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Historical Perspectives and Epidemiology of Bovine Tuberculosis

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ABSTRACT

Bovine tuberculosis is an important bacterial zoonotic disease of animals caused by Mycobacterium bovis which affect animal health, production and overall economy of the country. Among animals, the disease transmission occurs mainly through inhalation. In calves, the disease may occur via ingestion of contaminated milk from infected dam and rarely via congenital or cutaneous route. The disease transmits to human through ingestion of contaminated milk or milk products and also through inhalation. The disease has a slow progression which creates difficulty in early diagnosis and treatment. Conventional diagnostic methods should be used in combination with modern molecular and immunological techniques for early and accurate diagnosis. Treatment is usually not recommended in animals. Vaccination is being carried out in some countries as a preventive measure. For control of the disease, slaughter of the reactor animals in developed countries and segregation of the suspected or reactor animals in developing countries is preferred. This article focuses on the historical perspectives and epidemiology of bovine tuberculosis.

Key words: *Anergic, Mycobacterium bovis, Mycobacterium tuberculosis complex, Wildlife, zoonotic.*

INTRODUCTION

Bovine tuberculosis (bovine TB) is an OIE listed chronic, bacterial zoonotic disease caused by *Mycobacterium bovis (M. bovis)*, a member of the *Mycobacterium tuberculosis* complex (MTC). It is one of the major infectious disease among domestic animals and certain wildlife population[25,26]. World Health Organization (WHO) classified bovine tuberculosis among seven neglected zoonotic diseases having potential to infect man[15]. It is an economically significant disease because of trade restriction and serious public health consequences mainly in developing countries[35,45]. It is a known fact that humans and animals have had close interactions and this interaction is increasing due to the advancement in livestock production systems to meet out the demand for animal products. This contributes to the transmission of infectious diseases from animals to human[21,40].

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Cattle and buffalo are considered as the natural host of *M. bovis*[39]. Zebu cattle are much more resistant to tuberculosis than crossbred and exotic breeds. *Mycobacterium tuberculosis* has also been reported in a wide range of domestic and wildlife animal species, more frequently those living in close contact with human for a long time[2,28]. It is estimated that *M. bovis* is responsible for 5% of all TB infections in humans[10,22]. In developed countries, there is dramatic decline in the incidence of human tuberculosis due to *M. bovis* due to mandatory pasteurization of milk in combination with tuberculin skin test and slaughter of reactor cattle[29]. During the year 2015, estimated incidence of human TB due to *M. bovis* was 149,000 cases and 47,400 cases in worldwide and in South-East Asia respectively whereas mortality rate was 13,400 cases and 2,280 cases worldwide and in South-East Asia respectively[56].

Historical perspectives:

Tuberculosis has been co-evolved with human civilization. The earliest references related to TB can be found in the language *Samskritam* (*Sanskrit*). In ancient Chinese and Arabic literature, there is documentation of a TB-like disease[23,36]. Tuberculosis was referred to as *Yakshma*, in the ancient Indian scriptures, *The Vedas*. The word "tuberculosis" appears to have been derived from the Latin word *tubercula* i.e. a small lump[13]. The discovery of tubercle bacillus was announced by Robert Koch on 24th March 1882 during the monthly evening meeting of the Berlin Physiological Society. Since 1982, 24th March is being celebrated as "World TB Day" commemorating the centenary of this event[58]. The work of Villemin in 1865[11] and Koch in 1882[7] shown the cross adaptability of the tubercle bacilli from one species to other species and revealed that tuberculosis could be transmitted from animals to humans[11] This was supported by demonstration of *M. bovis* in a child with tuberculous meningitis by Ravenel in 1902. In developed countries, due to better public health practices, massive vaccination with Calmette-Guerin bacillus (BCG) vaccine and the advent of streptomycin, para-amino salicylic acid (PAS) and isoniazid, there was decline in morbidity and mortality rates due to TB during the 20th century but it continued to affect the developing countries like India. In the mid-1980s due to emergence of acquired immunodeficiency syndrome (AIDS), incidence of TB began to increase in both developed and developing world. In April 1993, WHO declared TB to be a "global emergency" after recognizing the global burden of TB[58]. During late 1990s, drug-resistant TB (DR-TB) with multidrug-resistant TB (MDR-TB) and during first decade of the 21st century, extensively drug-resistant TB (XDR-TB) was emerged[8]. There are reports of occurrence of extremely drug-resistant TB (XXDR-TB), super XDR-TB, totally drug-resistant TB (TDR-TB)[52] from some parts of the world including India[48].

The etiological agent:

The *Mycobacterium tuberculosis* complex (MTC) includes five *Mycobacterium* species- *M. tuberculosis, M. canettii, M. africanum, M. microti, M. bovis* and two subspecies- *M. caprae* and *M. pinnipedii*. These species are characterized by 99.9% similarity at nucleotide level and their identical 16S rRNA sequences[19,42]. but differs in terms of phenotype, host tropism and pathogenicity. The name *"Mycobacterium"*, meaning fungus like bacterium and is derived from mould-like appearance of *Mycobacterium tuberculosis* when grown on liquid media. They are characterized phenotypically as Gram positive bacilli or cocco-bacilli, strictly acid-fast, vary from 0.5-10µm × 0.2-0.6µm, non-motile, non-capsular, non-spore former and facultative intracellular aerobes. They are not heat-resistant, being readily killed by normal milk pasteurization conditions but resistant to drying and can persist in environment for long periods. Their cell wall is rich in lipids *i.e.* mycolic acid which is responsible for acid fastness, hydrophobicity and provides resistance to many disinfectants, laboratory stains, physical injuries, antibiotics and lysosomal enzymes[3]. The genome of MTC consists of double stranded circular

deoxyribonucleic acid (DNA), consisting of approximately 4.4 million base pairs. The genome has a high (G + C) content approximately 65%[4]. Almost all members of *M. tuberculosis* complex have 0-25 copies of IS6110 gene but only one copy of IS*6110* found in *M. bovis.* Among the various repetitive sequences only IS6110 and IS1081 are insertion sequences and others are short sequences with no known function. The direct repeat (DR) region in *M. tuberculosis* complex strains is composed of multiple direct variant repeat sequence (DVRS) each of which is composed of a 36 bp direct repeat and a non repetitive spacer sequence of similar size. It has been shown that there is extensive polymorphism in the direct repeat region by the variable presence of direct variant repeat sequence and this polymorphism is used for differentiation of mycobacterium species and to define epidemiological relationships[24].

Epidemiology:

Bovine TB has emerged as an economically significant disease affecting both animals and human. It is distributed globally except Antarctica, Caribbean islands, parts of South America and 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, the United States, Mexico, and some countries of Central and South America where it has been eradicated by following strict test and slaughter policies[47]. It is a major public health problem mainly in developing countries including India. Factors which influence the occurrence of disease are sex, breed and social management of livelihood conditions[1]. Hereditary and maternal factors also influence incidence of TB in cattle progeny. Infected cattle are the main source of infection for other animals and human beings also. Organisms are excreted in exhaled air, sputum, faces, milk, urine, vaginal and uterine discharges and discharges from open peripheral lymph nodes[55]. It is more prevalent in dairy workers because of their close association with animals.

Transmission:

The transmission between animals occurs mainly through aerosols. The transmission rate is increased by close contact among animals and intensive breeding[14]. Other rare routes like cutaneous, congenital and genital have also been reported. Suckling calves can get the infection through consumption of infected milk. Sometimes, tuberculosis can be foodborne also[22]. The infected bull may also transmit disease through artificial insemination with the use of infected semen[55]. Others factors like long survival period of the organism in environment also contribute to an increased risk of infection[2,12]. It is quite likely that animals can be infected with human-originated *M. tuberculosis*, becoming a reverse zoonosis, which in turn may act as a source of infection for humans.

Pathogenesis:

The mechanism of pathogenesis of tuberculosis caused by *M. tuberculosis* and *M. bovis* is almost same as they have 99.9% similarity at nucleotide level and identical 16S rRNA sequences[54]. After entry into the lungs, the bacteria are phagocytosed by the alveolar macrophages. Various chemokines and cytokines releases as a result of interaction of mycobacteria with macrophage receptors that serve as signals. By inhibiting phagolysosomal fusion, *M. tuberculosis*, *M. bovis* and *M. avium* can survive inside the macrophages[38,53]. It will result in migration of macrophages and dendritic cells to the site of infection. The dendritic cells engulf bacteria and migrate to the lymph nodes. In response to mediators produced by infected cells, primed T cells migrate back to infection site in lungs and leads to granuloma formation. The granuloma is formed by necrotic cells in the center of the tubercle surrounded by epitheloid cells and multinucleated giant cells, encapsulated by connective tissue[25,26]. The necrotic core of cells can often become calcified as the tubercle matures[5,16]. The infection can spread via hematogenous route to lymph nodes and to other organs[6].

Clinical signs and symptoms:

The disease usually take months to develop and can affect any part of the body but generally affect lungs and nearby lymph nodes. In early stages, the infected animals remain asymptomatic. Infection can reactivate during stress or in old age. During late stages, common symptoms include progressive emaciation, low grade fluctuating fever and capricious appetite. Pulmonary involvement characterized by moist cough that is worse in the morning and during cold weather[50]. In advanced cases, blood vessels, air passages or alimentary tract may be obstructed by enlarged lymph nodes. Sometimes affected lymph nodes may rupture and drain. Involvement of the digestive tract is manifested by intermittent diarrhea and constipation in some cases. In terminal stage, animals may become severely emaciated and develop severe respiratory distress[49]. Tuberculosis mastitis is of major public health importance and difficult to differentiate it from other forms of mastitis[31].

Diagnosis:

The increase in incidence of infection caused by *M. bovis* and *M. tuberculosis* in animals and humans has become the subject of investigation. The detection of early infection is dependent on cell mediated immunity as reflected in the response to diagnostic tests such as tuberculin test and cytokine assays. The introduction of molecular techniques has greatly reduced identification time and improved the level of detection in clinical specimens.

Smear microscopy and Histopathology:

Smear can be made from discharges of affected lymph nodes or nasal swabs and stained with Ziehl-Neelson'S (ZN) stain or auramine-rhodamine stain. The red coloured acid fast bacilli appears when examined under microscope after staining. But staining method have less sensitivity and specificity. During necropsy of suspected animal, tissue samples are collected and examined for histopathological lesions. The whitish hard caseous nodules present on different visceral organs and gritty sound will be there when slicing of these granulomas. Ziehl- Neelsen staining is done but to increase the sensitivity, auramine-rhodamine stain can also be used. The central to periphery of the nodule consists of necrosed mass, calcium deposits, acid fast bacilli, lymphocytes, epitheloid cells and fibrous connective tissue. The stained slides are observed under light microscope for the presence of red stained acid-fast bacilli[57].

Culture isolation:

The culture isolation and biochemical characterization of *M. bovis* from apparently healthy animals shows active transmission in the infected herds[20]. The egg based LJ media and stone brinks media are most commonly used in veterinary bacteriology. An agar-based medium such as middle brook 7H10 and 7H11 or blood based agar medium may also be used. *Mycobacterium tuberculosis, M. avium* and many of the atypical *mycobacteria* require glycerol for growth and *M. bovis* grows in absence of glycerol and its growth can be supported by pyurvate. The luxuriant growth of *M. tuberculosis* on glycerol containing media, giving the characteristic rough, tough and buff colonies is known as eugenic, the growth of *M. avium* on media containing glycerol is also eugenic but *M. bovis* has sparse, thin growth on glycerol containing media which is called as dysgenic[43].

Tuberculin skin test:

Affected animals develop immune response, which can be detected by tuberculin skin test. Tuberculin is a purified protein derivative made by growing TB bacteria, heat killed followed by filteration or chemical treatment. The purified protein derivative (PPD) should have potency of 2000IU. In cattle with diminished allergic sensitivity, a higher dose of PPD is needed and the volume of each injection dose must not exceed 0.2ml[32]. After 72 hours of tuberculin intradermal injection, characteristic swelling appears at the point of injection. The diameter of the swollen area more than 4mm is a positive test result, indicating exposure to one type of mycobacteria. Different types of tuberculin skin test could be used to increase the

specificity such as comparative tuberculin skin test, stormont test, short thermal test *etc*[17].

Interferon Gamma (IFN-gamma) assay:

This in-vitro assay detects specific cell mediated immune response by the circulating lymphocytes. The use of defined *Mycobacterium* antigens such as ESAT-6 and CFP-10 shows promise for improved specificity. The use of these antigens may also offer the ability to differentiate BCG-vaccinated from unvaccinated animals. The assay is based on the release of IFN-gamma from sensitized lymphocytes during 16-24 hours incubation period with specific antigen. The detection of bovine IFN-gamma is carried out with a sandwich ELISA that uses two monoclonal antibodies to bovine gamma-interferon[18].

Lymphocyte proliferation assay:

It is a more specific in-vitro assay which compares the reactivity of peripheral blood lymphocytes to PPD from *M. bovis* (PPD-B) and PPD from *M. avium* (PPD-A) and can be performed on whole blood or purified lymophocytes from peripheral blood samples. Results are usually analyzed as the difference in value obtained in response to PPD-B and PPD-A. It is not used for routine diagnosis because the test is time-consuming and the logistics are complex and the use of radio-active nucleotides[46].

Enzyme-linked immunosorbent assay (ELISA):

It appears to be the most suitable of antibody-detection tests and can be a complement, rather than an alternative, based on humoral immunity. It is a valuable complementary tool in-order to identify possible anergic cows that may be acting as reservoirs of the agent. Advantage lies in its simplicity, but limited sensitivity because of the late and irregular development of humoral immune response in cattle during the course of the disease. 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[32].

Polymerase chain reaction (PCR):

It is a very sensitive technique and can detect the presence of an organism when present at very low levels. It is used to detect the presence of genetic material (DNA) that is unique and specific to a particular organism and amplifies a portion of DNA that is specific for that organism and followed by gene sequencing[34].

Spoligotyping:

It is also called spacer oligonucleotide typing, a method for simultaneously detection and typing of *M. tuberculosis* complex bacteria. 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 36bp, which is interspersed by the non-repetitive DNA spacers of 35-41bp in length. Other MTC strains contain one or more IS6110 elements in DR-region[37].

Restriction fragment length polymorphism (RFLP):

It is considered as 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. However, this technique requires large amount of DNA and is therefore restricted to the mycobacterial cultures which take around 20 to 40 days to obtain sufficient DNA needed[27].

Treatment:

In animals, treatment is usually not recommended. Although anti-tuberculosis drug pyrazinamide is ineffective against *M. bovis*, the use of isoniazid and rifampicin could be used effectively. In developed countries, the reactor animals are sent to slaughter houses and in developing countries, the rector animals sent to gaushalas. But the reactor animals still remains a source of infection for other animals and human beings[30].

Prevention and control:

Bacillus Calmette and *Guerin* (BCG) vaccine developed in 1921 is the commonly available live attenuated vaccine for tuberculosis till date. But this vaccine has shown variable

efficacy in cattle. In support with an international venture, a number of vaccines capable of replacing BCG with primary immunogens and also as boosters for BCG are being studied[41]. To reduce the risk for tuberculosis transmission, most of the high-income countries implement tuberculosis control programs but such control programs are not routinely implemented in India. However, in spite of intensive test and slaughter policy in many countries, a low level tuberculosis problem still remains that is difficult to eradicate because of wildlife reservoirs[9]. Variations in the tuberculin testing frequency, tuberculin types used and the interpretation of the test results affect the apparent incidence and prevalence of the disease recorded. The identification and early disposal of infected animals form the basis of national bovine tuberculosis eradication programme worldwide.

CONCLUSION

It is necessary to diagnose the disease at an early stage to minimize the risk of transmission to animals and human beings both. New diagnostic techniques are being used for early and efficient disease diagnosis but the molecular mechanisms behind the pathogen escape from immune system are complex. Vaccines other than BCG vaccine are also under clinical trial. Working association between physicians, private sector, religious bodies and local nonprofit organizations should be strengthened for better dissemination of awareness about the disease.

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