances, sputum sampling in cattle is quite impossible thus, the common abattoir tissue lesions upon postmortem inspection were used to confirm *Mycobacterium bovis* by culture identification. Despite bacterial culture isolation being the 'gold standard' method of diagnosis in BTB in cattle, however, the need for sophisticated microbiological facilities that are used and applied in resource-poor settings was quite limited. The availability and cost of some of these conventional culture identification and advanced molecular techniques still pose challenges in diagnostics BTB in cattle. Based on the above conclusion, the following recommendations are forwarded:It seems that *bovine tuberculosis* persists, probably due to less attention given to it and the existing use of less efficient diagnostic methods, Public health awareness campaigns should be launched and raise community awareness about the risk of BTB transmission through the consumption of raw/ undercooked meat and the zoonotic implication of

BTB, A great interaction among the livestock owners, and medical and veterinary personnel is the prerequisite for the investigation of the zoonotic importance of BTB and further investigations for minimizing its devastating effect on animals and humans.

DECLARATION

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

All the datasets generated or analyzed during this study are included in this manuscript.

COMPETING INTERESTS

All authors have nothing to disclose in this work.

FUNDING

The current study was not funded by any institution.

AUTHORS' CONTRIBUTIONS

MHG and MY contributed to study conception, data collection, and writing; GDD and IAK contributed to revision, references search. All authors have approved the submission of the final manuscript.

REFERENCES

1. Dana TD, Dulo F, Tafere A, Hayesso S. (2018). A Review on *Bovine Tuberculosis* and its Diagnostic Approaches. Academic Journal of Animal Diseases. 7(3):59-67.
2. Dechassa T. (2014). Gross pathological lesions of *bovine tuberculosis* and efficiency of meat inspection procedure to detect-infected cattle in Adama municipal abattoir. Journal of Veterinary Medicine and Animal Health. 6(2):48-53.
3. OIE. (2009). OIE manual of diagnostic tests and vaccines for terrestrial animals. OIE, Paris, France. Available at: www.oie.int.
4. Helmy NM, Abdel-Moghney ARF, Atia MA. (2015). Evaluation of Different PCR-Based Techniques in Diagnosis of *Bovine Tuberculosis* in Infected Cattle Lymph Nodes. Am J Microbiol Biotechnol. 2(5):75-81.
5. Regassa A, Tassew A, Amenu K, Megersa B, Abunna F, Mekibib B, et al. (2010). A cross-sectional study on *bovine tuberculosis* in Hawassa town and its surroundings, Southern Ethiopia. Trop Anim Health Prod. 42(5):915-920.
6. Berg S, Garcia-Pelayo MC, Müller B, Hailu E, Asiimwe B, Kremer K, et al. (2011). African 2, a clonal complex of *Mycobacterium bovis* epidemiologically important in East Africa. J Bacteriol. 193(3):670-678.
7. Asseged B, Lübke-Becker A, Lemma E, Taddele K, Britton S. (2000). Bovine tuberculosis: a cross-sectional and epidemiological study in and around Addis Ababa. Bulletin of Animal Health and Production in Africa. 48(2):71-80.
8. Aman E, Dessalegn B, Masrie O, Debalke D, Enbiyale G. (2017). A Review on Current Diagnostic Techniques of Bovine Tuberculosis. Archiv Zool Stud. 1(001).
9. Kasper D, Fauci A, Hauser S, Longo D, Jameson J, Loscalzo J. (2015). Harrison's principles of internal medicine. 19e (Vol. 1, No. 2). New York, NY, USA: McGraw-Hill.
10. Chileshe J, Goosen WJ, Buss PE, van Helden PD, Warren R, Parsons SD, et al. (2019). A commercial ELISA for detection of interferon-gamma in white rhinoceros. J Vet Diagn Invest. 31(4):531-536.
11. Asil el TA, El Sanousi SM, Gameel A, El Beir H, Fathelrahman M, Terab NM, et al. (2013). *Bovine tuberculosis* in South Darfur State, Sudan: an abattoir study based on microscopy and molecular detection methods. Trop Anim Health Prod. 45(2):469-472.
12. Noussair L, Bert F, Leflon-Guibout V, Gayet N, Nicolas-Chanoine MH. (2009). Early diagnosis of extrapulmonary tuberculosis by a new procedure combining broth culture and PCR. J Clin Microbiol. 47(5):1452-1457.
13. Courcoul A, Moyen JL, Brugère L, Faye S, Hénault S, Gares H, et al. (2014). Estimation of sensitivity and specificity of bacteriology, histopathology and PCR for the confirmatory diagnosis of *bovine tuberculosis* using latent class analysis. PLoS One. 9(3):e90334.
14. Ojo O, Sheehan S, Corcoran GD, Okker M, Gover K, Nikolayevsky V, et al. (2008). *Mycobacterium bovis* strains causing smear-positive human tuberculosis, Southwest Ireland. Emerg Infect Dis. 14(12):1931-1934.
15. Palomino JC, Leão SC, Ritacco V. (2007). Tuberculosis 2007; from basic science to patient care. First Edition. TuberculosisTextbook.com.
16. Birhanu T, Mezgebu E, Ejeta E, Gizachew A, Nekemte E. (2015). Review on diagnostic techniques of *bovine tuberculosis* in Ethiopia. Rep Op. 7(1):7-14.
17. Waters WR, Nonnecke BJ, Palmer MV, Robbe-Austermann S, Bannantine JP, Stabel JR, et al. (2004). Use of recombinant ESAT-6: CFP-10 fusion protein for differentiation of infections of cattle by *Mycobacterium bovis* and by M. avium subsp. avium and M. avium subsp. paratuberculosis. Clin Diagn Lab Immunol. 11(4):729-735.

18. Borham M, Oreiby A, El-Gedawy A, Hegazy Y, Khalifa HO, Al-Gaabary M, et al. (2022). Review on Bovine Tuberculosis: An Emerging Disease Associated with Multidrug-Resistant Mycobacterium Species. Pathogens. 11(7):715.

19. Medeiros Ldos S, Marassi CD, Figueiredo EE, Lilenbaum W. (2010). Potential application of new diagnostic methods for controlling *bovine tuberculosis* in Brazil. Braz J Microbiol. 41(3):531-541.

20. Grange JM, Yates MD. (1994). Zoonotic aspects of *Mycobacterium bovis* infection. Vet Microbiol. 40(1-2):137-151.

21. Brückner GK. (2009). The role of the World Organisation for Animal Health (OIE) to facilitate the international trade in animals and animal products. Onderstepoort J Vet Res. 76(1):141-146.

22. Sichewo PR, Vander Kelen C, Thys S, Michel AL. (2020). Risk practices for *bovine tuberculosis* transmission to cattle and livestock farming communities living at wildlife-livestock-human interface in northern KwaZulu Natal, South Africa. PLoS Negl Trop Dis. 14(3):e0007618.

23. Sibhat B, Asmare K, Demissie K, Ayelet G, Mamo G, Ameni G. (2017). *Bovine tuberculosis* in Ethiopia: A systematic review and meta-analysis. Prev Vet Med. 147:149-157.

24. Lawn SD, Zumla AI. (2011). Tuberculosis. Lancet. 378(9785):57-72.

25. Ameni G, Vordermeier M, Firdessa R, Aseffa A, Hewinson G, Gordon SV, et al. (2011). Mycobacterium tuberculosis infection in grazing cattle in central Ethiopia. Vet J. 188(3):359-361.

26. Alemu J, Mamo G, Ameni G, Pal M. (2016). Molecular epidemiology of *bovine tuberculosis* in cattle and its public health implications in Gambella region, Ethiopia. Molecular Microbiology Research. 6(1):1-15.

27. Abebe F, Getachew S, Hailu T, Fesseha H. (2020). Assessment of Community Knowledge, Attitude, and Practice on Milk Borne Zoonotic Diseases in Jinka, Southern Ethiopia. Annual Public Health Research. 7:1096.

28. Radostits O, Gay CC, Hinchcliff KW, Constable PD. (2007). Veterinary Medicine: A Textbook of the Diseases of Cattle, Sheep, Pigs, Goats, and Horses (10th edn). Elsevier Saunders, Philadelphia, USA.

29. Verhagen LM, van den Hof S, van Deutekom H, Hermans PW, Kremer K, Borgdorff MW, et al. (2011). Mycobacterial factors relevant for transmission of tuberculosis. J Infect Dis. 203(9):1249-1255.

30. Schiller I, Oesch B, Vordermeier HM, Palmer MV, Harris BN, Orloski KA, et al. (2010). Bovine tuberculosis: a review of current and emerging diagnostic techniques in view of their relevance for disease control and eradication. Transbound Emerg Dis. 57(4):205-220.

31. Garner G, Saville P, Fediaevsky A. (2007). Manual for the recognition of exotic diseases of livestock: A reference guide for animal health staff [online]. Food and Agriculture Organization of the United Nations [FAO]; 2004. Equine viral encephalomyelitis.

32. Cousins DV. (2001). *Mycobacterium bovis* infection and control in domestic livestock. Rev Sci Tech. 20(1):71-85.

33. Byrne AW, Barrett D, Breslin P, Fanning J, Casey M, Madden JM, et al. (2022). *Bovine tuberculosis* in youngstock cattle: A narrative review. Front Vet Sci. 9:1000124.

34. Cousins DV, Florisson N. (2005). A review of tests available for use in the diagnosis of tuberculosis in non-bovine species. Rev Sci Tech. 24(3):1039-1059.

35. Monaghan ML, Doherty ML, Collins JD, Kazda JF, Quinn PJ. (1994). The tuberculin test. Vet Microbiol. 40(1-2):111-124.

36. Varello K, Pezzolato M, Mascarino D, Ingravalle F, Caramelli M, Bozzetta E. (2008). Comparison of histologic techniques for the diagnosis of *bovine tuberculosis* in the framework of eradication programs. J Vet Diagn Invest. 20(2):164-169.

37. Andrews A. (2003). Bovine medicine. 2nd edn. UK: Blackwell Science, Color of Atlas for the Diagnosis of Bacterial pathogens in animals. Germany: Paul Parey Scientific Publishers. pp. 108-118.

38. Alvarez J, Perez A, Bezos J, Marqués S, Grau A, Saez JL, et al. (2012). Evaluation of the sensitivity and specificity of *bovine tuberculosis* diagnostic tests in naturally infected cattle herds using a Bayesian approach. Vet Microbiol. 155(1):38-43.

39. World Organisation for Animal Health (OIE). (2012). Chapter 2.04.07: Bovine tuberculosis. Manual of diagnostic tests and vaccines for terrestrial animals. Available at: http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/2.04.07_BOVINE_TB.pdf.

40. Ameni G, Vordermeier M, Firdessa R, Aseffa A, Hewinson G, Gordon SV, Berg S. (2011). Mycobacterium tuberculosis infection in grazing cattle in central Ethiopia. Vet J. 188(3):359-361.

41. Tizard L. (2004). Veterinary immunology, an introduction. 7th edn. Texas A & M University: Sounders, Elsevier. Pp. 343-350.

42. Fentahun T, Luke G. (2012). Diagnostic techniques of bovine tuberculosis: a review. African Journal of Basic and Applied Sciences. 4(6):192-199.

43. Coad M, Hewinson RG, Clifford D, Vordermeier HM, Whelan AO. (2007). Influence of skin testing and blood storage on interferon-gamma production in cattle affected naturally with *Mycobacterium bovis*. Vet Rec. 160(19):660-662.

44. Kwaghe AV, Geidam YA, Egwu GO. (2011). Diagnostic Techniques for Bovine Tuberculosis: An Update. Journal of American Science. 7(11):204-215.

45. Wood PR, Jones SL. (2001). BOVIGAM: an in vitro cellular diagnostic test for bovine tuberculosis. Tuberculosis (Edinb). 81(1-2):147-155.

46. Cyrithia MK. (2003). Merck Veterinary manual. 9 edn. USA: Merial. pp: 549-553.

47. Ameni G, Miörner H, Roger F, Tibbo M. (2000). Comparison between comparative tuberculin and gamma-interferon tests for the diagnosis of *bovine tuberculosis* in Ethiopia. Trop Anim Health Prod. 32(5):267-276.

48. Keskin O. (1996). Sığır Tüberkülozisin teşhisinde ELİSAnın kullanılması ve allerjik yöntemle karşılaştırılması. Available at: https://teav.ankara.edu.tr/xmlui/bitstream/handle/20.500.12575/37727/tez.pdf?sequence=1&isAllowed=y.

49. Ramos DF, Silva PEA, Dellagostin OA. (2015). Diagnosis of bovine tuberculosis: review of main techniques. Brazilian Journal of Biology. 75(4):830-837.

50. Boadella M, Lyashchenko K, Greenwald R, Esfandiari J, Jaroso R, Carta T, et al. (2011). Serologic tests for detecting antibodies against *Mycobacterium bovis* and Mycobacterium avium subspecies paratuberculosis in Eurasian wild boar (Sus scrofa scrofa). J Vet Diagn Invest. 23(1):77-83.

51. Lilenbaum W, Ribeiro ER, Souza GN, Moreira EC, Fonseca LS, Ferreira MA, et al. (1999). Evaluation of an ELISA-PPD for the diagnosis of *bovine tuberculosis* in field trials in Brazil. Res Vet Sci. 66(3):191-195.

52. OIE. (2018). Reference Laboratories for *Bovine tuberculosis* of this Terrestrial Manual or consult the OIE website for the most up-to-date list: https://www.woah.org/en/scientific-expertise/reference-laboratories/list.

53. Wagari A. (2016). Review on diagnostic techniques of bovine tuberculosis. Int J Biochem Biophys Mol Biol. 1:11-17.

54. Lilenbaum W, Fonseca LS. (2006). The use of ELISA as a complementary tool for the tuberculosis eradication program in Brazil. Braz J Vet Res Anim Sci. 43(2):256-261.

55. Alelign A, Zewude A, Petros B, Ameni G. (2019). Tuberculosis at Farmer-Cattle Interface in the Rural Villages of South Gondar Zone of Northwest Ethiopia. Tuberc Res Treat. 2019:2106981.

56. Dalley D, Chambers MA, Cockle P, Pressling W, Gavier-Widén D, Hewinson RG. (1999). A lymphocyte transformation assay for the detection of *Mycobacterium bovis* infection in the Eurasian badger (Meles meles). Vet Immunol Immunopathol. 70(1-2):85-94.

57. Bernitz N, Kerr TJ, Goosen WJ, Chileshe J, Higgitt RL, Roos EO, et al. (2021). Review of Diagnostic Tests for Detection of *Mycobacterium bovis* Infection in South African Wildlife. Front Vet Sci. 8:588697.

58. Collins JD. (2006). Tuberculosis in cattle: strategic planning for the future. Vet Microbiol. 112(2-4):369-381.

59. Broughan JM, Judge J, Ely E, Delahay RJ, Wilson G, Clifton-Hadley RS, et al. (2016). A review of risk factors for *bovine tuberculosis* infection in cattle in the UK and Ireland. Epidemiol Infect. 144(14):2899-2926.

60. Good M, Duignan A. (2011). Perspectives on the History of Bovine TB and the Role of Tuberculin in Bovine TB Eradication. Vet Med Int. 2011:410470.

61. Alelign A, Zewude A, Petros B, Ameni G. (2019). Tuberculosis at Farmer-Cattle Interface in the Rural Villages of South Gondar Zone of Northwest Ethiopia. Tuberc Res Treat. 2019:2106981.

62. Asseged B, Woldesenbet Z, Yimer E, Lemma E. (2004). Evaluation of abattoir inspection for the diagnosis of *Mycobacterium bovis* infection in cattle at Addis Ababa abattoir. Trop Anim Health Prod. 36(6):537-546.

63. Biffa D, Bogale A, Skjerve E. (2010). Diagnostic efficiency of abattoir meat inspection service in Ethiopia to detect carcasses infected with *Mycobacterium bovis*: implications for public health. BMC Public Health. 10:462.

64. Corner LA. (1994). Post mortem diagnosis of *Mycobacterium bovis* infection in cattle. Vet Microbiol. 40(1-2):53-63.

65. Woldemariyam FT, Markos T, Shegu D, Abdi KD, Paeshuyse J. (2021). Evaluation of Postmortem Inspection Procedures to Diagnose *Bovine Tuberculosis* at Debre Birhan Municipal Abattoir. Animals (Basel). 11(9):2620.

66. Romha G, Gebru G, Asefa A, Mamo G. (2018). Epidemiology of *Mycobacterium bovis* and Mycobacterium tuberculosis in animals: Transmission dynamics and control challenges of zoonotic TB in Ethiopia. Prev Vet Med. 158:1-17.

67. Corner LA, Murphy D, Gormley E. (2011). *Mycobacterium bovis* infection in the Eurasian badger (Meles meles): the disease, pathogenesis, epidemiology and control. J Comp Pathol. 144(1):1-24.

68. Liebana E, Johnson L, Gough J, Durr P, Jahans K, Clifton-Hadley R, et al. (2008). Pathology of naturally occurring *bovine tuberculosis* in England and Wales. Vet J. 176(3):354-360.

69. Guirado E, Schlesinger LS. (2013). Modeling the Mycobacterium tuberculosis Granuloma - the Critical Battlefield in Host Immunity and Disease. Front Immunol. 4:98.

70. Patterson JS, Grooms D. (2000). Diagnosis of Bovine TB: Gross Necropsy, Histopathology, and Acid-fast Staining. Michigan State University Extension, United States.

71. Ejo M, Haile B, Tariku T, Nigatu S, Kebede E, Bitew AB, et al. (2021). Bacteriological and molecular characterization of *Mycobacterium bovis* isolates from tuberculous lesions collected among slaughtered cattle, Northwest Ethiopia. BMC Microbiol. 21(1):286.

72. Crawshaw TR, Griffiths IB, Clifton-Hadley RS. (2008). Comparison of a standard and a detailed postmortem protocol for detecting *Mycobacterium bovis* in badgers. Vet Rec. 163(16):473-477.

73. Nguyen TNA, Anton-Le Berre V, Bañuls AL, Nguyen TVA. (2019). Molecular Diagnosis of Drug-Resistant Tuberculosis; A Literature Review. Front Microbiol. 10:794.

74. Caviedes L, Lee TS, Gilman RH, Sheen P, Spellman E, Lee EH, et al. (2000). Rapid, efficient detection and drug susceptibility testing of Mycobacterium tuberculosis in sputum by microscopic observation of broth cultures. The Tuberculosis Working Group in Peru. J Clin Microbiol. 38(3):1203-1208.

75. Zou Y, Bu H, Guo L, Liu Y, He J, Feng X. (2016). Staining with two observational methods for the diagnosis of tuberculous meningitis. Exp Ther Med. 12(6):3934-3940.

76. Ei PW, Aung WW, Lee JS, Choi GE, Chang CL. (2016). Molecular Strain Typing of Mycobacterium tuberculosis: a Review of Frequently Used Methods. J Korean Med Sci. 31(11):1673-1683.

77. Algammal AM, Wahdan A, Elhaig MM. (2019). Potential efficiency of conventional and advanced approaches used to detect *Mycobacterium bovis* in cattle. Microb Pathog. 134:103574.

78. Soares Filho PM, Ramalho AK, de Moura Silva A, Hodon MA, de Azevedo Issa M, Fonseca Júnior AA, et al. (2019). Evaluation of post-mortem diagnostic tests' sensitivity and specificity for *bovine tuberculosis* using Bayesian latent class analysis. Res Vet Sci. 125:14-23.

79. Carvalho RC, Furlanetto LV, Maruyama FH, Araújo CP, Barros SL, Ramos CA, et al. (2015). Evaluation of the efficiency of nested q-PCR in the detection of *Mycobacterium tuberculosis complex* directly from tuberculosis-suspected lesions in post-mortem macroscopic inspections of bovine carcasses slaughtered in the state of Mato Grosso, Brazil. Meat Sci. 106:11-15.

80. Sharma R, Gupta V. (2011). Spoligotyping for the detection of *Mycobacterium tuberculosis complex* bacteria. A J Biochemistry. 6:29-37.

81. Soolingen van D. (2008). Molecular Epidemiology of Tuberculosis and Other Mycobacterial Infections main methodologies and achievements. J Intern Med. 249(1):1-26.

82. Gori A, Bandera A, Marchet G, Esposti AD, Catozzi L, Piero G, et al. (2005). Spoligotyping and Mycobacterium tuberculosis. Global Veterinaria. 5(5):255-258.

83. Iwnetu R, van den Hombergh J, Woldeamanuel Y, Asfaw M, Gebrekirstos C, Negussie Y, et al. (2009). Is tuberculous lymphadenitis over-diagnosed in Ethiopia? Comparative performance of diagnostic tests for mycobacterial lymphadenitis in a high-burden country. Scand J Infect Dis. 41(6-7):462-468.

84. Zijenah LS. (2018). The World Health Organization recommended TB diagnostic tools. Tuberculosis. 2:71-90.

85. Susha C. (2019). Restriction Fragment Length Polymorphism (RFLP) Technique. News-Medical, viewed 08 May 2023.

86. Bouzouita I, Draoui H, Mahdhi S, Essalah L, Saidi LS. (2021). Evaluation of PCR pncA-restriction fragment length polymorphism and PCR amplification of genomic regions of difference for the identification of M. bovis strains in lymph nodes cultures. Afr Health Sci. 21(3):985-989.