Review on Diagnostic Techniques of Bovine Tuberculosis in Ethiopia

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Abstract Bovine tuberculosis is a major infectious disease of cattle, other domesticated animals and certain wildlife populations. It is one of the zoonotic diseases and can be diagnosed with different techniques. The currently used techniques are acid fast staining which is a cost-effective tool for diagnosing tuberculosis case and to monitor the progress of treatment and it has also drawbacks such as the low sensitivity in the immune-suppressed individuals; Immunological diagnostic techniques like tuberculin skin tests: Single Intradermal Test, Comparative Intradermal Test, Short Thermal Test and Stormont Test; Blood based diagnostic techniques like gamma interferon assays, Enzyme-Linked Immunosorbent Assays and Lymphocyte Proliferation Assay, Culture of Mycobacterium and Molecular diagnostic techniques which involves Polymerase Chain Reaction, is a method that allows direct identification of the *Mycobacterium tuberculosis Complex*. Spoligotyping, Restriction Fragment Length Polymorphism, Variable Number Tandem Repeats typing are the techniques used for concurrent detection and typing of mycobacterium species at strain level. Its clinical usefulness over the other techniques is determined by its rapidity, both in identifying causative bacteria and in providing molecular epidemiologic information on strains. However, it holds drawbacks of being expensive, and requiring well-equipped laboratory and skilled laboratory personnel, which are not always available in endemic areas. Thus, both conventional and molecular tools should be effectively used to diagnosis Tuberculosis.

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1. Introduction

Diagnosis of Tuberculosis (TB) remains a significant challenge in developing countries especially Sub Saharan Africa, including Ethiopia, where a high rate of Human immune Deficiency Virus (HIV) infection and shortage of diagnostic techniques are found. TB is an infectious disease that affects all age groups and a major global public health problem. It is caused by the genus of mycobacterium which includes many pathogens known to cause serious diseases in mammals [1, 2]. This genus is characterized phenotypically as non-motile, non-capsular, non-spore forming, obligate aerobic, thin rod usually straight or slightly curved having 1-10μm length and 0.2-0.6μm width, facultative intracellular microbe and has a slow generation time about 15-20 hours. Its cell wall is rich in lipids (mycolic acid) that provide it the thick waxy coat which is responsible for acid fastness and hydrophobicity. This waxy coat (mycolic acid) is also greatly contributing for the bacterium resistance to many disinfectants, common laboratory stains, antibiotics and physical injuries. It probably also contributes to the slow growth rate of some species by restricting the uptake of nutrients [3].

Similarly, Bovine Tuberculosis (BTB) is a major zoonotic infectious disease of cattle, other domesticated animals and certain wildlife populations [4]. It is mainly caused by Mycobacterium *(M.) bovis*, which is highly similar to *M. tuberculosis*. Of *Mycobacterium tuberculosis* Complex (MTC), *M. tuberculosis, M. bovis and M. africanum* can cause BTB [5]. Despite the different species tropisms, the MTC is characterized by 99.9% or greater similarity at the nucleotide level and possess identical 16S `rRNA sequence [7].(Smith *et al*., 2006). In human, it is the most frequent cause of zoonotic TB which is clinically indistinguishable from TB caused by *M. tuberculosis*. Before milk pasteurization, *M. bovis* was an important cause of human TB especially intestinal TB in children. The development of the Polymerase Chain Reaction (PCR) and other molecular tools to identify *M. bovis* and differentiate it from other members of the MTC have allowed the discovery of more cases in retrospective studies and have suggested new forms of transmission [6].

Although the presumptive diagnosis of TB is often based on clinical suspicion and radiological data, a definitive diagnosis of the disease requires microbiological assays. Laboratory diagnosis of TB has been based on smear microscopy, culture and phenotypic identification. While the quickest, easiest and cheapest method available is acid-fast staining, its low sensitivity (45%-80% of positive cultures) has limited its usefulness, especially in geographical areas of lower incidence, in BTB [1].

A further point is that despite having good overall specificity the smear has a low positive predictive value (50%-80%) in areas of higher incidence of Non-Tuberculous Mycobacterium (NTM) clinical isolates [8]. By contrast, the culture technique is still regarded as the reference method due to its sensitivity and many studies were conducted with the isolated mycobacterium (identification, sensitivity and epidemiological typing). However, the slow growth of the tubercle bacillus is a major obstacle to rapid disease diagnosis. Indeed, while the last two decades have witnessed spectacular improvements to the culture method through the use of new media and several weeks are still required to obtain the final laboratory confirmation, and even longer in the case of conventional phenotypic identification procedures. Therefore, in recent years new methods have been developed for the rapid diagnosis of active TB, the best alternative being the molecular or genotypic techniques [9].

The current diagnostic techniques are either inadequate to detect TB cases with precision, or are time consuming, expensive, and require highly equipped laboratories which are not available in developing countries, where the disease is endemic. Even though the current diagnostic tools are a key to diagnose TB cases accurately, only a few Research Institutes like Aklilu Lemma Institute Pathobiology and Armauer Hansen Research Institute and Jimma University are practicing the molecular techniques. Hence, there is a lack of the techniques and reviewed information on this regard in Ethiopia. Thus, the objectives of this seminar are to review on the current BTB diagnostic techniques and recommend on the techniques.

2. BTB Diagnostic Techniques

2.1 Clinical Signs

It is usually a chronic debilitating disease in cattle, but it can occasionally be acute and rapidly progressive. Early infections are often asymptomatic. In countries with eradication on programs, most infected cattle are identified early and symptomatic infections are uncommon. In late stages, common symptoms included progressive emaciation allows grade fluctuating fever, weakness and in appetence. Animals with pulmonary involvement usually have a most cough that is worse in the morning, during cold weather of exercise and may have dyspnea or tachypnea. In terminal stage, animals may become extremely emaciated and develop act respiratory distress [10]. In brush- tailed opossums, BTB is usually a fulminating pulmonary disease that typically lasts two to six months. In the final stage of the disease, animals become disoriented, can't climb, and may be seen wandering about in day light. In contrast, most infected badgers have no visible lesion (NVL) and can survive for many years asymptomatic badgers, the disease is primary a respiratory distress [10, 11].

2.2 Postmortem lesions

It is characterized by the formation of granulomas (tubercles) where bacteria have localized. These granulomas are usually yellowish and either caseous, or calcified, they are often encapsulated. In some species such as deer, the lesion tends to resemble abscesses rather than typical tubercles. Some tubercles are small enough to be missed by the naked eye unless the tissue is sectioned. In cattle, tubercles are found in the lymph nodes, particularly those of the head and thorax. It is also common in the lungs, spleen, liver and the surfaces of body cavities. In disseminated case, lesions are sometimes founds on the female genitalia, but are rare on the male genitalia. In countries with good control programs, infected cattle typically have few lesions at necropsy. Most of those lesions found in lymph nodes associated with the respiratory system. However, small lesions can often be discovered in the lungs of these animals if the tissues are sectioned [4, 10].

2.3 Conventional techniques

***Acid-fast staining*:** It is used to detect acid fast bacilli (AFB) in clinical specimens by Ziehl-Neelsen (ZN) or fluorescent staining. It is a cost-effective tool for diagnosing of BTB and to monitor the progress of treatment especially in developing countries. However, there are many drawbacks such as the difficulty of obtaining the swab sample and the low sensitivity especially in immune-compromised patients with AFB smear positivity ranging from 31 to 90 percent. Culture is the gold standard for BTB diagnosis with an excellent sensitivity and specificity. The traditional method of inoculating solid medium such as Lowenstein-Jensen (LJ) or 7H10/7H11 media is slow and takes 6-8 weeks of incubation to diagnose the infection and further more time to determine the susceptibility patterns that results in delay in initiation of appropriate therapy [9]. The cell walls of these acid-fast bacteria contain approximately equal amounts of polysaccharide. The high lipid content, which ranges from 20-40% of the dry cell weight, is largely responsible for the ability of these bacteria to resist decolorization with acidified organic solvents. The bacteria that take up this stain, including *M. bovis*, will appear as short red or pink rods when examined under a microscope [11, 12, 13]. Preliminary examination of tissues suspected of being TB should include the preparation of suitably stained smears. The identifiable smear can be made on a new slide from scrapings of the cut surface of tissue. The smear should be air dried and fixed by flaming for one to two seconds. The smears are treated as with concentrated carbolfuchsin by heating and then decolorized with a sulfuric acid and alcohol solution. Malachite green or methylene blue is commonly used counter stains [14]. The stained slides are observed with an ordinary light microscope for the presence of acid-fast an ordinary light microscope for the presence of acid-fast bacilli, which appear as red, colloidal or bacillary cells 1-3 microns in length occurring singly or in clumps [11, 15, 16].

Mycobacterium culture: In the isolation of mycobacterium by culture, the ideal medium should be able to support rapid and luxuriant growth, and allow the determination of its characteristic features. For example, colony morphology, growth rate and pigment production. The luxuriant growth of *M. tuberculosis* on glycerol containing media, giving the characteristic 'rough, tough and buff' colonies is known as eugenic while the growth of *M. avium* on media containing glycerol is also described as eugenic whereas *M. bovis* has sparse, thin growth on glycerol containing media that is called dysgenic, however, grows well on pyruvate-containing media without glycerol [12].

Most of mycobacterial culture media fall into egg-potato-base media and agar-base media The most popular egg-based media are the LJ buffered egg-potato medium and the American Trudeau Society egg yolk-potato flour medium. Among the agar based media, Middle brook 7H10, Middle brook 7H11, and Dubose oleic-albumin agar are recommended. The advantages of egg-based media are the long shelf life (1 year when refrigerated) and the low cost of preparation. Egg media require heat for solidification, which, along with the presence of albumin, inactivates certain antituberculous drugs [1, 17].

Similarly in veterinary bacteriology, the egg based LJ and stone brinks media are most commonly used. LJ medium can be obtained commercially. An agar-based medium such as middle brook 7H10 and 7H11 or blood based agar medium may also be used [1]. The media are prepared as solid slants in screw-capped bottles. Malachite green dye (0.025g/100ml) is commonly used as selective agent. *M. tuberculosis, M. avium* and many of the atypical mycobacteria require glycerol for growth. However, glycerol is inhibitory to *M. bovis* while sodium pyruvate (0.4%) enhances its growth. Thus, the media with glycerol and without glycerol (but with sodium pyruvate) should be inoculated. The media can be made more selective by the addition of cycloheximide (400µg/ml), lincomycin (2µg/ml) and nalidixic acid (35µg/ml). Each new batch of culture medium should be inoculated with the stock strains of mycobacteria to ensure that the medium supports satisfactory growth. The inoculated media may have to be incubated at 37°C for up to 8 weeks and preferably for 10 to 12 weeks with or without carbon dioxide for the Mycobacteria in the TB group. *M. tuberculosis* and *M. avium* prefer the caps on the culture media to be loose while *M. bovis* grows best in airtight containers [16, 18].

2.4 Immunological techniques

Tuberculin skin test: The tuberculin skin test based on a delayed type hypersensitivity to mycobacterial tuberculoprotein, is the standard ante mortem test both in human and cattle. It is convenient, cost effective method for assessing cell mediated responses to a variety of antigens and it is "gold standard" for diagnostic screening for detection of new or asymptomatic MTC infection [19].

The reaction in cattle is usually detectable 30-50 days after infection. The tuberculin is prepared from cultures of *M. tuberculosis or M. bovis* grown on synthetic media. The tuberculin test is usually performed between the mid necks, but the test can also be performed in the caudal fold of the tail [20, 21].

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. Bovine tuberculin is more potent and specific and potency of tuberculins must be estimated by biological methods, based on comparison with standard tuberculins and potency is expressed in the international unit (IU). In several countries, bovine tuberculin is considered to be of acceptable potency if its estimated potency guarantees per bovine dose at least 2000 IU in cattle. In cattle with diminished allergic sensitivity, a higher dose of bovine tuberculin is needed and the volume of each injection dose must not exceed 0.2ml. Cell mediated hypersensitivity, acquired through infection can be demonstrated systematically by fever or ophthalmically by conjunctivitis, or dermally by local swelling, when tuberculin test or its purified protein derivative (PPD) is given by the subcutaneous, conjuctival or intradermal route, respectively [21].

***Single Intradermal Test*:** It is applied by the intradermal injection of 0.1ml of bovine tuberculin PPD into a skin fold at the base of the tail or into the cervical fold and the subsequent detection of swelling as a result of delayed hypersensitivity. The reaction is read between 48 and 96 hours after injection with a preference for 48-72 hours for maximum sensitivity and at 96 hours for maximum specificity. The positive reaction constitutes a diffuse swelling at the site of injection. The main disadvantage of the SID test is its lack of specificity and the number of reactor lesion occur. Mammalian tuberculin is not sufficiently specific to differentiate between reactions due to infection with *M. bovis* and infection with *M. avium,* *M. tuberculosis a*nd *M. paratuberculosis* including vaccination or *Nocardia farcinicus* [21]. The other disadvantages of SID test include failure to detect cases of minimal sensitivity, in old cows and in cows which have recently calved as well as in early infection, in some cattle in an unresponsive state, referred to as anergy which is developed due to antigen excess or immunosuppression which in turn caused by non specific factors such as malnutrition and stress [18, 22, 23].

Comparative Intradermal Test (CID): two sites on the mid neck, 10-12 cm apart, are shaved and the thickness is measured in millimeters with caliper before the injection of tuberculin. In the CID test, 0.1ml of Purified Protein Derivatives from Mycobacterium avium (PPD-A) and 0.1ml of Purified Protein Derivatives from Mycobacterium bovis (PPD-B) are injected intradermally into separate clipped sites on the side of the neck. Care must be taken in placing the injection as varied from place to place in the skin. After 72 hours the thickness of the skin at the sites is measured again [4, 18].

When the change in skin thickness is greater at the PPD- A injection site, the result is considered negative for BTB. When the change in skin thickness increased at both sites, the difference between the two changes is considered. Thus, if the increased in the skin thickness at the injection site for the bovine (B) is greater than the increase in the skin thickness at the injection site at the avian (A) and (B-A), is less than 1mm, between 1 and 4 mm, or a 4 mm and above, the result is classified as negative, doubtful, or positive for BTB, respectively and the animal with the evidence of infection is termed as reactor. The comparative test is used to differentiate between animals infected with *M. bovis* and those responding to bovine tuberculin as a result of exposure to other Mycobacterium. This sensitization can be attributed to the antigenic cross reactivity among mycobacterial species and related genera [24].

Short thermal test: In this test, intradermal tuberculin (4ml) is injected subcutaneously into the neck of cattle which have a rectal temperature of not more than 39°C at the time of injection and for 2 hours later. If the temperature at 4, 6 and 8 hours after injection rises above 40°C, the animal is classed as a positive reactor. The temperature peak is usually at 6-8 hours and is generally over 41°C [25].

***Stormont test*:** This test is a more sensitive test than short thermal test of TB in cattle. This test relies on the increased sensitivity of the test site, which occurs after a single injection. The test is performed similarly to the SID test in the neck with a further injection at the same site seven days later. An increase in the thickness of 5 mm or more, 24 hours after this second injection, is a positive result. The loss of sensitivity is probably due to the general immunological hyporeactivity that occurs associated with parturition [26].

Table 1. Comparison of Tuberculin tests

S. no	Tests	Advantages	Disadvantages
1	Single Intradermal Test	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 an animal

Source: [26].

Gamma Interferon Assays: It is a laboratory based test detecting specific cell mediated immune responses by circulating lymphocytes. In this test, the release of the lymphokine gamma interferon (IFN-) is measured in a whole-blood culture system. The assay is based on the release of IFN- from sensitized lymphocytes during a 16-24 hours incubation period with specific antigen. The test makes use of comparison of IFN-production following stimulation with PPD-A and PPD-B. The detection of bovine IFN- is carried out with a sandwich ELISA that uses two monoclonal antibodies to bovine gamma-interferon. It is recommended that the blood samples be transported to the laboratory and the assay set up as soon as practical, but not later than the day after blood collection. Because of the IFN- test capability of detecting early infections, the use of both tests in parallel allows the detection of a greater number of infected animals before they become a source of infection for other animals as well as a source of contamination of the environment.

The use of defined mycobacterium antigens such as ESAT-6 and CFP-10 shows promise for improved specificity [28, 29]. The use of these antigens may also offer the ability to differentiate Bacillus Calmette Guerin (BCG)-vaccinated from unvaccinated animals.

In animals that are difficult or dangerous to handle, such as excitable cattle or other bovidae, the advantage of the IFN- test over the skin test is that the animals need be captured only once. The IFN- test is used for serial testing (to enhance specificity) and parallel testing (to enhance sensitivity). The advantages of the IFN- assay are its increased sensitivity, the possibility of more rapid repeat testing and no need for a second visit to the farm and more objective test procedures. The limitations of IFN- comprise a reduced specificity, high logistical demands (culture start is required within 24 hours after blood sampling), an increased likelihood of non specific response in young animals [owing to natural killer (NK) cell activity] and its high cost [1, 30].

2.5 Enzyme-Linked Immunosorbent Assays (ELISA)

The ELISA 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]. It is a valuable complementary tool in order to identify possible anergic cows that may be acting as reservoirs of the agent [31]. An advantage of the ELISA is its simplicity, but sensitivity is limited mostly because of the late and irregular development of humoral immune response in cattle during the course of the disease. Specificity is also poor in cattle when complex antigens such as tuberculin or culture filtrates are used. *M. bovis* has been shown to be useful in increasing specificity in the ELISA. Improvement may be possible by using a combination of different antigens including proteins such as MPB 70 and MPB 83, which are specific but lack sensitivity [30, 31].

Immune responses to MPB 70 and MPB 83, proteins derived from pathogenic strains of *M. bovis* culture filtrates, are both representative of a strong antigen- induced cell mediated immune response in the early stages of the tuberculosis infection. MPB 70 is the major secreted antigen of *M. bovis*, while MPB 83 is a cell wall lipoprotein [32]. The ELISA may also be useful for detecting *M. bovis* infections in wildlife. For example, a lateral flow-based rapid test has been shown to be useful for detecting tuberculous 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. However; its sensitivity in cattle is relatively low [33].

2.7 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 endeavors 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, meaning it requires long incubation times and the use of radio-active nucleotides. As with the IFN- test, the lymphocyte proliferation assay should be performed shortly after blood is collected. The test is relatively expensive and has not been subjected to inter-laboratory comparisons [3, 21, 34].

2.8 Molecular techniques

Polymerase Chain Reaction (PCR): With the advance of molecular diagnosis, various PCR methods in diverse clinical specimens have been introduced to identify *M. tuberculosis* more easily and quickly. Owing to the limitations of the traditional microbiological methods, puacibacilary nature of the specimen and the extensive differential diagnosis in extra-pulmonary tuberculosis, a rapid, sensitive and specific diagnosis is needed in developing countries. PCR has several advantages over culture, including confirmation of the presence of *M. tuberculosis* within 1 to 3 days as compared to 6 weeks with conventional culture techniques [3]. Additional advantages of PCR over conventional methods include its high sensitivity, performance in few hours, and depending on the assay design, ability to differentiate between MTC and mycobacterial species other than TB, and identification of gene mutations associated with drug resistance [21, 35].

Observing *M. tuberculosis* in tissues or smears using ZN staining or fluorescence method allows faster diagnosis. Unfortunately, these methods are insensitive and non-specific. This is the cause for development of a new and sensitive diagnostic technique like PCR. Key mycobacterial targets for PCR amplification are: the insertion sequence (IS) IS6110, 65KD (kilodalton) heat shock protein, 38KD protein, and ribosomal RNA. IS6110 is considered to be a good target for amplification as this is found in almost all members in high copy number in most strains of the MTC. PCR methods allow direct identification of the MTC and can detect less than ten bacteria in a clinical specimen. PCR’s sensitivity ranges from 70-90% compared to the results of culture and its specificity varies between 90 and 95%. In smear of positive cases, the sensitivity of PCR is greater than 95%, but in smear of negative cases, it is only 50 to 60%. Therefore; at present amplification methods should not replace diagnostic convectional culture [36].

For the diagnosis of BTB, PCR is used to identify *M. bovis* in tissue collected at necropsy from animals suspected of being infected with BTB. PCR is only used on tissues that have histological (microscopic) evidence compatible with BTB. The result can typically be obtained within seven days and are classified as either positive or negative. A positive test obtained on PCR is highly suggestive that the animal is infected with BTB. PCR has been widely evaluated for the detection of MTC in clinical samples, mainly sputum in human patients and has recently been used for the diagnosis of TB in animals [37]. The introduction of the BACTEC-TB 460 radiometric system (Becton Dickinson, Sparks, MD, USA) in the 1980s allowed the detection of *M. tuberculosis* in a few days as compared with the conventional culture media. Introductions of the rapid and automated systems have increased the sensitivity of isolation of mycobacterium from clinical samples and have brought down the time required for positive culture substantially (9-10days). Faster culture results in *M. tuberculosis* infected patients can result in evidence-based therapy and faster implementation of the planned treatment [3, 38].

Although a combination of traditional solid and liquid media is recommended and helpful for the primary isolation of mycobacterium, the process still requires several days and expensive infrastructure. The Gen-Probe assay specific for MTC is a rapid detection, nucleic acid amplification (NAA) test, where results can be obtained as fast as in two hours (provided if a positive culture is present); it also has a high sensitivity of 99 percent and specificity of 99.2 percent. It holds the disadvantage of needing of positive culture that can take several days [40].

Spoligotyping: It also called spaceroligonucleotides typing is a novel or new method for simultaneously detection and typing of MTC bacteria, has been recently developed. This method is based on polymerase chain reaction (PCR) amplification of highly polymorphic direct repeat (DR) locus in the *M. tuberculosis* genome. The DR region in *M. bovis* BCG contains direct repeat sequences of 36 bp, which is interspersed by the non-repetitive DNA spacers of 35-41 bp in length. Other MTC strains contain one or more IS6110 elements in DR-region [38, 39].

It applied to culture is simple, robust and highly reproducible. 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. 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. The specificity and sensitivity of this technique has been found to be 98 and 96%, respectively with the clinical samples. One of the clearest advantages 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 [40, 41, 42].

Restriction Fragment Length Polymorphism (RFLP): The gold standard molecular technique that is used for the molecular typing of *M. tuberculosis* due to its high discriminative power and reproducibility. It can also be used for outbreaks identification and can facilitate contact tracing of tuberculosis [39, 46]. However, this technique requires large amount of DNA and is therefore restricted to the mycobacterial cultures which take around 20-40 days to obtain sufficient DNA needed and for the combined process of probe labeling, DNA fragmentation, electrophoresis, blotting, hybridization, washing and auto-radiograph. Moreover, this technique is also technically demanding, slow, cumbersome, expensive and requires sophisticated analysis soft ware for result analysis [12, 39, 41].

Variable Number Tandem Repeats Typing: Genetic loci containing variable numbers of tandem repeats (VNTR) loci form are the basis for human gene mapping, forensic analysis, and paternity testing. In this technique, DNA containing VNTR sequences is amplified by PCR and the size of the product determined by gel electrophoresis identified six VNTR loci (ETR-A to F) for typing the MTC. When compared to the RFLP-IS6110 fingerprinting, VNTR was demonstrated to be less discriminatory for strains with a high copy number of IS6110, but allowed improved discrimination for strains with only one or two copies of IS6110. The usefulness of this technique has not yet been fully assessed for *M. bovis*, although its evaluation is underway for isolates from the Great Britain at the Veterinary Laboratories Agency. Preliminary results suggest that although VNTR gives a higher degree of discrimination than spoligotyping, best results are obtained by combining the two techniques [39, 42, 43, 44].

Conclusions

Extra pulmonary TB including BTB remains a significant problem in low-income countries and there is a diagnostic challenge in sub Saharan Africa, where a high rate of HIV infection found. The combinations of conventional, immunological and molecular techniques are the best diagnostic tools of TB. But, molecular techniques are limited in developing countries especially in Ethiopia. Thus, Veterinarians and government should have to cooperate to widen the availability and accessibility of these diagnostic techniques as much as possible.

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