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Insights into Bovine Tuberculosis (bTB), Various Approaches for its Diagnosis, Control and its Public Health Concerns: An Update

¹Amit Kumar Verma, ²Ruchi Tiwari, ³Sandip Chakraborty, ¹Neha, ⁴Mani Saminathan, ⁴Kuldeep Dhama and ⁵Shoor Vir Singh

¹Department of Veterinary Epidemiology and Preventive Medicine,

²Department of Veterinary Microbiology and Immunology, College of Veterinary Science and Animal Husbandry, Uttar Pradesh Pandit Deen Dayal Upadhyay Pashu Chikitsa Vigyan Vishwavidyalay Evum Go-Anusandhan Sansthan (DUVASU), Mathura, 281001, India

³Department of Animal Resources Development, Pt. Nehru Complex, Agartala, 799006, India

⁴Division of Pathology, Indian Veterinary Research Institute (IVRI), Izatnagar, Bareilly (U.P.), 243122, India

⁵Microbiology Laboratory, Animal Health Division, Central Institute for Research on Goats (CIRG), Makhdoom, PO-Farah, Mathura (U.P.), 281122, India

Corresponding Author: Amit Kumar Verma, Department of Veterinary Epidemiology and Preventive Medicine, College of Veterinary Science and Animal Husbandry, Uttar Pradesh Pandit Deen Dayal Upadhyay Pashu Chikitsa Vigyan Vishwavidyalay Evum Go-Anusandhan Sansthan DUVASU, Mathura (UP), 281001, India

ABSTRACT

Bovine tuberculosis (bTB) is a zoonotic disease transmitted from animals to human and makes significant economic impacts due to high cost of eradication programs, trade restriction and serious consequences regarding public health thereby causing human tuberculosis. *Mycobacterium bovis* is the main etiological agent of bTB which is an acid fast staining bacterium due to waxy substance (mycolic acid) present in its bacterial cell wall. The bacteria can be transmitted by both aerogenous and enterogenous routes. Disease causes development of miliary tubercular lesions, chronic cough, obstructions of air passages and alimentary tract or blood vessels and enlargement of lymph nodes. A spectrum of Cell-Mediated Immune responses (CMI) predominate infection, projecting the role of macrophages and T-cell populations. In advanced stage, there is increased humoral response. Polymerase Chain Reaction (PCR) and real time quantitative PCR (RT-qPCR) have been widely used for the detection of *M. tuberculosis* complex in clinical samples. Single intradermal test, short thermal test and Stormont tests are the valuable delayed type of hypersensitivity tests. Gamma interferon assay, lymphocyte proliferation assay, Enzyme Linked Immune Sorbent Assay (ELISA), multiantigen print immunoassay (MAPIA), Fluorescent Polarization Assay (FPA), immunochromatographic lateral flow test, single antigen as well as multiplex chemiluminescence assays are the various blood-based laboratory tests. Attenuated bovine-strain of tuberculosis bacterium, known as Bacillus of Calmette and Guerin (BCG) is used as vaccine. The present review addresses important insights into the bovine TB, a complex and multi-species disease, the etiological agent, advances and trends in its diagnosis, vaccine development and treatment options and the public health significance of this important disease which would altogether help devising effective strategies for prevention and control of tuberculosis in cattle as well as in wildlife.

Key words: Bovine tuberculosis, *Mycobacterium bovis*, epidemiology, diagnosis, vaccine, treatment, public health concerns, zoonosis

INTRODUCTION

Tuberculosis (TB) is a complex and multi-species disease which can be of three types, bovine, avian and human TB (Dhama *et al.*, 2011). Among mycobacteria, there are around 120 species, most of them are saprophytic but few are major pathogens. *M. bovis* is a member of Mycobacterium Tuberculosis Complex (MTC) and based upon 16S ribosomal RNA sequence studies it shared more than 99.95% identity with other members of MTB complex (Rastogi *et al.*, 2001; Garnier *et al.*, 2003; Smith *et al.*, 2009; Le Roex *et al.*, 2013). Bovine tuberculosis is a chronic debilitating highly contagious disease of cattle, buffaloes and many wild species (Le Roex *et al.*, 2013; Hardstaff *et al.*, 2013). *Mycobacterium bovis*, the causative organism is an aerobic, gram-positive, non-motile, non-sporulating, acid-fast, rod shaped slow-growing organism, obligate intracellular parasite. The disease is characterised by progressive development of tubercles (or nodular granuloma) with resultant caseations and calcification in many of the vital organs in host species except skeletal muscles.

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 (Le Roex *et al.*, 2013; Dhama *et al.*, 2013a; Rodriguez-Campos *et al.*, 2014). World Health Organization (WHO) classified bovine tuberculosis among seven neglected zoonotic diseases having potential to infect man (Ereqat *et al.*, 2013). A study conducted between 1998-2005, over european badger (*Meles meles*) and cattle populations showed close association between *M. bovis* strain types isolated from cattle and associated badgers indicates intraspecific transmission (Menzies and Neill, 2000; Skuce *et al.*, 2011). Bovine TB is a chronic infectious disease which affects a broad range of mammalian hosts including cattle, pigs, goats, sheep, badgers, possums, domestic cats, deer, camelids, omnivores and wild carnivores (O'Reilly and Daborn, 1995; Mathews *et al.*, 2006; Carslake *et al.*, 2011). Other than domestic animals various wildlife species such as Badgers (*Meles meles*), brushtail possums (*Trichosurus vulpecula*), deer (*Odocoileus virginianus*), bison (*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 (Hardstaff *et al.*, 2013). Being maintained in the wildlife communities *M. bovis* may act as principal source of infection for domestic, captive and wide range of protected wildlife species (De Lisle *et al.*, 2001; Hardstaff *et al.*, 2013; Delahay *et al.*, 2003; Scantlebury *et al.*, 2004; Witmer *et al.*, 2010; O'Brien *et al.*, 2006; 2011; Le Roex *et al.*, 2013). In addition to a broad host range, mycobacterium also occurs in numerous biotopes including water, soil, protozoa, aerosols, and fresh tropical vegetation (Biet *et al.*, 2005) Bovine tuberculosis is endemic disease of cattle (Aylate *et al.*, 2013). Around 30 years ago as carriers of *M. bovis* badgers (*Meles meles*) were identified for the first time and they have been found to make an essential contribution in spreading *M. bovis* between cattle herds. Between animal conservationists (who are keen for saving badgers) and farmers (who are interested in culling badgers for reducing losses of livestock) there is a battle currently for this reason (Krebs *et al.*, 1997; Delahay *et al.*, 2007; Ward *et al.*, 2009).

The present review addresses important insights of the bovine TB (*Mycobacterium bovis*), a complex and multi-species disease with the aims of dealing various approaches for controlling tuberculosis in cattle as well as in wildlife.

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 (Corner, 1994). 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 (Cousins *et al.*, 2003; Atkins and Robinson, 2013). 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 (Corner, 1994). In the environment *M. bovis* can survive for various months especially in cold as well as dark and conditions which is 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 (Biberstein and Holzworth, 1987). The incubation period of *M. bovis* is 3 weeks.

EPIDEMIOLOGY

The disease tuberculosis (TB) is chronic in nature affecting a wide range of mammals that include: humans and cattle, deer, llamas, pigs, domestic cats, carnivores (wild like foxes and coyotes), as well as omnivores (possums as well as mustelids and rodents). Equids and sheep are comparatively more resistant to the disease. All species including humans with various age groups are susceptible. The bacteria primarily affect the cattle and other domestic and wild animals along with man may also get affected. Disease is found throughout the world including India but more prevalent in Africa, parts of Asia and America. The prevalence of disease is high in the tropical and sub-tropical countries. Bovine TB is distributed globally except Antarctica and those countries such as Caribbean islands, parts of South America and Australia where it has been eradicated by following strict test and slaughter policies. It is major health problem in India. Chances and severity of infection depend upon several predisposing factors like environmental variables, topographic causes, anthropogenic variables, seasonality, immunosuppression, long antibiotic therapy, working conditions and environmental factors. Infected cattle are the main source of infection for other cattle. Organisms are excreted in exhaled air, sputum, faeces, milk, urine, vaginal and uterine discharges and discharges from open peripheral lymph nodes (Gilbert *et al.*, 2005). Epidemiology of bovine TB is influenced from many risk factors as genetic, behavioural, biological or environmental which have effect on transmission, establishment of infection and expression of disease (Gordejo and Vermeersch, 2006; Vial *et al.*, 2011). Male badgers are more affected as compare to female. Male have higher risk of mortality which promote gender influence while risking transmission of TB to cattle (Graham *et al.*, 2013). It is more prevalent in dairy workers exposed to poor control areas of bovine tuberculosis. The pulmonary form of disease is more developed in occupational groups working with animals infected from *M. bovis* on farm or slaughter house, than the alimentary form of disease. Bovine tuberculosis herd prevalence was positively related with Mycobacterium Tuberculosis Complex (MTC) and also correlated positively with size of island, number of imported cattle and presence of wild host but not with isolation of cattle as well as density of cattle. Incidence of TB in cattle progeny is also affected by hereditary and maternal

influences (Petukhov, 1981). The factors associated with tuberculosis which influence the occurrence of disease are sex, breed and social management of livelihood conditions (O'Reilly and Daborn, 1995; Biffa *et al.*, 2012; Acevedo *et al.*, 2013; Torres-Gonzalez *et al.*, 2013).

Routes or sources of infection and its transmission: In various ways the disease can be transmitted. For instance in air that is exhaled; sputum and urine, faeces as well as pus the bacteria can spread. Either direct contact or contact with infected animal excreta, aerosol inhalation can spread the disease that depends on the involvement of the species (O'Reilly and Daborn, 1995; Phillips *et al.*, 2003; Delahay *et al.*, 2002). The common mode of transmission is inhalation or ingestion. Aerogenous or inhalation: it is mainly by droplet infection, inhalation of dust contaminated by sputum, faeces, urine of infected animals. Thus close housing and overcrowding along with improper management predisposes to the disease. Zebu (Brahman) type cattle are thought to be much more resistant to tuberculosis than European cattle. The dynamics of *M. bovis* transmission from an effective disseminator tuberculous animal to susceptible hosts are currently not clearly identified and are meagrely understood. Though, infection is principally confined to the respiratory system as bacteria aim to establish the infection within the lung. Infected cattle are considered as possible source of infection as they shed significant amount of *M. bovis* through droplet nuclei in to the environmental and may act as source of intra-herd transmission (Perumaalla *et al.*, 1999; Van Rhijn *et al.*, 2008; Humblet *et al.*, 2009; Raviglione and Krech, 2011). Primarily, tuberculosis is a respiratory disease and is transmitted through mainly by air born route within and between species during close contact (O'Reilly and Daborn, 1995). In case of enterogenous route or ingestion through buccal mucosa, pharyngeal mucosa and intestinal mucosa the organism may enter into the animal body. It is also through the congenital route but is less common mode of acquiring infection (O'Reilly and Daborn, 1995). The infected bull may also transmit disease or through artificial insemination with the use of infected semen (Roumy, 1966). The *M. bovis* is transmitted from animal to man through ingestion of unpasteurized dairy product, milk of infected cattle and undercooked meat which was recognised as a major public health problem (O'Reilly and Daborn, 1995).

ECONOMIC IMPORTANCE

Tuberculosis occurs in almost every country of the world and is of major importance in dairy cattle due to high morbidity and loss of production as infected animals lose 10-25% of their productive efficiency. Apart from these, advance tuberculosis may lead to death of the animals. WHO declared tuberculosis as global emergency. About one third of human population of the world are suffering from tuberculosis infection (Joardar *et al.*, 2002; 2003). Tuberculosis has great importance regarding the economy of the livestock industry of India because it can infect the human population due to its zoonotic nature, therefore it is an important public health issue (O'Reilly and Daborn, 1995). It is listed disease by World Organisation for Animal Health formerly Office International des Epizooties (OIE). Tuberculosis also has significance to the international trade of animals and animal product (Cousins, 2001; Rodriguez-Campos *et al.*, 2014).

PUBLIC HEALTH RISKS

Human tuberculosis due to *M. bovis* is usually underestimated or underdiagnosed because of no clinical, radiographical and histopathological differentiation of tuberculosis caused by *M. tuberculosis* and *M. bovis* (Perez-Lago *et al.*, 2013). *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 (Dhama *et al.*, 2013b; Malama *et al.*, 2013), or by inhaling infective droplets or direct exposure to infected animals (Perez-Lago *et al.*, 2013). In an estimate, about 10% cases of human tuberculosis are caused by *M. bovis*, while majority are caused by *M. tuberculosis* (Perez-Lago *et al.*, 2013). In countries wherein 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 (Fritsche *et al.*, 2004; Corner, 2006; Etter *et al.*, 2006; Evans *et al.*, 2007; Malama *et al.*, 2013). 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. (Asseged *et al.*, 2004; Torgerson and Torgerson, 2010). 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 (Alexandroff *et al.*, 1999; Atkinson *et al.*, 2000; Vento and Lanzafame, 2011).

IMMUNE RESPONSES AGAINST BOVINE TB PATHOGEN

Understanding of the immune response following infection of *M. bovis* in bovines elaborates the knowledge of disease pathogenesis and development of nodular lesions (Wang *et al.*, 2013). Among the immunity which persist spectrum of Cell-Mediated Immune response (CMI) predominate projecting the role of macrophages and T-cell populations. As the tuberculosis progresses a shift from Cell-Mediated Immune (CMI) responses to increased humoral response develops which is evident from change of dominance from a T helper type 1 (Th1) cell towards a Th2 type immune response signifying suppressed CMI and amplified humoral immune response. Cytokine analysis indicates deviation in CMI and interleukin responses as the pathological form of disease advances. Reduction in *in vitro* CMI responses, elevated levels of IL-10 expression and augmented HI responses involving production of anti-*M. bovis* immunoglobulin G1 (IgG1) isotype have been noticed with increased pathology and disease (Villarreal-Ramos *et al.*, 2003). Knowledge of interactions of *M. bovis* with products of immune response provides prospects for development of immune-dependent tools as of diagnostics, vaccines and in evolving better methods/policies for combating and controlling the disease (Pollock *et al.*, 2001; Welsh *et al.*, 2005; Spencer, 2011).

BOVINE TUBERCULOSIS-THE DISEASE

Clinical signs: Basic pathogenic mechanisms are more or less same in case of human TB and bovine tuberculosis. Developing technologies support the fact of identical pathogenesis, because of unusually high conserved sequence similarity in genome of TB causing bacteria in more than 99.95% animals (Smith *et al.*, 2006; Smith *et al.*, 2009; Thye *et al.*, 2010; Verhagen *et al.*, 2011). TB is a chronic debilitating disease occurs in cattle. No symptoms occur in early stage of disease that is asymptomatic. However, in late stage, there is progressive emaciation, a mild fluctuating fever, weakness and in-appetence. When infection is present in the lung then dyspnoea, moist cough or trachypnoea may occur. In the terminal stage, animal become extremely emaciated and develop

acute respiratory distress. Involvement of respiratory tract and its role in pathogenesis of disease is evidenced by the predominant distribution of lesions present in upper respiratory tract, lung and tonsils in affected human as well as in animals. Some cows with extensive miliary tubercular lesions are clinically normal but in most cases progressive emaciation unassociated with other clinical signs occur, inspite of good appetite. A capricious appetite and fluctuating temperature are commonly associated with disease. The hair coat may be rough. Affected animal tend to become more docile and sluggish but eyes remain bright and alert. These general signs often become more pronounced after calving. Pulmonary involvement is characterized by chronic cough due to bronchopneumonia. Cough occurs only once or twice at a time and is low suppressed and moist which is easily stimulated by squeezing the pharynx or by exercise and is most common in morning and in cold weather.

In advanced cases, air passages, alimentary tract, or blood vessels may be obstructed by enlargement of lymphnodes. Lymph nodes of the head and neck may become visibly affected and sometimes rupture and drain. Involvement of the digestive tract is manifested by intermittent diarrhoea and constipation in some instances. There may be chances of bloat occur due to pressure of enlarged mediastinal glands on the oesophagus. The enlargement of retropharyngeal glands results in dysphagia. Extreme emaciation and acute respiratory distress may occur during the terminal stages of tuberculosis. Lesions on the female genitalia may occur, while male genitalia are seldom involved. Tuberculosis mastitis is of major importance because of danger to public health and of spread of disease to calves and difficulty of differentiating it from other forms of mastitis (Radostits *et al.*, 2000). Experiments have shown that lions may also become susceptible to bovine TB (Trinkel *et al.*, 2011).

With the help of descriptive statistic and regression model on data analysis, indicates that tuberculosis lesion are mostly occur in lung and lymph node of respiratory system (Biffa *et al.*, 2012).

Disease usually has a prolonged course, and symptoms take months or years to appear. The usual clinical signs include.

Weakness, loss of appetite, weight-loss, fluctuating fever, intermittent hacking cough, diarrhoea, large prominent lymph nodes, anorexia, induration of udder.

Post mortem lesions: Disease causes a chronic granulomatous, caseous-necrotising inflammation in lungs and associated lymph nodes (Domingo *et al.*, 2014). On post mortem examination, tubercles are commonly found in bronchial, mediastinal, retropharyngeal and portal lymph nodes along with tissue affected. Apart from this, lung, liver, spleen and the surfaces of body cavities are commonly affected. Tuberculous granuloma usually has a yellowish appearance with caseous, caseo-calcareous, or calcified in consistency. The efficiency of meat inspection procedure should be evaluated by conducting detailed post-mortem examination which determines the gross lesions distribution in cattle infected with *M. bovis* (Asseged *et al.*, 2004).

DIAGNOSIS

Diagnosis of this disease has various challenges and difficulties. Tentative and presumptive diagnosis can be made by *ante mortem* examination on the basis of clinical signs. However, disease can be diagnosed more clearly after post mortem examination based on the presence of gross lesions compatible with BTB in the lungs and/or associated lymph nodes and these are not confirmatory (Malama *et al.*, 2013). Typical lesion or gross lesions are found at necropsy in macroscopic detection

and histopathological examination of lesion may confirm the diagnosis but the definitive diagnosis is done only by isolation of *Mycobacterium bovis* from lesion, bacteriologically (Corner, 1994). The confirmation of disease requires certain laboratory examination.

Laboratory diagnosis

Identification of agent: Organism in clinical samples and tissue samples collected after post-mortem examination may be demonstrated by examination of stained smears or tissue sections and confirmed by cultivation of the organism on primary isolation medium.

Microscopic examination: *M. bovis* can be demonstrated microscopically on direct smears from clinical samples (blood stained purulent exudates i.e., cough and sputum, pleural fluid) and on prepared tissue materials (lung biopsy). The acid fastness of *M. bovis* is normally demonstrated with the classic Ziehl-Neelsen stain and a fluorescent acid-fast stain may also be used. The tentative diagnosis can be made by observing caseous necrosis, mineralisation, epithelioid cells, multinucleated giant cells and macrophages in the tissue samples on histopathology.

Culture of *M. bovis*: The tissue sample is homogenised using a pestle and mortar, followed by decontamination with either detergent, acid or an alkali, such as 0.375-0.75% hexadecylpyridiumchloride (HPC), 5% oxalic acid or 2-4% sodium hydroxide. The mixture is shaken for 10 min at room temperature and then neutralised. After that centrifuge the suspension and discard the supernatant. Sediment is used for culture and microscopic examination. For primary isolation, the sediment is usually inoculated on to a set of solid egg-based media such as Lowenstein-Jensen, Coletsos base or Stonebrinks; these media should contain either pyruvate or pyruvate and glycerol. Cultures are incubated for a minimum of 8 weeks (and preferably for 10-12 weeks) at 37°C with or without CO₂. The media should be in tightly closed tubes to avoid desiccation. Slants should be examined at regular intervals for presence of any growth. When growth is visible, smears are prepared and stained by the Ziehl-Neelsen technique.

Nucleic acid recognition methods: PCR has been widely used for the detection of *M. tuberculosis* complex in clinical samples (mainly sputum) in human cases and has recently been used for the diagnosis of tuberculosis in animals. The real time PCR determine the status of infection in cattle for bovine tuberculosis as compare to the IFN gamma mRNA in blood culture. Another useful diagnostic method for bovine tuberculosis in cattle is RT-qPCR (Palmer and Waters, 2006; Collins, 2011; Gan *et al.*, 2013).

Bacterial culture and post-mortem confirmation of tuberculosis is insufficiently sensitive. So, veterinarians and other health researchers have evaluated other diagnostic approach i.e. immunological including lateral-flow devices and Enzyme-linked Immunosorbent Assay (ELISA), tuberculin skin test and interferon-gamma release assay (Chambers, 2013).

Delayed type hypersensitivity reaction

Skin test or Single Intra Dermal test (SID): This is standard test for detection of bovine tuberculosis and involves the intradermal injection of bovine tuberculin PPD (purified protein derivative) and the subsequent detection of swelling (delayed hypersensitivity) at the site of injection 72 h later. Generally, this test is conducted on middle neck and the alternate site may be the caudal fold of the tail. However, skin of the neck is preferred over tail due to higher sensitivity

of skin on neck. During initial stage of infection i.e. 3-6 weeks after infection, this test may give negative reaction. After a SID test, the animals giving a suspicious result should not be tested again before 60 days (Costello *et al.*, 1997). This test has poor specificity due to cross-reactions with other non-pathogenic mycobacteria (Praud *et al.*, 2014). False-negative reactions may be given by:

- Animals in advanced stage of disease
- In initial stages i.e. 6 weeks after infection
- Cows within 6 weeks after calving
- Animals that were tested within 8-60 days after single intradermal testing
- Old cattle
- Low-potency tuberculin or bacterial contamination of the tuberculin
- Variable dose with multi dose syringes

Short thermal test: Tuberculin (4 mL) is injected subcutaneously into the neck of cattle which have a rectal temperature of not more than 102°F at the time of injection and for 2 h later. If the temperature at 4, 6 and 8 h after injection rises above 104°F, the animal is considered as positive. The temperature peak is usually at 6-8 h and is generally over 105.8°F. In some cases, death may occur due to anaphylaxis.

Stormont test: 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 (Whelan *et al.*, 2003).

Blood based laboratory tests: These include gamma interferon assay, lymphocyte proliferation assay, ELISA etc and require well equipped laboratory facilities with skilled laboratory personnel (Coad *et al.*, 2008; Whelan *et al.*, 2008). Interferon gamma assay was initially developed circa 1990 for diagnosis of bovine tuberculosis in Australian tuberculosis eradication programme (Waters *et al.*, 2014). As an ancillary test the interferon-gamma test is used for diagnosing bovine tuberculosis at ante-mortem. This helps in measuring the cellular response to antigens of mycobacteria and thereby helps in measuring broadly similar kind of immune response as that of intradermal tests. If there is release of interferon-gamma (a pivotal cytokine) preferentially a positive result is indicated (Wood *et al.*, 1991; Wood and Jones, 2001). Printing of several antigens is done onto a membrane by means of multiantigen print immunoassay (MAPIA) and for each animal measurement of antibody response is done (Waters *et al.*, 2006). Use of tracer which is the target antigen or part of it is done in case of Fluorescence Polarisation Assay (FPA) where in a fluorescent molecule is added to serum. There is binding of antibody (if present) in the serum. There is increase in the measurable fluorescent polarisation because of the increase in size of the antigen-antibody complex in combination (Jolley *et al.*, 2007). There is use of latex beads (coloured and antigen-impregnated) mixed with serum in case of immunochromatographic lateral flow test. Along a membrane the material flows by means of capillary action across a line where impregnation of antigen is done onto the membrane. There is formation of a coloured line where there is binding of coated latex beads to serum antibody (Lyashchenko *et al.*, 2006). There is use of magnetic iron beads coated with antigen of choice in case of single antigen chemiluminescence

wherein magnetic collection is done. Detection of any bound antibody is then done by the use of an anti-bovine antibody specifically followed by identification of by the use of a chemiluminescent reaction (Green *et al.*, 2009; Foddai *et al.*, 2010a; 2010b). There is printing of individual antigens in small dots on a multi-well plate (96 well) in case of multiplex chemiluminescence immunoassay viz., Enferplex. To each of the well serum samples are used individually and detection of antibody to each spotted antigen is done by immunoassay detection method. There is colour change in each spot that indicates specific antibody's presence to that antigen (Whelan *et al.*, 2008). The assessment of the performance of such newer tests just began in recent years but on their potential value certain optimisms have been followed cautiously by seeing preliminary results.

Antibody detection in milk: In dairy animals, milk samples can also be used for measuring antibodies against *M. bovis* antigens such as MPB70 and MPB83 using ELISA kits (Buddle *et al.*, 2013). Milk samples can be pooled from 10-20 animals but this may result in 50% decrease in sensitivity. This test is unlikely to be useful in nations with low prevalence of disease and large herd sizes.

Animal inoculation test: Mostly Guinea pig and rabbit are used. The clinical sample (exudates, CSF, sputum or centrifuge milk) is deposit in the thigh region of Guinea Pig produces or results into typical tuberculosis lesion of spleen, liver and lymph nodes of infected animal.

Genetic fingerprinting technique: The different strains of *M. bovis* can also be distinguished with the help of genetic fingerprinting technique, VNTR typing, spoligotyping and current molecular diagnostic techniques. Variable Number Tandem Repeat (VNTR) typing and spoligotyping are DNA typing schemes developed in 1990 for *M. bovis* (Kamerbeek *et al.*, 1997; Filliol *et al.*, 2002; Boehme *et al.*, 2010; Dhama *et al.*, 2012). The genomic detection analysis and spoligotyping is another method used in identification of *M. bovis* (Biffa *et al.*, 2012).

The primarily screening test for bovine tuberculosis is tuberculin skin test having low sensitivity. In comparison to tuberculin test the interferon gamma assay is found to be more sensitive (Schiller *et al.*, 2011). The necessity of new-fangled and improved diagnostic for Bovine tuberculosis (TB) has driven researchers to develop specific tests with significant sensitivity (Pollock and Neill, 2002; Singh *et al.*, 2014).

TREATMENT

In human tuberculosis, drugs like isoniazid, combinations of streptomycin and para-aminosalicylic acid and other acids are commonly used. The treatment of animals with tuberculosis is not a favoured option in eradication-conscious countries. Being long term therapy chances of development of multidrug resistant (MDR), extremely drug resistant (XDR) and even Totally Drug Resistant (TDR) bacterial strains are more if treatment regime is not properly followed. Alternative therapeutic approaches involve panchgavya therapy, cytokine therapy, egg yolk antibody, herbal medication, immunomodulation and bacteriophage therapy etc. (Mahima *et al.*, 2012; Dhama *et al.*, 2013c; Tiwari *et al.*, 2014c; Rahal *et al.*, 2014). The fruit extracts of black pepper (*Piper longum*) and way bread, a perennial herb (*Plantago major*) is known for its significant valid anti-mycobacterial and antitubercular activity even against MDR strains of *Mycobacterium tuberculosis* also (Stuckler *et al.*, 2008; Ford *et al.*, 2011; Mahima *et al.*, 2012; Dhama *et al.*, 2013d, 2013e; Tiwari *et al.*, 2013; 2014a, 2014d). Being equipped with peptidoglycan

hydrolase, lipolytic action and growth inhibition activity endolysins and mycobacteriophage have broad spectrum of demolishing efficiency against mycobacteria. Functional analysis of mycobacteriophage Ms6 revealed presence of lysis gene lysine A which encodes endolysin, lysine B and the holin-like enzymatic proteins with lipolytic activity (Garcia *et al.*, 2002; Gil *et al.*, 2008; Payne *et al.*, 2009; Catalao *et al.*, 2011a,b; Tiwari *et al.*, 2014b).

PREVENTION AND CONTROL

Control of bovine tuberculosis can be done by test-and-slaughter or test-and-segregation methods. There is periodic re-testing of affected herds for eliminating cattle shedding the organism. For this purpose the test which is generally used is tuberculin test. Quarantine programme is followed for the infected herds thereby helping in tracing the animals found in contact with reactors. From domesticated animals only test-and slaughter policy can eradicate bovine tuberculosis. There may be reduction in the spread of the agent within the herd by means of sanitation as well as disinfection. To disinfectants *M. bovis* is relatively resistant thereby requiring long contact time for inactivating. 5% phenol, iodine solutions (having presence of iodine at high concentration), glutaraldehyde as well as formaldehyde are found to be effective disinfectants. If the concentration of organic material is low in environment the efficacy of 1% sodium hypochlorite with a long contact time is efficacious. Moist heat of 121°C (250°F) for a minimum period of 15 min can kill *M. bovis*. In affected farms it is also advisable to perform control of rodents. Experimental infection of meadow voles as well as house mice can be done and in vole feces the organism can be shed (USDA/APHIS, 1995; DEFRA, 2003; Ryan *et al.*, 2006).

In 1906, Albert Calmette and Camille Gueringot success for the first time in immunization against tuberculosis, a serious zoonotic disease using attenuated bovine-strain tuberculosis, which was later known as Bacillus of Calmette and Guerin (BCG) (Waters *et al.*, 2014). BCG is a live, laboratory-attenuated vaccine (*M. bovis*) strain derived from a virulent French *M. bovis* field isolate which has been in use against *M. tuberculosis* for nearly a century (Dhama *et al.*, 1998; 1999; 2004). It is noteworthy that BCG provides solid protection against military TB (generalized form of human TB) in young children but is less effective in adults against pulmonary form of human TB. Contribution of BCG vaccination in global TB control programme is still controversial because mostly TB infection occurs in pulmonary form (Segal-Maurer and Kalkut, 1994; Orme, 2010; Margaret and Anthony, 2011).

Trials have been conducted on a number of deletion mutants and other vaccines and none has been shown to induce a superior protection to BCG (Dhama *et al.*, 2008). Experimental vaccine trials have shown that vaccine prepared from *M. bovis* strain Amce2 (*M. bovis* strain deleted in *mce2A* and *mce2B* genes) tested in cattle calf have proved this strain as promising vaccine candidate in controlling bovine TB pathogenesis in cattle. Post-vaccination challenge immune response was assessed by measuring IFN- γ concentration using an Interferon-gamma Release Assay (IGRA), cytometry and cytokine responses of bovine Purified Protein Derivative (PPD) re-stimulated Peripheral Blood Mononuclear Cells (PBMCs) (Blanco *et al.*, 2013). Based upon several trials most effective vaccination strategy against bovine TB has been suggested to first prime the immune system with BCG vaccine and after initiation of immune response booster dose should be administered with subunit, DNA or protein vaccines containing any protective antigen which was a component of BCG vaccine. This vaccination strategy is referred as heterologous prime-boost strategy (Skinner *et al.*, 2003; 2005; Wedlock *et al.*, 2005; Vordermeier *et al.*, 2004; 2006).

In countries where test and slaughter control scheme is not possible, vaccination may be used but before implementing vaccination programme, the vaccination schedule must be optimised according to local conditions. In countries like UK, BT persisted in cattle herds even after following test and slaughter policies due to close proximity with wildlife reservoir. Due to maintenance of bacteria in wild life, there is difficulty in eradicating this disease using well proved control strategies, so there is need of some alternate control strategies (Le Roex *et al.*, 2013; Hardstaff *et al.*, 2013). Hence, immunological approaches of developing an effective vaccine and careful strategies for its delivery for the control of *M. bovis* infection in wildlife may act as potential alternative tools (Buddle *et al.*, 2000; 2003; Woodroffe *et al.*, 2009; Blanco *et al.*, 2013; Hutchings *et al.*, 2013; Malama *et al.*, 2013). Ideally, the dose of vaccine is 10^4 to 10^6 Colony-Forming Units (CFU) through subcutaneous route. It is important to recognise that use of vaccine will compromise tuberculin skin tests or other immunological tests. Thus, in countries where control or trade measures are based on this testing, the vaccine should not be used. This fact indicates limitations that still a long road has to be crossed to achieve full protection against bovine TB (Kao *et al.*, 1997; Clifton-Hadley and Hewinson, 2003). The results show that the vaccine combination of BCG and the vaccinating moiety (adjuvant subunit, DNA vaccines) are more effective or superior in protection as compared to the use of BCG alone. DIVA vaccines are also under development for bovine tuberculosis. Specific antigens such as prototype DIVA antigens ESAT-6, CFP-10 and others should be identified to facilitate the differentiation of BCG-vaccinated and *M. bovis* infected bovines for differential diagnosis of infected from vaccinated cattle and to define differentiating infected from vaccinated animals (DIVA) antigens. (Buddle *et al.*, 2011; Vordermeier *et al.*, 2011a; 2011b). Another important factor that can affect the pathogen is the host's genome. The identification of genetic variability responsible for susceptibility and resistance to bacteria may be useful in selection of drug target and development of disease resistant animals (Le Roex *et al.*, 2013). Because the bovine tuberculosis is a notifiable or reportable disease so if bovine tuberculosis is suspected in animals then notification should be given to higher authorities to take recommended action against it.

Control measures: The methods of controlling *M. bovis* in wildlife are limited and dependent on sound disease control principles and judicious use of diagnostic tests. Though population control and vaccination are potential alternative control methods but not applicable in all the situations:

- The primary tool used for screening of bovine tuberculosis is the tuberculin test (Schiller *et al.*, 2011). The standard control measure applied to tuberculosis is test and slaughter or abattoir surveillance (Schiller *et al.*, 2010). Screening of meat at slaughterhouses along with detection of slaughtered animal's herd of origin will be helpful in reducing the disease (Smith *et al.*, 2014)
- Slaughter of diseased cattle can be an effective policy for tuberculosis eradication, if no other reservoirs of infection are maintained in near surroundings (Verma *et al.*, 2014)
- In early stage of disease, test and segregation method is followed while in later or terminal stage of disease, test and slaughter method is recommended
- The animal which is import from other state or country should be strictly quarantine
- DNA fingerprinting is an epidemiological tool in cattle for control measure (O'Reilly and Daborn, 1995)
- Quality control produces quality product (Duignan *et al.*, 2012)
- Novel diagnostic biomarkers as specific antigens should be identified to support the development of DIVA skin tests (Vordermeier *et al.*, 2011a)

- The Bovigam assay is an in vitro diagnostic test, based on the measurement of interferon gamma (IFN-gamma) after stimulation of blood with avian and bovine tuberculin PPD used for diagnosis of bovine TB (Rothel *et al.*, 1990; 1992)
- It is difficult to eradicate and control where wild population is established and also when once it spread into the ecosystem with free ranging maintenance host. (Miller and Sweeney, 2013)
- The routine inspection of abattoir also play important role for national surveillance. (Probst *et al.*, 2011; Aylate *et al.*, 2013)
- Reintroduction of bovine tuberculosis and its eradication is done by premovement testing which act as central tool for eradication (Schiller *et al.*, 2011)
- Post mortem examination, meat inspection, intensive surveillance, gamma interferon assay, systematic individual testing of animals, followed by removal of infected and in contact animals for reducing or eliminating the disease (De La Rua-Domenech, 2005)
- Ancillary diagnostic techniques, herd testing, health surveillance, ante mortem diagnosis including tuberculin testing and immunization are effective in controlling the TB incidences (De La Rua-Domenech *et al.*, 2006; Torgerson and Torgerson, 2010)
- Post mortem meat inspection of animals looks for the tubercles in the lungs and lymph nodes. Detecting these infected animals prevent unsafe meat from entering the food chain and allows veterinary services to trace-back to the herd of origin of the infected animal which can then be tested and eliminated if needed
- Pasteurisation of milk of infected animals
- Treatment of infected animals is not economically feasible because of the high cost, lengthy time and the larger goal of eliminating the disease.
- Hygienic measures to prevent the spread of infection should be instituted as soon as the first group of reactors is removed. Feed troughs should be cleaned and thoroughly disinfected with hot, 5% phenol or equivalent cresol disinfectant. Water troughs and drinking cups should be emptied and similarly disinfected (Woolhouse *et al.*, 1997)
- It is important that calves being reared as herd replacements be fed on tuberculosis-free milk, either from known free animals or pasteurized
- Farm attendants should be checked as they may provide a source of infection
- Rodent population should be decreased on herd which also help in transmission of disease
- Bio-security measures should be followed on herd/farm which helps in decreasing or reducing the interaction between domestic animals and wildlife animals
- Carcasses of infected animals should be buried at least four feet under the ground
- Notification, surveillance by effective implementation of TB control programmes like 'Revised National Tuberculosis control programme'
- Vaccination of animals along with vector control may be more effective than only vaccination
- Wear protective cloth during handling of the diseased animal and infected carcasses
- In the control and eradication programmes, must consider or incorporated the data collection and epidemiological analysis of disease so that progress and the constraints to progress may be evaluated
- Epidemiological approaches, including case-control studies are helpful to provide the information regarding various sources of *M. bovis* and further cost-effective techniques as control measures can be designed for eradication of tuberculosis

- Monitoring of the control and eradication programme should be done continuously to know the progress of the programme and for the implementation of necessary modification as required to the programme. Application of advanced genotyping tools and co-operation and co-ordination in human as well as veterinary health care professionals will ultimately help in eradication of bovine tuberculosis especially in developing nation like India (Dhama *et al.*, 2013f; Verma *et al.*, 2014)

CONCLUSION AND FUTURE PERSPECTIVES

In almost every country of the world, bovine tuberculosis is prevalent and accounts for 10-25% loss in productivity. It is a listed disease in world organization for Animal Health. The disease has an important public health issue due to its zoonotic significance. In certain species of animals, antimicrobial treatment has been attempted but as long term treatment is required so in eradication conscious countries practicing anti-tubercular treatment is not a wise option. Programs involving eradication of the disease consists of: inspection of meat at post mortem and conducting surveillance programme intensively. This include, on-farm visits, individual testing of cattle systematically along with infected as well as in contact animals' removal and control of movement. The advances in development of bovine tuberculosis diagnostics and vaccines for cattle are offering valuable insights in the use of vaccination for the control of tuberculosis in a range of bovines and captive wildlife species. In human medicine, vaccination is practiced but as a preventive measure it is not widely used in animals. There is variation in the efficacy of existing vaccines in animals thereby interfering with testing for elimination of the disease. Testing of new candidate vaccines is underway. Combination of BCG vaccine along with vaccine moiety viz., adjuvant subunit and DNA vaccines are more efficacious or superior in providing protection in comparison to BCG alone. DIVA vaccines are also under development. Application of advanced genotyping tools and co-operation and co-ordination in human as well as veterinary health care professionals will ultimately help in eradication of bovine tuberculosis especially in developing nations.

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