Global Veterinaria 21 (6): 316-327, 2019
ISSN 1992-6197

DOI: 10.5829/idosi.gv.2019.316.327

Review on Molecular and Conventional Diagnostic Techniques of Bovine Tuberculosis

[1]Yosef Deneke and [2]Garoma Desa

[1]Jimma University, School of Veterinary Medicine, Jimma, Ethiopia
[2]National Institute for Control and Eradication of Tsetse Fly and Trypanosomosis,
Kaliti Tsetse fly Mass Rearing and Irradiation Center, P.O. Box 19917, Addis Ababa, Ethiopia

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 diagnose Tuberculosis.Detection of Bovine Tuberculosis in Ethiopia is carried out most commonly on the basis of tuberculin skin testing, abattoir meat inspection and very rarely on bacteriological techniques.

Key words: Bovine · Conventional · Diagnostics · Molecular · Techniques · Tuberculosis

INTRODUCTION

Bovine tuberculosis (TB) is a chronic disease of animals caused by bacteria called *Mycobacterium bovis*, (*M.bovis*) which is closely related to the bacteria that cause human and avian tuberculosis. This disease can affect practically all mammals, causing a generalstate of illness, coughing and eventual death. The name Tuberculosis comes from the nodules, called 'tubercles', which form in the lymph nodes of affected animals. Until the 1920s when control measures began in developed countries, it was one of themajor diseases of domestic animals throughout the world. Bovine tuberculosis (BTB) is the zoonotic disease transmitted from animal to human and makes a significant economic impact due to high cost of eradication programs and has serious consequences for movements of animals and their products, biodiversity, public health and significant economic effect [1-3].

World Health Organization (WHO) classified bovine tuberculosis among seven neglected zoonotic diseases having potential to infect man [4]. The disease affects a broad range of mammalian hosts including cattle, pigs, goats, sheep, badgers, possums, domestic cats, deer, camelids, omnivores and wild carnivores [5]. Other than domestic animals various wildlife species such as Badgers (*Meles meles*), brushtail possums (*Trichosurus vulpecula*), deer (*Odocoileus virginianus*), bison

Corresponding Author: Garoma Desa, National Institute for Control and Eradication of Tsetse Fly and Trypanosomosis, Kaliti Tsetse fly Mass Rearing and Irradiation Center, P.O. Box: 19917, Addis Ababa, Ethiopia.

(*Bison bison*) and African buffalo (*Syncerus caffer*) also play role of maintenance hosts of *M. bovis*. Among these wild boar has been identified in having the highest ability to transmit the disease to cattle [6]. This genus is characterized phenotypically as non-motile, noncapsular, 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 [7].

Of *Mycobacterium tuberculosis* Complex (MTC), *M.tuberculosis, M. bovis and M. africanum* can cause BTB [8]. 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. In human, it is the most frequent cause of zoonotic TB which is clinically indistinguishable from TB caused by *M. tuberculosis* [9].

More recently, several molecular methods have been developed that provide clear criteria for the identification of *Mycobacterium bovis*. These comprise a variety of polymerase chain reaction (PCR) methods, which is based on DNA sequence variations in the direct repeat region of mycobacterium tuberculosis complex strains. Ethiopia is one among the nations that possesses the largest livestock population in the African continent. The distribution and the quantity of each species are different according to the type of prevailing animal production systems and agro-ecological zones. Therefore, the objective of this paper was to give review onmolecular and conventional diagnostic techniquesof bovine tuberculosis.

Literature Review

Etiology: *Mycobacterium bovis* is the main etiological agent of bovine tuberculosis. It is an acid-fast bacteria having characteristic feature of acid fast staining which is due to waxy substance (mycolic acid) present in their bacterial wall. The recovery of *M. bovis* is not enhanced by addition of carbon dioxide in the incubation atmosphere. However, now other members of *M. tuberculosis* complex have also been accepted as new species. These include *M. caprae*(mostly infect goats) and *M. pinnipedii* (usually infect fur seals and sea lions). Badgers also act as reservoir for spreading of bovine tuberculosis [10, 11].

It is found that *M. bovis* best survive in frozen tissue and there is adverse effects of tissue preservative i.e. sodium tetraborate on viability. In the environment *M. bovis* can survive for various months especially in cold as well as dark and conditions which are moist. The survival period varies from 18-332 days at 12-24°C (54-75°F) which is dependent of sunlight exposure. From soil or grazing pasture there is infrequent isolation of this organism. It has been found that culture of the organism can be done for approximately two years in samples that are stored artificially. The viability of the organism has been found more recently to be between 4-8 weeks in 80% shade whereas it can get destroyed in either summer or winter on New Zealand pastures [12].

Clinical Diagnosis: 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 [13].

In some animals, the retropharyngeal or other lymph nodes enlarge and may rupture and drain. Greatly enlarged lymph nodes can also obstruct blood vessels, airways, or the digestive tract. If the digestive tract is involved, intermittent diarrhea and constipation may be seen [14]. The symptoms of bovine tuberculosis usually take months to develop in cattle. Infections can also remain dormant for years and reactivate during periods of stress or in old age. Therefore, Bovine Tuberculosis (BTB) can be difficult to diagnose based only on the clinical signs, especially in developed countries, where the number of severe cases of animals with clinical evidence may be limited or absent and most are diagnosed by routine testing or found at the slaughterhouse [15].

Molecular Diagnostic 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, paucibacillary 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. 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 [16].

A PCR is a powerful tool that is used in a wide variety of diagnostic procedures. The PCR is used to detect the presence of genetic material (DNA) that is unique and specific to an organism of interest. PCR works by amplifying a portion of DNA that is specific for that organism. This product can be easily visualized using standard laboratory procedures. The PCR test is very sensitive and can detect the presence of an organism when present at very low levels [17].

For the diagnosis of bovine tuberculosis, PCR is used to identify *M. bovis* in tissue collected at necropsy from animals suspected of being infected with bovine tuberculosis. PCR is the only used on tissue that have histological (microscopic) evidence compatible with bovine tuberculosis. The result can typically be obtained within 7 days and are classified as either positive or negative. A positive test obtained on PCR is highly suggestive that the animal is infected with bovine tuberculosis. PCR has been widely evaluated for the detection of Mycobacterium tuberculosis complex (MTC) in clinical samples, mainly sputum in human patients and has recently been used for the diagnosis of tuberculosis in animals [18].

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 () 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 [19].

Spoligotyping: Spoligotyping also called spacer oligonucleotides typing is a novel or new method for simultaneously detection and typing of mycobacterium tuberculosis complex 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 [19].

It applied to culture is simple, robust and highly reproducible. Results can be obtained from *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. 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 [20].

Restriction Fragment Length Polymorphism (RFLP): RFLP, a gold standard molecular technique, is used for the molecular typing of *M. tuberculosis* dueto its high discriminative power and reproducibility. Itcan also be used for outbreaks identification and canfacilitate contact tracing of tuberculosis.However, this technique requires large amount ofDNA and is therefore restricted to the mycobacterialcultures which take around 20-40 days to obtainsufficient DNA needed and for the combined processof probe labeling, DNA fragmentation, electrophoresis, blotting, hybridization, washing andauto-radiograph. Moreover, this technique is also technically

demanding, slow, cumbersome and expensive and requires sophisticated analysis soft ware for result analysis [19].

Variable Number Tandem Repeats Typing (VNTRPT): 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 [21].

Biochemical Tests: The definitive identification of the species of *Mycobacteria* is largely based on biochemical criteria [22].

Niacin Production Test: The commercially available niacin test strips (Difco) are easier and safer to use as this avoids employing toxic BrCN solution used in convectional tests. *M. tuberculosis* is positive and *M. avium* is negative in this test [23].

Nitrate Reduction: Place a few drops of sterile distilled water in a screw-caped tube and add a loop full of a young culture of the *mycobacterium*. Use un-inoculated tube as a negative control. Add 2 ml of NaNo solution (0.01 M solution of NaN03 in 0.022M phosphate buffer, pH 7). Shake and incubate in a water bath at 37°C for 2 hours. Add a drop of 1:2 dilution of concentrated HCl, 2 drops of 0.2% aqueous solution of sulphanilamide and then 2 drops of 0.1% aqueous N-(1-naphthyl) ethylenediamine dihydrochloride. Examine for the development of a pink to red color and compare with the negative control. As strong red indicates nitrate reduced to nitrite. Add a pinch of powdered zinc to all negative tubes (converts nitrate to nitrite). The production of a red color indicates a negative test (nitrates not reduced). The commercial paper strip method can be used but a negative result should be confirmed by test [24].

Deamination of Pyrazinamide: The medium is a broth base containing 0.1g pyrazinamide, 0.2g of pyruvic acid and 15.0g agar per liter. Dispense in 15 ml amounts in screw-capped tubes. Autoclave at 121°C for 15 minutes and solidifies in an upright position. Incubate the agar with a heavy suspension of a young culture and incubate at 37°C for 4 days. A positive reaction is given by a pink band in the agar. Use an uninoculated tube and *M. avium* tube as negative and positive controls, respectively [25].

Urease Test: Mix one part of urea-agar base concentrate with nine parts of sterile water. Dispense in 4 ml amounts in screw-capped tubes (16 x 125 mm). Emulsify a loopful of young culture in the tube of substrates. Incubate at 37°C. A color change from amber to pink or red is a positive reaction. Discard after three days [26].

Inhibition and Tolerance Test: Reagents such as 5% NaCl and thiophen-2-carbonic acid hydrazide (TCH) 10µg/ml are usually incorporated into a media such as are usually incorporated into a media such as Lowenstein-Jensen [22].

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 are 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 [27].

The pathologic diagnosis, or post mortem, the BTB, while performing autopsies or sanitary inspection of carcasses in slaughterhouses refrigerated presents considerable difficult. The conventional post mortemexamination has detected approximately 47% of presumptive BTB lesions in carcasses of cattle slaughtered. Despite this, the anatomic pathology analysis has been crucial for the diagnosis of BTB in the control programs [28].

In recent years, in developed countries, the inspection of carcasses for evidence of BTB has come increasingly to be regarded as an extension of the national animal health program rather than solely as a control point in the prevention of human cases of tuberculosis caused by *M. bovis*, as was so often the case a century or more ago. Now, however, it is important to focus also on the contribution which efficient meat inspection, coupled with other diagnostic techniques and supported by advanced diagnostic bacteriology including DNA-based strain typing, that can provide the epidemiological data for to the eradication and control of this zoonotic disease [29].

Conventional Diagnostic Techniques

Culture of Mycobacteria: To process specimens for culture, the tissue is first homogenized using a mortar and pestle, stomacher or blender, followed by decontamination with either detergent (such as 0.375-0.75% hexadecylpyridiniumchloride [HPC], an alkali (2-4% sodium hydroxide) or an acid (5% oxalic acid). The alkali or acid mixture is shaken for 10-15 minutes at room temperature and then neutralized. Neutralization is not required when using HPC. The suspension is centrifuged, the supernatant is discarded and the sediment is used for culture and microscopic examination. It is recommended that, as a minimum, pooled lymph node samples from the head and thorax be cultured when no visible lesions are detected in tuberculin or interferon test positive animals at post-mortem examination [30].

Media for Mycobacteria: The egg based Lowenstein-Jensen and stone brinks media are most commonly used in veterinary bacteriology. Lowenstein-Jensen 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 [17]. The media are prepared as solid slants in screw-capped bottles. Malachite green dye (0.025g/100ml) is commonly used as selective agent. *Mycobacterium tuberculosis*, *Mycobacterium avium* and many of the atypical *Mycobacteria* require glycerol for growth. However, glycerol is inhibitory to *Mycobacterium 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 [22].

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 tuberculosis group. *Mycobacterium tuberculosis* and *Mycobacteriumavium* prefer the caps on the culture media to be loose while *Mycobacterium bovis* grows best in airtight containers [30].

Colonial Morphology: The luxuriant growth of *Mycobacterium tuberculosis* on glycerol containing media, giving the characteristic 'rough, tough and buff' colonies is known as eugenic while the growth of *Mycobacterium avium* on media containing glycerol is also described as eugenic. *Mycobacterium bovis* has sparse, thin growth on glycerol containing media that is called dysgenic. *Mycobacterium bovis*, however, grow well on pyruvate-containing media without glycerol [31].

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 [32].

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 [27, 31, 33].

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. 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 singlyor in clumps [22].

Pigment Production and Response to Light: The *Mycobacteria* that produce yellowish-orange carotenoid pigments are called chromogenic. The term photochromogenic is applied to those *Mycobacteria* that produce pigment only if exposed to light. The scotochromogenic *Mycobacteria* produce pigment when incubated either in light or in the dark. Pigment formation is tested with young, well-developed colonies on Lowenstein-Jensen medium. The cultures are exposed to a 100 Watt, clear electric light bulb, at a distance of 50 cm, for at least an hour and then incubated again in darkness for a further 1-3 days. After this treatment the photochromogens will develop pigment. Older colonies of *Mycobacteria* in the tuberculosis group often have a yellowish hue but they are described as non-chromogenic [22].

Immunological Techniques

Tuberculin Skin Test (Delayed Hypersensitivity 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. 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 [34].

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 tuberculin must be estimated by biological methods, based on comparison with standard tuberculin 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, conjunctival or intradermal route, respectively [16].

Single Intradermal (SID) Test: It is applied by the intradermal injection of 0.1ml of bovine tuberculin Purified Protein Derivative(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 [18].

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* and *M. paratuberculosis* including vaccination or *Nocardia farcinicus* [16]. 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 energy which is developed due to antigen excess or immunosuppression which in turn caused by non specific factors such as malnutrition and stress [35, 36].

Comparative Intradermal (CID) Test: 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 [30, 37].

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 [38].

Short Thermal Test: In this test, intradermal tuberculin (4ml) is injected subcutaneously into the neck of cattlewhich 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 [38].

Stormont Test: This test is a more sensitive test than short thermal test of TB in cattle. This test relieson the increased sensitivity of the test site, which occurs after a single injection. It is performed in the same way as single intradermal test in the neck with a second injection at the same site but after 7 days of first injection. After 24 h of second injection, an increase in skin thickness of 5 mm or more should be considered as positive. It is more accurate than the Single Intra Dermal (SID) test but a practical difficulty is the necessity for three visits to the farm [39]

Blood Based Diagnostic Techniques: Besides the classical intradermal tuberculin test, a number of blood tests have been used. Due to the cost and more complex natures of laboratory based assays they are usually used as ancillary tests to maximize the detection of infected animals (parallel testing), or to confirm or negate the result of an intradermal skin test (serial testing). There is also evidence that when an infected animal is skin tested, an enhanced blood test can occur during the following week. This allows for better separation of in-vitro blood test responses leading to greater test accuracy [17].

Gamma Interferon Assays: Since 2006, the IFNγ assay (Bovigam®, Prionics, Switzerland) is an assay through which it is possible to verify the existence of cell-mediated immune response developed by the body of the animal in response to mycobacterial infection. IFNγ produced by T lymphocytes of the infected animal is detected, using monoclonal anti-IFNγ. The lack of detection of IFNγ characterizes the negativity of the animal to infection *M. bovis* since lymphocytes from uninfected cattle do not produce this cytokine in specific ways. As this is an in vitro test that has the advantage of not interfering with the immune status of the animal and may be repeated in the same animal is the need to respect the period of desensitization. This assay showed the increase in the sensitivity and the possibility of more rapid repeat testing, no need for a second visit to the farm and more objective test procedures and interpretation in comparison to the TST [23, 29, 40].

The strategic application of the IFNγ assay, as an adjunct to the tuberculin test, can facilitate the early removal of infected animals in problem herds that are otherwise negative to the tuberculin test. Recognition that the objective of the assay is to identify high-risk animals that are potentially infectious for other cattle can generate confidence in herd-owners that rational decisions can be made based on sound scientific principles and that effective schemes can be devised to make more rapid progress in the elimination of the infection from affected herds [41].

The assay is based on the release of IFNγ from sensitized lymphocytes during a 16-24 hours incubation period with specific antigen and makes use of comparison of IFNγ production following stimulation with avium and bovine PPD [42]. Besides high logistical demands (culture start is required within 24 h after blood sampling) and its high costs, showed the same difficulties in the standardization already discussed in relation to the TST with the tuberculin [29, 43]. ESAT6 and CFP10, *M. tuberculosis* complex specific antigens, have also been used to improve IFNγ assay specificity, especially in population groups testing positive to the TST. The use of these antigens may also offer the ability to differentiate BCG-vaccinated from unvaccinated animals[22, 40].

In this test, the release of a lymphokine gamma interferon (IFN-γ) is measured in a whole-blood culture system. The assay is based on the release of IFN-γ from sensitized lymphocytes during a 16-24-hour incubation period with specific antigen (PPD-tuberculin) [44]. The test makes use of the comparison of IFN-γ production following stimulation with avian and bovine PPD. The detection of bovine IFN-γ 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 [45, 46].

In some areas, especially where 'nonspecificity' is prevalent, some concerns about the accuracy have been expressed. However, because of the IFN-γ test capability of detecting early infections, the use of both tests in parallel allows 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 [41].The use of defined mycobacterial antigens such as ESAT 6 and CFP-10 shows promise for improved specificity [47] and these antigens are employed in a number of countries such as the United Kingdom and New Zealand for serial testing.

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. It is a valuable complementary tool in order to identify possible energic cows that may be acting as reservoirs of the agent. 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 [48].

In order to diagnose cattle infected by *M. bovis*, antigens usually employed are the PPD and single or associated purified antigens from *M. bovis* such as antigens of the Ag85 that complex represents a major part of the secreted proteins and MPB70 and it highly homologous protein MPB83, secreted mycobacterial proteins with limited species distribution. Most of these antigens have achieved a sensitivity and specificity of around 90% and their recommendations are based on the existence of energic animals, as well as increased antibody titres in more advanced stages of the disease [40, 49].

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 [50].

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 radioactive 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 interlaboratory comparisons [18].

Futures of Bovine Tuberculosis in Ethiopia

Bovine Tuberculosis: the Status in Ethiopia: Ethiopia is one among the nations that possesses the largest livestock population in the African continent with an estimated 30-33 million cattle [51], 24 million sheep, 18 million goats and 7 million equines [52]. The distribution and the quantity of each species are different according to the type of prevailing animal production systems and agro-ecological zones. In contrast to the huge livestock resource, the livestock productivity is, however, found to be very low. The major biological and socio-economical factors attributing to the low productivity includes: the low genetic potential and performance, poor nutrition (in quality and quantity terms), the prevailing of different diseases, traditional way of husbandry systems and inadequate skilled manpower among others. Ethiopia is one of the African countries where BTB is considered as protruding disease in animals.

Detection of BTB in Ethiopia is carried out most commonly on the basis of tuberculin skin testing, abattoir meat inspection and rarely on bacteriological techniques. However, the current status on the actual prevalence rate of BTB at a national level is yet unknown. In Ethiopia, screening of cattle by the tuberculin skin test was sporadic until 1984. But, while the higher prevalence rate of the disease has been observed after the two year survey in government and some "parastatal" dairy farms, then it was decided to embark on a routine BTB survey, on these dairy farms, in particular using single and comparative intradermal skin tests [53]. Since then tuberculin skin test and abattoir meat inspection surveillances have been undertaken in different parts of the country at various times.

Most of the surveys carried out in Ethiopia have been based on tuberculin skin testing and abattoir inspection reports of animals in a particular locality. BTB is one of the endemic infectious diseases that have long been recorded in Ethiopia and the infection has been detected in cattle in Ethiopia and rarely in other species of domestic animals [53].

The disease is considered as one of the major livestock diseases that results in high morbidity and mortality. In Ethiopia, based on the detection of tuberculous lesions, condemnation of carcasses totally or partially is a standard practice for the control of zoonotic infections at abattoirs. Detection of tuberculous lesions in slaughterhouses takes place by observation of the visible tuberculous lesions in infected cattle; however, the level of the quality of such practices may vary from place to place and/or abattoir to abattoir in the country. Hence, the probability of carcasses to escape the abattoir inspection is likely to be high when a large number of animals are examined in large city abattoirs in particular. Very few studies in Ethiopia have indicated that not all cattle infected with *M. bovis* have visible tuberculous lesions at slaughter [54].

This may limit the sensitivity of this detection technique at abattoirs, although detection of tuberculous lesions through abattoir inspection is so far the common procedure in Ethiopia. In Ethiopia the routine abattoir inspection for any infection including BTB applies the method developed by the Meat Inspection and Quarantine. It involves visual examination and palpation of intact organs like the liver and kidney as well as palpation and incision of the head, lung and pleural lymph nodes. Other lymph nodes are incised if lesions are detected in one of these tissues [55].

Public Health Risks: Human tuberculosis due to *M. bovis* is usually underestimated or underdiagnosed because of no clinical, radiographicaland histopathological differentiation of tuberculosis caused by *M. tuberculosis* and *M. bovis* [56]. *M. bovis* is not the major cause of human tuberculosis but it can infect human beings too either by consuming raw milk, meat and their products from infected animals [57, 58], or by inhaling infective droplets or direct exposure to infected animals. In an estimate, about 10% cases of human tuberculosis are caused by *M. bovis*, while majority are caused by *M. tuberculosis* [56]. In countries where in milk is pasteurized and there is effective implementation of bovine tuberculosis programme tuberculosis in human due to *M. bovis* is very rare. But in areas where the disease in bovine is poorly controlled the reporting of the disease is more frequently done. In farmers as well as abattoir workers and others the incidence rate is higher.

Exposure to other species apart from cattle can cause infection in human. It has been documented that goats as well as seals, farmed elk and rhinoceros can also act as sources of bovine tuberculosis. A source of infection may be wildlife especially in countries where people use to take bush meat [58-60]. If the whole carcass is condemned then it indicates a high degree of tuberculosis infection and its transmission so it requires immediate attention from both the economic and public health point of view. [61]. Being cause of chronic granulomatous disease tubercle bacilli increases susceptibility to bladder and lung cancer. Though BCG induced cytotoxicity of bladder has paved the way towards initiation of BCG immunotherapy for treatment of bladder cancer [57].

CONCLUSIONS

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 is 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.Eradication programs based on tuberculin testing and subsequent slaughter of positive animals have been successful in many developed countries. However, a tuberculin test is limited in its specificity and sensitivity, so culture should be used to confirm the presence of *M. bovis*. Molecular techniques like PCR can also detect *M. bovis* directly in clinical samples. Moreover, genetic fingerprinting techniques (e.g. spoligotyping) can distinguish different strains of *M. bovis*.

REFERENCES

1. Le Roex, N., P.D. van Helden, A.P. Koets and E.G. Hoal, 2013. Bovine TB in livestock and wildlife: What's in the genes? Physiol. Genom., 45: 631-637.
2. Kuldeep, D., A.K. Verma, T. Ruchi, C. Sandip, V. Kranti, K. Sanjay, D. Rajib, K. Karthik, S. Rajendra, M. Muhammad and N. Senthilkumar, 2013. A perspective on applications of Geographical Information System (GIS): an advanced tracking tool for disease surveillance and monitoring in veterinary epidemiology. Advances in Animal and Veterinary Sciences, 1(1): 14-24.

3. Rodriguez-Campos, S., N.H. Smith, M.B. Boniotti and A. Aranaz, 2014. Overview and phylogeny of Mycobacterium tuberculosis complex organisms: implications for diagnostics and legislation of bovine tuberculosis. Research in Veterinary Science, 97: S5-S19.
4. Pesciaroli, M., J. Alvarez, M.B. Boniotti, M. Cagiola, V. Di Marco, C. Marianelli, M. Pacciarini and P. Pasquali, 2014. Tuberculosis in domestic animal species. Research in Veterinary Science, 97: S78-S85.
5. Macdonald, D.W., P. Riordan and F. Mathews, 2006. Biological hurdles to the control of TB in cattle: a test of two hypotheses concerning wildlife to explain the failure of control. Biological Conservation, 131(2): 268-286.
6. Hardstaff, J.L., G. Marion, M.R. Hutchings and P.C. White, 2014. Evaluating the tuberculosis hazard posed to cattle from wildlife across Europe. Research in Veterinary Science, 97: S86-S93.
7. Zegeye, E.D., 2013. Biochemical Characterization of the DNA Helicases RecG, XPB and DinG in Mycobacterium tuberculosis.
8. Ameni, G., M. Vordermeier, R. Firdessa, A. Aseffa, G. Hewinson, S.V. Gordon and S. Berg, 2011. Mycobacterium tuberculosis infection in grazing cattle in central Ethiopia. The Veterinary Journal, 188(3): 359-361.
9. Birhanu, T., E. Mezgebu, E. Ejeta, A. Gizachew and E. Nekemte, 2015. Review on diagnostic techniques of bovine tuberculosis in Ethiopia. Report and Opinion, 7: 7-14.
10. Smith, N.H., K. Kremer, J. Inwald, J. Dale, J.R. Driscoll, S.V. Gordon, D. van Soolingen, R.G. Hewinson and J.M. Smith, 2006. Ecotypes of the Mycobacterium tuberculosis complex. Journal of Theoretical Biology, 239(2): 220-225.
11. Atkins, P.J. and P.A. Robinson, 2013. Bovine tuberculosis and badgers in Britain: relevance of the past. Epidemiology & Infection, 141(7): 1437-1444.
12. Upadhayay, U.P.P.D.D. and P.C.V. Vishwavidyalay, 2014. Insights into bovine tuberculosis (bTB), various approaches for its diagnosis, control and its public health concerns: an update. Asian Journal of Animal and Veterinary Advances, 9(6): 323-344.
13. Garner, G., P. Saville and A. Fediaevsky, 2004. Manual for the recognition of exotic disease of livestock. A reference guide for animal healthy staff (on line).
14. UNE, Y. and T. MORI, 2007. Tuberculosis as a zoonosis from a veterinary perspective. Comparative Immunology, Microbiology and Infectious Diseases, 30(5-6): 415-425.
15. Cousins, D.V. and J.L. Roberts, 2001. Australia's campaign to eradicate bovine tuberculosis: the battle for freedom and beyond. Tuberculosis, 81(1-2): 5-15.
16. Radostits, O., D. Blood and C. Gray, 2007. Veterinary Medicine, a Text Book of the Diseases of cattle, sheep, Pigs, Goats and Houses. 8th ed., London: Ballier Tindals, pp: 830-838.
17. OIE, 2009. 'Bovine tuberculosis', in Manual of diagnostic tests and vaccines for terrestrial animals. Viewed 11 July2013, fromTerrestrial Manual, pp: 1-16.
18. Regassa, A., A. Tassew, K. Amenu, B. Megersa, F. Abuna and B. Mekibib, 2010. A cross-sectional study on bovine tuberculosis in Hawassa town and its surrounding Southern Ethiopia: Tropical Animal Health Productio, 42: 915-920.
19. Sharma, R. and V. Gupta, 2011. Spoligotyping for the detection of mycobacterium tuberculosis complex bacteria. Asian J. Biochem, 6: 29-37.
20. Iwnetu, R., J. Van Den Hombergh, Y. Woldeamanuel, M. Asfaw and C. Gebrekirstos, 2009. Comparative Performance of Diagnostic Tests for MycobacterialLymphadenitis in a High-Burden Country. Scand J. Infect Dis., 41: 462-468.
21. Van Soolingen, D., 2008. Molecular Epidemiology of Tuberculosis and other mycobacterial infections: Main methodologies and achievements. J. Vet. Diagn. Invest., 249: 1-26.
22. Fentahun, T. and G. Luke, 2012. Diagnostic techniques of bovine tuberculosis: a review. African Journal of Basic and Applied Sciences, 4(6): 192-199.
23. Wood, P. and S. Jones, 2009. An in vitro cellulardiagnostic test for Bovine TB, Animal Health, Parkville, Victoria, Australia, 81: 147-155.
24. Kubica, T., R. Agzamova, A. Wright, G. Rakishev, S. Rushgerdes and S. Niemann, 2006. Mycobacterium bovis isolates with Mycobacterium tuberculosis specific characteristic. Global Veterinaria, 2: 146-218.
25. Varges, R., C.D. Marassi, W. Oelemann and W. Lilenbaum, 2009. Interference of intradermal tuberculin tests on the serodiagnosis of paratuberculosis in cattle. Research in Veterinary Science, 86(3): 371-372.

26. Probst, C., C. Freuling, I. Moser, L. Geue, H. Köhler, F.J. Conraths, H. Hotzel, E.M. Liebler-Tenorio and M. Kramer, 2011. Bovine tuberculosis: making a case for effective surveillance. Epidemiology & Infection, 139(1): 105-112.
27. Rieder, H.L., A. Van Deun, K.M. Kam, S.J. Kim, T.M. Chonde, A. Trebucq and R. Urbanczik, 2007. Priorities for tuberculosis bacteriology services in low-income countries. 2nd edition International Union against Tuberculosis and Lung Disease, Paris, France, pp: 28-36.
28. Biet, F., M.L. Boschiroli, M.F. Thorel and L.A. Guilloteau, 2005. Zoonotic aspects of Mycobacterium bovis and Mycobacterium avium-intracellulare complex (MAC). Veterinary Research, 36(3): 411-436.
29. Schiller, I., B. Oesch, H.M. Vordermeier, M.V. Palmer, B.N. Harris, K.A. Orloski, B.M. Buddle, T.C. Thacker, K.P. Lyashchenko and W.R. Waters, 2010. Bovine tuberculosis: a review of current and emerging diagnostic techniques in view of their relevance for disease control and eradication. Transboundary and Emerging Diseases, 57(4): 205-220.
30. Quinn, P.J. and B.K. Markey, 2003. Concise review of Veterinary Microbiology. UK: Blackwell Publishing, pp: 34-37.
31. Patterson, J. and D. Grooms, 2000. Diagnosis of bovine tuberculosis Gross necropsy, histopathology and acid fast staining. Michigan State University Extension, 35: 1-2.
32. Vargas, D., L. García, R.H. Gilman, C. Evans, E. Ticona, M. Ñavincopa, R.F. Luo, L. Caviedes, C. Hong, R. Escombe and D.A. Moore, 2005. Diagnosis of sputum-scarce HIV-associated pulmonary tuberculosis in Lima, Peru. The Lancet, 365(9454): 150-152.
33. Sharma, S.K., A. Mohan, A. Sharma and D.K. Mitra, 2005. Miliary tuberculosis: new insights into an old disease. Lancet Infect Dis., 5: 415-430.
34. Lee, E. and R.S. Holzman, 2002. Evolution andcurrent use of the tuberculin test. ClinInfect Dis., 34: 365-370.
35. Hirsh, C. and C. Zee, 2000. Mycobacteria species; the agent of animal tuberculosis. Veterinary Microbiology. Blackwell Science, pp: 158-164.
36. Andrews, A., 2003. Bovine medicine, 2nd Ed, UK: Blackwell Science, Color of Atlas for the Diagnosis of Bacterial pathogens in animals, Germany: Paul Parey Scientific Publishers, pp: 108-118.
37. Mamo, G., F. Abebe, Y. Worku, N. Hussein, M. Legesse, G. Tilahun, G. Medhin, G. Bjune and G. Ameni, 2013. Bovine tuberculosis and its associated risk factors in pastoral and agro-pastoral cattle herds of Afar Region, Northeast Ethiopia. Journal of Veterinary Medicine and Animal Health, 5(6): 171-179.
38. Diaz, F., 2012. Manual of diagnostic tests and vaccines for terrestrial animals.
39. Whelan, A.O., J.C. Hope, C.J. Howard, D. Clifford, R.G. Hewinson and H.M.Vordermeier, 2003. Modulation of the bovine delayed-type hypersensitivity responses to defined mycobacterial antigens by a synthetic bacterial lipopeptide. Infect. Immun., 71: 6420-6420.
40. Faye, S., J.L. Moyen, H. Gares, J.J. Benet, B. Garin-Bastuji and M.L. Boschiroli, 2011. Determination of decisional cut off values for the optimal diagnosis of bovine tuberculosis with a modified IFNgamma assay (Bovigam®) in a low prevalence area in France. Veterinary Microbiology, 151(1-2): 60-67.
41. Gormley, E., M.B. Doyle, T. Fitzsimons, K. McGill and J.D. Collins, 2006. Diagnosis of Mycobacterium bovis infection in cattle by use of the gamma-interferon (Bovigam) assay. Veterinary Microbiology, 112(2-4): 171-179.
42. Alito, A., J. Mcnair, R.M. Girvin, M. Zumarraga, F. Bigi, J.M. Pollock and A. Cataldi, 2003. Identification of Mycobacterium bovis antigens by analysis of bovine T-cell responses after infection with a virulent strain. Brazilian Journal of Medical and Biological Research, 36(11): 1523-1531.
43. Vordermeier, H.M., A. Whelan and G. Hewinson, 2008. The scientific case for the gamma interferon "BOVIGAM™" assay. Government Veterinary Journal, 19: 38-43.
44. Wood, P.R., L.A. Corner, J.S. Rothel, J.L. Ripper, T. Fifis, B.S. McCormick, B. Francis, L. Melville, K. Small, K. De Witte and J. Tolson, 1992. A field evaluation of serological and cellular diagnostic tests for bovine tuberculosis. Veterinary Microbiology, 31(1): 71-79.
45. Schiller, I., H.M. Vordermeier, W.R. Waters, A.O. Whelan, M. Coad, E. Gormley, B.M. Buddle, M. Palmer, T. Thacker, J. McNair and M. Welsh, 2010. Bovine tuberculosis: effect of the tuberculin skin test on in vitro interferon gamma responses. Veterinary Immunology and Immunopathology, 136(1-2): 1-11.

46. Buddle, B.M., T.J. Ryan, J.M. Pollock, P. Andersen and G.W. de Lisle, 2001. Use of ESAT-6 in the interferon-γ test for diagnosis of bovine tuberculosis following skin testing. Veterinary Microbiology, 80(1): 37-46.
47. Buddle, B.M., D.N. Wedlock and M. Denis, 2006. Progress in the development of tuberculosis vaccines for cattle and wildlife. Veterinary Microbiology, 112(2-4): 191-200.
48. Corbett, E.L., C.J. Watt, N. Walker, D. Maher, B.G. Williams, M.C. Raviglione and C. Dye, 2003. The growing burden of tuberculosis: global trends and interactions with the HIV epidemic. Arch Intern Med., 163: 1009-1021.
49. Welsh, M.D., R.T. Cunningham, D.M. Corbett, R.M. Girvin, J. Mcnair, R.A.D.G. Skuce, Bryson and J.M. Pollock, 2005. Influence of pathological progression on the balance between cellular and humoral immune responses in bovine tuberculosis. Immunology, 114(1): 101-111.
50. Lilenbaum, W., D.C. Marassi and S.L. Medeiros, 2011. Use of MPB-70 ELISA as a complementary test for bovine tuberculosis in the field in Brazil. Journal of British Veterinary Association, 168: 167-168.
51. Shitaye, J.E., W. Tsegaye and I. Pavlik, 2007. Bovine tuberculosis infection in animal and human populations in Ethiopia: a review. Veterinarni Medicina-praha, 52(8): 317.
52. Admasu, P., W. Berihun and A. Niguse, 2014. Prevalence of bovine tuberculosis in dairy cattle of Yeki District, Southern Ethiopia. Afr J. Basic Appl. Sci., 6(5): 135-40.
53. Shitaye, J.E., B. Getahun, T. Alemayehu, M. Skoric, F. Treml, P. Fictum, V. Vrbas and I. Pavlik, 2006. A prevalence study of bovine tuberculosis by using abattoir meat inspection and tuberculin skin testing data, histopathological and IS6110 PCR examination of tissues with tuberculous lesions in cattle in Ethiopia. Veterinarni Medicina-praha, 51(11): 512.
54. Asseged, B., Z. Woldesenbet, E. Yimer and E. Lemma, 2004. Evaluation of abattoir inspection for the diagnosis of Mycobacterium bovis infection in cattle at Addis Ababa abattoir. Tropical Animal Health and Production, 36: 537-546.
55. Teklu, A., B. Asseged, E. Yimer, M. Gebeyehu and Z. Woldesenbet, 2004. Tuberculous lesions not detected by routine abattoir inspection: the experience of the Hossana municipal abattoir, southern Ethiopia. Revue Scientifiqueet Technique-Office International Des Epizooties, 23(3): 957-964.
56. Pérez-Lago, L., Y. Navarro and D. García-de-Viedma, 2014. Current knowledge and pending challenges in zoonosis caused by Mycobacterium bovis: a review. Research in Veterinary Science, 97: S94-S100.
57. Upadhayay, U.P.P.D.D. and P.C.V. Vishwavidyalay, 2014. Insights into bovine tuberculosis (bTB), various approaches for its diagnosis, control and its public health concerns: an update. Asian Journal of Animal and Veterinary Advances, 9(6): 323-344.
58. Malama, S., J.B. Muma and J. Godfroid, 2013. A review of tuberculosis at the wildlife-livestock-human interface in Zambia. Infect. Dis. Poverty, pp: 2.
59. Etter, E., P. Donado, F. Jori, A. Caron, F. Goutard and F. Roger, 2006. Risk analysis and bovine tuberculosis, a re-emerging zoonosis. Annals of the New York Academy of Sciences, 1081(1): 61-73.
60. Evans, J.T., E.G. Smith, A. Banerjee, R.M. Smith, J. Dale, J.A. Innes, D. Hunt, A. Tweddell, A. Wood, C. Anderson and R.G. Hewinson, 2007. Cluster of human tuberculosis caused by Mycobacterium bovis: evidence for person-to-person transmission in the UK. The Lancet, 369(9569): 1270-1276.
61. Torgerson, P.R. and D.J. Torgerson, 2010. Public health and bovine tuberculosis: what's all the fuss about?. Trends in Microbiology, 18(2): 67-72.