Academic Journal of Animal Diseases 7(3): 59-67, 2018
ISSN 2079-200X

DOI: 10.5829/idosi.ajad.2018.59.67

A Review on Bovine Tuberculosis and its Diagnostic Approaches

Tesfahun Demeke Dana, Fitsum Dulo, Arega Tafere and Samuel Hayesso

Wolaita Sodo University, School of Veterinary Medicine, Wolaita Sodo, Ethiopia

Abstract: Bovine tuberculosis is recognized as one of the most important threats to human and animal health causing significant mortality, morbidity and economic losses in the world, particularly in developing nations. *M. bovis* is very closely related to *M. Tuberculosis* but causes tuberculosis in cattle and other animals as well as in humans. It is spread by respiratory aerosols between animals and from animals to humans. The commonest clinical signs include fever, chills,weight loss, loss of appetite and chronic cough but other symptoms present depending on the organs involved. Its pathology is characterized by the formation of granulomatous lesions, which can within the course of the disease regress or exhibit extensive necrosis, calcify or liquefy and subsequently lead to cavity formation. Many methods exist to diagnose cattle suffering from tuberculosis. Among them in vivo test like tuberculin test, bacterial culture, postmortem examination, enzyme linked immunosorbent assay (ELISA) and it is tentatively diagnosed by clinical sign. In recent years new methods have been developed for the rapid diagnosis of active TB, however the best alternative being the molecular or genotypic techniques. Controlling tuberculosis caused by *M. bovis* in its principal reservoir and cattle, is the best method of preventing transmission to other species, including man.

Key words: Bovine Tuberculosis · Diagnosis · *M. bovis*

INTRODUCTION

Bovine Tuberculosis is a highly infectious bacterial zoonosis, which is recognized as one of the most important threats to human and animal health causing significant mortality, morbidity and economic losses in the world, particularly in developing nations [1, 2]. This disease is a prominent disease found in cattle, is caused by *M. bovis*, which is a member of Mycobacterium tuberculosis complex (MTBC). A member of the Mycobacterium tuberculosis complex (MTBC) includes *M. tuberculosis*, *M. africanum*, *M. bovis*, *M. canetti*, *M. microti*, *M. caprae* and *M. pinnipedii* [3, 4]. *M. bovis* is very closely related to *M. tuberculosis* but causes tuberculosis in animals and humans. *M. bovis* is spread by respiratory aerosols between animals and from animals to humans. *M.bovis* can also be transmitted to humans by milk and, to a lesser extent, by meat from tuberculous animals [5]. As [6] referred that, in most species, the disease is characterized by formation of granulomatous nodules called tubercles, caseation and calcification in the parenchyma of affected organ.

There are several methods has being used for the diagnosis of bovine tuberculosis where as the standard method for the detection of bovine tuberculosis in live animal is the tuberculin test (the prescribed test for international trade), which involves the intradermal injection of bovine tuberculin purified protein derivative (PPD) and the subsequent detection of swelling (delayed hypersensitivity) at the site of injection 72 hours later [7].

In many countries, routine meat inspection forms part of the surveillance programme for bovine tuberculosis. Tuberculin testing followed by isolation and slaughter of reactor has been implemented as the basis of eradication programme. However, wild animals which act as important reservoir of bacilli may pose a serious threat in control and eradication programme [8]. The present paper delineates the organism's characteristics, pathogenesis, clinical feature, epidemiology, diagnosis and management of *M. bovis* which is a bacterial zoonosis of global significance.

The Organism and its Characteristics: Mycobacterium species are generally non-fastidious, Gram-positive, non spore forming, pleomorphic aerobes 1-4 μm in length.

Corresponding Author: Tesfahun Demeke, Wolaita Sodo University, School of Veterinary Medicine, Wolaita Sodo, Ethiopia. Tel +251932660888; E-mail: tesfahundemeke0@gmail.com.

M. bovis is mesophilic and is not heat-resistant, being readily killed by normal milk pasteurization conditions [5], Tuberculous mycobacteria are alcohol and acid-resistant. This Mycobacterial species are also resistant to many disinfectants, desiccation and other adverse environmental factors because the cell wall has high lipid content [9].

Pathogenesis and Clinical Feature: The Organism may enter in to the host through respiratory, alimentary, cutaneous and genital routes. It may be self-limiting to fulminating disease with extensive tissue destruction [8]. When the organism enter through respiratory tract; the bacilli are taken by alveolar macrophages to the circulation and establishes in the lymph nodes. Cellular response attempting to control the disease results in accumulation of large number of phagocytes and lead to the formation of a macroscopic lesion referred as tubercle. Cell mediated immunity (CMI) emerges 10 -12 days after infection and triggers release of cytokines from T-lymphocytes that activate the bacteriostatic effect of macrophages and accelerates the recruitment of additional blood borne mononuclear cells into the site resulting into delayed type of hypersensitivity reaction. The gross appearance of the tubercle is usually firm yellow and rarely a yellowish caseous necrotic material or calcified tissue is observed [8, 10].

Tubercles are typical granulomas containing a central core of caseous necrotic tissue. The tubercles are typically pale orange in color. The centers of tubercles may later calcify. When bacteria escape from this original focal point, they can spread to other parts of the body via the lymphatic ducts and lymph nodes or the blood stream. If many organisms find their way into the bloodstream in this way, general dissemination throughout the body takes place and multiple lesions are formed which can lead to toxemia, debility, weakness and death. Sometimes lesions are limited to such an extent by the dense connective tissue that further spread does not take place and the disease is limited to that area. Lesions usually also develop in the lymph nodes, which drain the lymph of the affected part of the body or organ and therefore the lymph nodes are usually examined to determine the presence of TB [11].

The interactions between host and pathogen within granuloma determine the disease outcome. Based on microscopic evaluation of cellular composition of granulomas, morphological distinct of granulomas is categorized into four stages as stage I (initial), stage II (solid), stage III (necrotic) and stage IV (necrotic and mineralized). Such classification of granuloma and the diverse cytokine production at different stages of granuloma may facilitate to understand bovine TB pathogenesis [12, 13].

Animals with advanced stage IV granulomas revealed high reactivity to IFN-γ and TGF-β in caseous necrosis areas, whereas animals with stage III granuloma also revealed high reactivity to IFN- γ, but moderate reactivity to TNF-á, IL-10 and TGF-β. The stage I and stage II granulomas were found in few bovines and exhibited low cytokine production [14].

M. bovis is an intracellular bacteria which can induce both pulmonary and extra pulmonary symptoms in humans and animals [15]. The symptoms of bovine tuberculosis usually take three weeks or months to develop in cattle [16]. In early stage of disease, symptoms are absent and asymptomatic. However, in late stage, there is progressive emaciation, a mild fluctuating fever, weakness and inappetence [17].

An animal which is infected with this strain of mycobacterium can show different symptoms depending on where the infection is taking place. If the infection is in the GI tract for example a main symptom is extreme abominable discomfort whereas an infection in the lungs causes dyspnoea, moist cough or trachypnoea may occur. In the terminal stage, animal become extremely emaciated and develop acute respiratory distress [17, 18].

Epidemiology: The distribution of Bovine TB is worldwide and it is found in all continents except in Antarctica [19]. Although bovine tuberculosis was once found worldwide, control programs have eliminated or nearly eliminated this disease from domesticated animals in many countries. Nations currently classified as tuberculosis-free include Australia, Iceland, Denmark, Sweden, Norway, Finland, Austria, Switzerland, Luxembourg, Latvia, Slovakia, Lithuania, Estonia, the Czech Republic, Canada, Singapore, Jamaica, Barbados and Israel. Eradication programs are in progress in other European countries, Japan, New Zealand and the United States [20].

However in several countries bovine TB remains a major, costly infectious disease of cattle and other domesticated animals such as, feral and wild animal populations, including badgers, possums, deer, goats, sheep, camelids and the disease poses a significant problem to the economy of the livestock sub-sector and remains a potential public health threat in developing countries where controlling programs are lacking. In Africa, approximately 85% of cattle and 82% of human population lives in areas where BTB is partly or not controlled at all in animals [21, 22].

Several factors are mentioned for distribution of BTB in Africa, but the major one is distribution of the domestic ruminant population and dairy production varies within the ecological zones of Africa. This factor, together with the differences in the production systems under which livestock are managed on the African continent (i.e. pastoralism, 'agro-pastoralism', mixed farming and intensive dairy farming), may have a significant influence on the distribution of animal tuberculosis [23]

The occurrence of tuberculosis within a country varies widely between different areas. South American reports show that the highest levels of tuberculosis circumstance are in the surrounding areas of larger cities where intensive dairy production is most common [24]. Bovine TB tends to be a low-incidence infectious disease with an apparently low transmission rate in countries where advanced test and control programmes (a comprehensive set of surveillance and control measures to address cattle-cattle transmission) [25].

Transmission of the disease among cattle can be either direct, through close contact, or indirect from exposure to viable bacteria in a contaminated environment [26]. Direct' routes of transmission require close and mostly sustained contact with an infectious case, whereas 'indirect' routes would include transmission via, for example, a contaminated external or internal environment, contaminated feed, water, equipment etc. On balance, direct ('speaking distance') contact would seem to be farm more significant than transmission potentially supported by 'indirect' routes [25].

Cattle serve as the principal reservoir of *M. bovis*. Human can be infected with *M. bovis* is mainly through the consumption of raw milk and milk products and less frequently through air route. Children are most commonly affected with *M. bovis.* The organism infects tonsils present as "scrofula" (Cervical lymphadenitis) or intestinal tract causing abdominal tuberculosis. The extrapulmonary site of *M. bovis* is usually intestinal tract, kidneys, brain and bones [8].

Bovine Tuberculosis Diagnostic Approaches

Clinical Diagnosis: Bovine tuberculosis is tentatively diagnosed by its clinical symptoms [27]. Clinical signs 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 [16, 20]. Clinical signs such as low-grade fever, chronic debility, moist cough and enlargement of local lymph nodes may suggest infection with TB [28].

Post Mortem Diagnosis: During post mortem examination, the lymph nodes especially of those associated with head, thorax and abdomen are closely examined for the identification of tubercle [8, 29]. The gross pathological change in the carcass is characterized by progressive development of tubercles in any tissue/organ of the body called granuloma [2, 6 & 30].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 common in the lungs, spleen, liver and the surfaces of body cavities [31].

Tuberculin Skin Test: Tuberculin skin test comprises intradermal injection of *M. bovis* purified protein derivative (PPD) tuberculin and the subsequent detection of a swelling (delayed hypersensitivity) at the site of injection three days later [32]. The tuberculin skin test (TST) has been a useful diagnostic and epidemiological tool in live animal for control of bovine tuberculosis for several decades [33]. The injectable solution is known as tuberculin, tuberculin is a solution of protein material extracted from the cell wall of the Mycobacteria organism. When this protein solution is injected into the skin of an infected animal, the body's sensitized immune response will cause a localized inflammatory reaction that leads to the typical signs of a positive tuberculin test. Animals that have not been exposed to tuberculosis will not mount an inflammatory reaction [11].

Comparative Intra-Dermal (CID) Test: Comparative intra-dermal test is taken in the mid neck, 10-12cm apart is shaved and the thickness is measured in millimeters with caliper before the injection of tuberculin [34]. In the CID test, 0.1ml of avian PPD and 0.1ml of bovine PPD 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. When the change in skin thickness is greater at the avian PPD injection site, the result is considered negative for Bovine tuberculosis and if the change in skin thickness is lesser at the PPD injection site, the result is considered positive for Bovine tuberculosis [35].

Caudal Fold Tuberculin (CFT) Test: The CFT test is the primary screening test used to identify cattle infected with bovine TB. This test measures the immune response to *M. bovis* using an intradermal **l** injection of a Purified Protein Derivative (PPD) of tuberculin into the skin of the caudal fold (the fold of skin at the base of the tail). If the animal has been exposed to mycobacteria, the immune system responds with inflammatory cells at the injection site, causing swelling and/or discoloration of the skin. The veterinarian evaluates the response to the CFT injection by inspecting and palpating the injection site 72 hours later. Any abnormality at the injection site classifies the animal as a responder [36].

Gamma Interferon Test: Gamma interferon test 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 [37].

Enzyme-Linked Immunosorbent Assays (ELISA): 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 [7, 37].The indirect ELISA technique measures the binding of specific antibodies to an antigen [38, 39]. Antigens usually employed to diagnose cattle infected by *M. bovis* 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 is highly homologous protein MPB83, secreted mycobacterial proteins with limited species distribution. 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 [39]. The ELISA is relatively inexpensive and can be easily automated to process large numbers of samples [40].

Lymphocyte Proliferation Assay: Lymphocyte Proliferation,It is an in-vitro assay which 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 shortly after blood collection. 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 radioactive nucleotides [39].

Bacterial Isolation: M. bovis can be isolated by culturing on media, Lowenstein-Jensen medium can be used for isolation of mycobacterium. 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 [41].

In the determination of mycobacterial infections, culture is still considered the international gold standard. However, due to dysgenic and slow growth characteristics, the identification of *M. bovis* by culture and biochemical methods is cumbersome and time consuming. Furthermore, application of molecular techniques is expensive as it demands availability of adequate laboratory resources and trained personnel [42, 43].

Histopathological Diagnosis: In the course of postmortem examination of cattle suspected of being infected with BTB, tissue samples are collected and examined for histopathological (microscopic) lesions that are compatible with M. bovis [44]. The sample for histopatology examination can be taken from the following: fine needle aspiration of lymph nodes, affected peripheral lymph node particularly cervical nodes can be aspirated [37]. The presumptive diagnosis of mycobacteriosis can be made if the tissue has characteristic histological lesions such as caseous necrosis, mineralisation, epithelioid cells, multinucleated giant cells and macrophages [45, 46].

Moreover to looking for specific lesions under the microscope, pathologist can use special stain in order to identify organisms that are compatible with M. bovis, the bacterium that causes BTB. This is called an acid-fast stain. This Acid -fast stain is used to diagnose the presence of acid fast bacilli (AFB) in clinical specimens by using Ziehl-Neelsen staining followed by light microscopy or auramine O staining and fluorescence microscopy [47].

Molecular Diagnosis: The sequences of usual tests for *M. bovis* can be slow, bulky, erroneous, not reproducible and time-consuming, can give indefinite result and cannot be performed in any laboratory. However, molecular technics like PCR can also detect *M. bovis* directly in clinical samples. PCRhas been successfully applied to detect members of the *M. tuberculosis* complex and is especially useful for the direct detection of *M. bovis* in bovine tissue samples [48, 39]. Furthermore, genetic fingerprinting techniques (e.g. spoligotyping) can distinguish different strains of *M. bovis* [39].

Spoligotyping: Spoligotyping also called spacer oligonucleotides typing is a novel method for simultaneous detection and typing of mycobacterium tuberculosis complex bacteria, has been recently developed. This method is based on 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 [49].

Hence the clinical efficacy 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 [31]. Spoligotyping is highly sensitive and highly specific, thus the specificity and sensitivity has been found to be 98 and 96%, respectively with the clinical samples [50]. The advantage of this technique is its speed, reliability and species differentiation of *M. tuberculosis* and *M. bovis*. It also enables the molecular study of spoligotypes geographical dispersion [51, 39].

Variable Number Tandem Repeat (VNTR): Tandem repeats are allelic variations that occur as repetitions of sequences of nucleotides found in intergenic regions of the genome. These repeated sequences are known in eukaryotic cells. The hyper variability of these repetitions in human and animal cells generates the Variable number tandem repeat (VNTR). In the diagnosis of mycobacterial isolates, the polymorphism may be analyzed by repetitions of sequences of nucleotides, or even variation in the number of repeating units [52, 53]. 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 [54]. The usefulness of this technique has not yet been fully being assessed for M.*bovis* although its evaluation is underway for isolates from 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 [49].

Restriction Fragment Length Polymorphism (RFLP): It is considered as a gold standard 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 [44]. Strain differentiation by using RFLP analysis has proven to be a very useful tool for epidemiologic studies of tuberculosis. RFLP based on the presence of the insertion sequence IS6110 has been widely used as a genetic marker [55].The insertion sequence IS6110 is considered specific to the members of the M. tuberculosis complex and the difference in location and number of copies of the insertion sequence is a source of polymorphism between isolates [51].

Restriction fragment length polymorphism (RFLP) method utilizing the hupB gene, encoding a histone-like protein of M. tuberculosis, as a target for detection and identification of M. tuberculosis and M. bovis from other members of the MTC and non-tuberculous mycobacterial. and non-mycobacterial species [56]. One limitation of the RFLP-based typing systems is that they require a well-grown culture for DNA extraction. The time lag between

isolation of M. bovis from a clinical sample and the growth of a mycobacterial culture is often too long which takes 20 to 40 days to obtain sufficient DNA needed. This problem can be circumvented with the use of several complementary biomarkers such as those based in the polymorphic IS6110 region and by using direct repeats (DR) and polymorphic GC-rich repeat sequences (PGRS) as probes [57, 58 & 59].

Management: Evidence is strong that control measures against zoonotic BTB, where applied rigorously, work well even if the disease in livestock persists, e.g. where there is a wildlife reservoir in the environment and re-infection. For effective control practices of BTB, infected cattle need to be identified accurately in the early stages of the disease. [60] Best management practices are largely focused on reducing the incidence in livestock (preferably eradication) [42, 61], through test and slaughter policies, movement controls and on improving hygiene, through meat inspection at slaughter and control of the food chain to ensure meat and milk are safe. There have also been great successes with BTB control in New Zealand where culling of wildlife coupled with control in livestock has proved largely effective [62]. Also, appropriate diagnosis of the disease and provision of useful information for use by public health and agricultural officials is important in the control practices of bovine tuberculosis [63]. These are not all possible in poor settings. The current best practices for poor communities includes such as education and awareness about; health risks of contact with and eating, uncooked meat and unpasteurized milk from infected livestock (and bush meat) and an appreciation of the density dependent nature of the disease and its association with poor nutrition and/or co-infection [64].

CONCLUSIONS

Bovine tuberculosis is the most important disease of intensification with a serious effect on animal production and also has a significant public health importance. Eradication programs based on tuberculin testing and subsequent slaughter of positive animals have been successful in many developed countries. On the contrary, this disease is still common in developing countries and severe economic losses can occur from livestock deaths, chronic disease and trade restrictions.

Based on the above conclusion, the following recommendations are forwarded:-

- Health risks of contact with and eating, uncooked meat and unpasteurized milk from infected livestock should be avoided.
- Further research should be done on knowledge gaps in the epidemiology of BTB in mixed species systems through one health approach
- Government should widen the availability and accessibility of effective diagnostic techniques as much as possible.
- Improving biosecurity of sedentary herds (reduced infection) in rural areas and where possible testing and BCG vaccination to reduce spread.

REFERENCES

1. Pal, M., N. Zenebe and M.T. Rahman, 2014. Growing significance of Mycobacterium bovis in human health. Microbes Infect., 3: 21-34.
2. Alemu, J., G. Mamo, V. Kandi and M. Pal, 2017. Prevalence of Tuberculosis in Gambella Regional Hospital, Southwest Ethiopia: A Retrospective Study to Assess the Progress towards Millennium Development Goals for Tuberculosis (2006-2015). Am J. Public Health Res., 5: 6-11.
3. Du, Y., Y. Qi, L. Yu, J. Lin, S.Liu, H. Ni, H. Pang, H. Liu, W. Si, H. Zhao and C. Wang, 2011. Molecular characterization of Mycobacterium tuberculosis complex (MTBC) isolated from cattle in northeast and northwest China. Res. Vet. Sci., 90, 385-391.
4. Alhaji, I., 2009. Mycobacteriological study of bovine granulomatain four Northern states of its environmental implications in the Niger Delta. Toxicol. Environ. Chem., 91: 619-25.
5. Adam, M.R. and M.O. Moss, 2008. Food Microbiology. 3rd ed. Royal Society of Chemistry, Cambridge, pp: 231-233.
6. Terefe, D., 2014. Gross pathological lesions of bovine tuberculosis and efficiency of meat Inspection procedure to detect infected cattle in Adama municipal abattoir. JVMAH 6: 48-53.
7. OIE, 2009.Bovine tuberculosis: Terrestrial manual., pp: 1-16.
8. Pal, M. and B.G. Boru, 2012. Zoonotic significance of Mycobacterium bovis. J. Natural Hist., 8: 86-89.
9. Acha, P.N. and B. Szyfres, 2001. Zoonotic Tuberculosis. In: Zoonoses and Communicable Diseases Common to Man and Animals: Bacterioses and Mycoses. Pan American Health Organization, Washington, D.C. 3rd ed. pp: 285-99.

10. Quinn, P.J., M.E. Carter, G.R.Markey and G.R. Carter, 1995. Clinical Veterinary Microbiology. Equine Veterinary Journal, 27: 1.
11. AFFRSA., 2006. Agriculture, Forestry and fisheries Republic of South Africa Bovine Tuberculosis Manual.
12. Wangoo, A., L. Johnson, J. Gough, R. Ackbar and S. Inglut, 2005. Advanced granulomatous lesions in Mycobacterium bovis-infected cattle are associated with increased expression of type I procollagen, gammadelta (WC1+) T cells and CD 68+ cells. J Comp Pathol., 133: 223-234.
13. Palmer, M.V., W.R. Waters and T.C. Thacker, 2007. Lesion development and immuno histochemical changes in granulomas from cattle experimentally infected with Mycobacterium bovis. Vet. Pathol., 44: 863-874.
14. Mehta, P.K., R. Dharra and Z. Thakur, 2017. Cattle as Experimental Model to Study Immunopathogenesis of Tuberculosis. Mycobact Dis., 7:248. Microbiology.Mosby International Publishers, London.
15. Fitzgerald, S.D. and J.B. Kaneene, 2013. Wildlife reservoirs of bovine tuberculosis worldwide: hosts, pathology, surveillance and control. Vet. Pathol., 50: 488-499.
16. Palmer, M.V., M.D. Welsh and J.M. Hostetter, 2011. Mycobacterial diseases of animals, Vet. Med. Int., 5: 1-2.
17. Van Rhijn, I., J. Godfroid, A. Michel and V. Rutten, 2008. Bovine Tuberculosis as a Model for Human Tuberculosis: Advantages over Small Animal Models. Microbes and Infection, 10, 711-715. http://dx.doi.org/10.1016/j.micinf.2008.04.005
18. Koehler, A., 2010. Mycobacterium bovis. Microbe wiki, n.d. Web. 18 Feb. 2010. Available at [http://microbewiki.kenyon.edu/index.php/Mycobacterium_bovis] (accessed on March, 2018).
19. Verma, A.K., R. Tiwari, S. Chakraborty, Neha, M. Saminathan, K. Dhama and S.V. Singh, 2014. Insights into Bovine Tuberculosis (BTB), Various Approaches for Its Diagnosis, Control and Its Public Health Concerns: An Update. AJAVA., 9: 323-344.
20. Center for Food Security and Public Health (CFSPH), 2009.Bovine tuberculosis
21. Carslake, D., W. Grant and L.E. Green, 2011. Endemic cattle diseases: comparative epidemiology and governance Philos. Trans. Royal Soc. B., 366(1573): 1975-1986.
22. Cosivi, O., J.M. Grange, C.J. Dabron, M.C. Raviglione, T. Fujikura, D. Cousins, R.A. Robinson, H.F. Huchzermeyer, I. de Kantor and F.X. Meslin, 1998. Zoonotic tuberculosis due to Mycobacterium bovis in developing countries. Emerg. Infect. Dis., 4: 1-17.
23. Cosivi, O., F.X. Meslin, C.J. Daborn and J.M. Grange, 1995. Epidemiology of Mycobacterium bovis infection in animals and humans, with (particular reference to Africa Rev. sci. tech. Off. int. Epiz., 14(3): 733-746.
24. Szyfres, B., 1972. The status of animal tuberculosis in the Americas. In First International Seminar on Bovine Tuberculosis for the Americas. Santiago, Chile, 21-25 September 1970. Pan American Health Organization (PAHO) Scientific Publication No. 258. PAHO, Washington, D, 27-43.
25. Skuce, R.A., A.R. Allen and S.W.J. McDowell, 2011. Bovine tuberculosis (TB): a review of cattle-to-cattle transmission, risk factors and susceptibility. http://www.dardni.gov.uk
26. Gallager, J. and R.S. Clifton-Hadley, 2000. Survival of Mycobacterium bovis in defined environmental conditions. Vet. Microbiol., 10: 193-197.
27. Seifert, H.S.H., 1996. Tropical Animal Health. Kluwer Publishers, The Netherlands.
28. OIE, 2004. Manual of Standards for Diagnostic Tests and Vaccines. 5th ed, OIE, Paris, France.
29. Corner, L.A., 1994. Postmortem diagnosis of Mycobateriun tuberculosis infection in cattle. Veterinary Microbiology, 40: 53-63.
30. Hlokwe, T.M., P. van Helden and A. Michel,2013.Evaluation of the discriminatory power of variable number of Tandem Repeat Typing of Mycobacterium bovis isolates from Southern Africa. Transbound Emerg Dis., 60: 111-120:
31. Ameni, G., M. Vordemeier, R. Firdessa, A. Assefa, G. Hewinson, V. Gordon and S. Berg, 2010. Mycobacterium tuberculosis infection in grazing cattle in central Ethiopia Veterinary Journal of Lancet Infect Dis., 5: 415-430.
32. Cousins, D.V. and N. Floriss, 2005. A review of tests available for use in the diagnosis of tuberculosis in non-bovine species Rev. Sci. Tech., 24: 1039-1059.
33. Monaghan, M.L., M.L. Doherty, J.D. Collins, J.F. Kazda and P.J. Quinn, 1994. The tuberculin test. Vet. Microbiol., 40(1-2): 111-124.

34. Fauci, S., E. Braunwald, L. Dennis, S. Kasper, L. Stephen, J. Hauser, L. Jameson and J. Loscalzo, 2008. Harrison's Principles of Internal Medicine, 17th ed, McGraw-Hill Companies, USA, pp: 1543-1571
35. Iwnetu, R., J. Van Den Hombergh, Y. Woldeamanuel, M. Asfaw and C. Gebrekirstos, 2009. Comparative Performance of Diagnostic Tests for Mycobacterial Lymphadenitis in a High-Burden Country. Sc. and J. of Infect. Dis., 41: 462-468.
36. California Department of Food and Agriculture (CDFA),2016. Bovine Tuberculosis Testing Information for Cattle Producers, USA
37. Birhanu, T., E. Mezgebu, E. Ejeta, A. Gizachew and E. Nekemte, 2015. Review on Diagnostic Techniques of Bovine Tuberculosis in Ethiopia Rep. Opinion, 7: 7-14.
38. De la Rua-Domenech, R., 2006. Human Mycobacterium bovis infection in the United Kingdom: Incidence, risks, control measures and review of the zoonotic aspects of bovine tuberculosis. Tube, 86(2): 77-109.
39. Ramos, D.F., L. Tavares, P.E. da Silva and O.A. Dellagostin, 2015. Molecular typing of Mycobacterium bovis isolates: a review. Braz. J. Microbiol, 45: 365-72.
40. Boadella, M., K. Lyashchenko, Greenwald, R.J. Esfandiari, R. Jaroso, T. Carta, J.M. Garrido, J. Vicente, J.de la Fuente and C. Gorta´zar1, 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: 77-83.
41. Patterson, J. and D. Grooms, 2000. Diagnosis of Bovine Tuberculosis: Gross Necropsy, Histopathology and Acid Fast Staining. Michigan State University Extension.
42. Ayele, W.Y., S.D. Neill, J. Zinsstag, M.G. Weiss and I. Pavlik, 2004. Bovine tuberculosis: an old disease but a new threat to Africa. Int J. Tuberc Lung Dis., 8: 924-37.
43. Araujo, C.P., C.Q. Leite, K.A. Prince, S. Jorge Kdos and A.L. Osorio, 2005. Mycobacterium bovis identification by a molecular method from post-mortem inspected cattle obtained in abattoirs of MatoGrosso do Sul, Brazil. Mem. Inst. Oswaldo Cruz., 100(7): 749-752.
44. Varello, K., M. Pezzolato, D. Mascarino, F. Ingravalle, M. Caranelli and E. Bozzeta, 2006. Comparison of histological techniques for the diagnosis of bovine tuberculosis in the framework of eradication programs. AJBAS, 1(1-2): 26-30.
45. 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. Transbound Emerg Dis., 57(4): 205-220.
46. Corner, L.A., D. Murphy and E. Gormley, 2011. Mycobacterium bovis infection in the Eurasian badger (Meles meles): the disease, pathogenesis, epidemiology and control. J. Comp Pathol., 144(1): 1-24.
47. Marais, B.J., W. Brittle, K. Painczyk, A.C. Hesseling, N. Beyers, E. Wasserman, D. Van Soolingen and R.M. Warren, 2008. Use of light- emitting diode fluorescence microscopy to detect acid-fast bacilli in sputum. Clin. Infect. Dis., 47(2): 203-207.
48. Zumárraga, M.J.., V. Meikle, A. Bernardelli, A. Abdala, H. Tarabla, M.I. Romano and A. Cataldi, 2005. Use of touch-down polymerase chain reaction to enhance the sensitivity of Mycobacterium bovis detection J. Vet. Diagn. Invest., 17(3): 232-8.
49. Sharma, R. and V. Gupta, 2011. Spoligotyping for the Detection of Mycobacterium tuberculosis complex bacteria. Asian J. Biochem., 6: 29-37.
50. Rieder, H.L., A. Van Deun, K.M. Kam, S.J. Kim, T.M. Chonde, A. Trébucq and R. Urbanczik, 2007. Priorities for tuberculosis bacteriology services in low-income countries. 2nd ed International Union against Tuberculosis and Lung Disease, Paris, France. pp: 28-36.
51. Haddad, N., M. Masselot and B. Durand, 2004. Molecular differentiation of Mycobacterium bovis isolates. Res. Vet. Sci., 76: 1-18.
52. Romero, B., A. Aranaz, L. Juan, J. Álvarez, J. Bezos, A. Mateos, E.G. Mampaso and L. Domínguez, 2006. Molecular Epidemiology of Multidrug-Resistant Mycobacterium bovis Isolates with the Same Spoligotyping Profile as Isolates from Animals. J. Clin. Microbiol., 44(9): 3405-3408.
53. Rocha, V.C., S.C. Figueiredo, C.A. Rosales, J.H. Grisi Filho, L.B. Keid and R.M. Soares, 2013. Molecular discrimination of Mycobacterium bovis in São Paulo, Brazil. Vector Borne Zoonotic Dis., 13: 17-21.
54. Tadesse, B., M. Ebisa, E. Eyasu and G. Ayele, 2015. Review on Diagnostic Techniques of Bovine Tuberculosis in Ethiopia. Rep Opinion, 7(1): 7-14.
55. Otal, I., C. Martín, V. Vincent-Lévy-Frebault, D. Thierry and B. Gicquel, 1991. Restriction fragment length polymorphism analysis using IS6110 as an epidemiological marker in tuberculosis J. Clin. Microbiol., 29(6): 1252-4.

56. Saddam, M., B. Girma, A. Bemrew, S. Anmaw and Y. Habitamu, 2015. Review on Polymerase Chain Reaction and its Diagnostic Merit Over Conventional Techniques in Animal Disease African Journal of Basic & Applied Sciences, 7(5): 262-281.
57. Tewodros F. and L. Girja, 2012. A Review in Diagnostic Techniques of Bovine Tuberculosis African Journal of Basic & Applied Sciences, 4(6): 192-199.
58. Cousins, D.V., R.A. Skuce, R.R. Kazwala and J.D. van Embden, 1998. Towards a standardized approach to DNA fingerprinting of Mycobacterium bovis. International Union against Tuberculosis and Lung Disease, Tuberculosis in Animals Subsection. Int. J. Tuberc. Lung. Dis., 2(6): 471-8.
59. Van Embden, J.D., D. Van Soolingen, H.F. Heersma, A.J. De Neeling, M.E. Jones, M. Steiert, V. Grek, F.R. Mooi and J. Verhoef, 1996. Establishment of a European network for the surveillance of Mycobacterium tuberculosis, MRSA and penicillin-resistant pneumococci. J Antimicrob Chemother., 38(5): 905-7.
60. Hazem H.R., H.N. Adel El-Gohary, A.M. Amro and A.N. Essam, 2012. Detection of Mycobacterium Bovis and Mycobacterium Tuberculosis from Clinical Samples by Conventional and Molecular Techniques in Egypt Global Veterinaria, 9(6): 648-654.
61. Cousins, D.V., 2001. Mycobacterium bovis infection and control in domestic livestock. Rev. Sci. Tech., 20: 71-85.
62. O'Brien, D.J., S.M. Schmitt, B.A. Rudolph and G. Nugent, 2011. Recent advances in the management of bovine tuberculosis in free-ranging wildlife. Veterinary Microbiology, 151: 23-33.
63. Bemrew Admassu, Elias Kebede and Anmaw Shite Review on Bovine Tuberculosis European Journal of Biological Sciences, 7(4): 169-185.
64. World Health Organisation, 2007. Improving the diagnosis and treatment of smear-negative pulmonary and extrapulmonary tuberculosis among adults and adolescents: Recommendations for HIV-prevalent and resource-constrained settings.