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Bovine tuberculosis diagnosis: An overview

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Abstract
Bovine TB (bTB) is chronic bacterial zoonosis disease. Humans are the primary host of *M. tuberculosis* (TB), but *M. bovis* causes bovine tuberculosis, which affects humans, domestic animals, and wildlife. Over 9 million people are affected with tuberculosis (TB), which continues to be the world's top cause of death. National control programs in developed countries have effectively managed bovine TB, but complete eradication and maintaining a bTB-free status pose challenges due to the risk of spillover from wildlife reservoir hosts. Changes in climate, human and animal movements, closer interspecies interactions, and animal management have led to the re-emergence of bovine TB in many developed countries. Approximately 94% of the world's population is either poor or absent in regulation of bTB in cattle or buffaloes, and in many developing countries, bTB regulations programmes has not been economically or socially acceptable. Due to India's lack of a control mechanism poses a possible threat for infection and transmission of bTB around the world.

Keywords: Bovine tuberculosis (bTB), bacilli calmette guerin (BCG) vaccine, reservoir host

Introduction
One major, highly contagious bacterial zoonotic illness that affects people worldwide is tuberculosis (TB). The term "tubercles," which are non-vascular nodules that mostly develop in lymph nodes and the lungs, eventually spreading to the bones, joints, and other body parts of body (Trail, 1970) [20]. German bacteriologist Robert Koch identified the tubercle bacillus as the infectious agent of tuberculosis on March 24, 1882. Hence, every year, 24th March is celebrated as World TB day. Global TB report estimated TB incidence in countries (WHO, 2019) [12] (Fig 1).

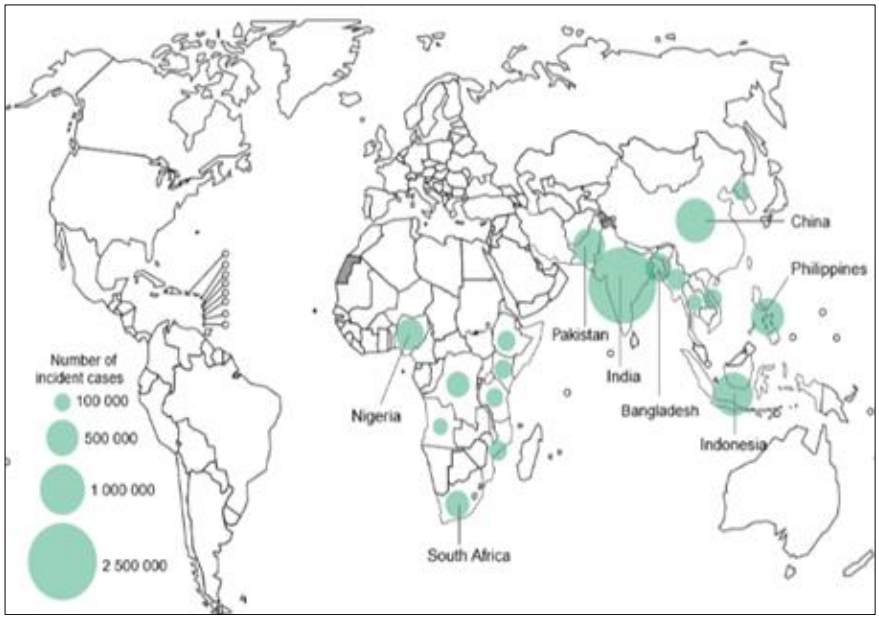

Fig 1: Global tuberculosis report (WHO, 2019)

M. tuberculosis mostly affects humans, whereas *M. bovis* causes bovine tuberculosis and affects wide host range involving domestic animal, human as well as wild animals (Verma *et al.*, 2014) [21]. Members of MTC includes *M. tuberculosis* (MTB), *M. bovis*, *M. microti*. *M. caprae*, *M. pinnipedii*, *M. africanum*, *M. mungi*, *M. canettii*, *Dassie bacillus* and *M. orygis* (Afzal *et al*., 2016) [1]. Bovine tuberculosis (bTB) zoonotic disease that is primarily caused by *M. bovis* and is easily transmitted to humans by the intake of unpasteurized infected milk or aerosol inhalation (Prasad *et al*., 2005) [14]. *M. avium* and *M. tuberculosis* is closely related to *M. bovis*. *M. bovis* shared 99.95% identical with *M. tuberculosis* (Taylor *et al*., 2007) [19]. *M. bovis* is thought to be responsible for about ~10% of all cases of human tuberculosis in developing nations, which makes it a significant threat to global health (Olea-Popelka *et al*., 2017) [13]. Approximately 25% of childhood TB cases were caused by *M. bovis* prior to the mandatory pasteurization of milk in several countries (Roswurm & Ranney, 1973) [16]. Mostly infections are chronic and may persist for a long time in the subclinical stage. The illness leads to 10-20% loss of weight and milk production, 10-25% decrease of reproductive efficiency, infertility and condemnation of meat (Lilenbaum *et al*., 2001) [10].

History

M. bovis was identified in 1898. In 1909, Griffith isolated *M. bovis* from the sputum of a butcher who had pulmonary tuberculosis. It was demonstrated clearly in 1911 that *M. bovis* could infect humans with all types of tuberculosis and that a greater number of infants got the infection through milk. It was one of the most common diseases affecting domestic animals worldwide up to 1920. By 1937, it was clearly shown that inhaling dust from *M. bovis* sheds causes pulmonary infections in humans.

Epidemiology

M. bovis may live for several months in the environment, especially in cold, dark, and wet environments. Depending on how much sunlight it gets at 12-24 °C, it can survive for 18-332 days (FAO, 1993) [6]. Extra-pulmonary forms of tuberculosis are mostly caused by *M. bovis*, which is eliminated by exhaled air, sputum, faeces, urine, milk, vaginal discharges, and uterine discharges (Verma *et al*., 2014) [21]. According to data from 1942 to 2016, a systemic review and meta-analysis revealed that the prevalence of bTB in different states of India ranged from 0.4 to 51.2% (Srinivasan *et al*. 2018) [18].

Hosts

Cattle is primary host of *M. bovis.* Mainly two type of hosts, maintenance host which include wild animal like feral pig, possums, ferrets, badgers, bison, elk, kudu, white tailed deer, feral water buffalo (Corner *et al.*, 2006) [5] and spillover host which includes sheep, goat, horse, pigs, dogs, others (Fig 2).

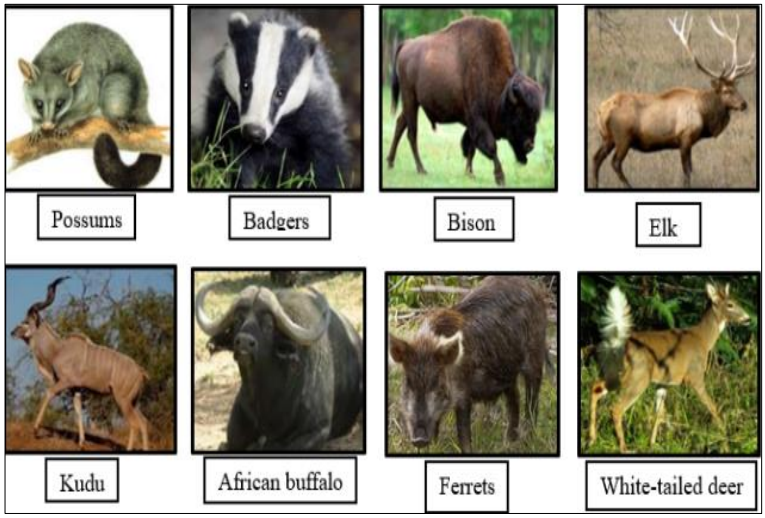

Fig 2: Maintenance host of bTB

Mode of Transmission

Animal to Animal: Transmission- vertically congenital infections, which are extremely rare in developed countries and horizontally ingestion and inhalation of aerosols (90%).

Animal to Human: Transmission-pulmonary TB, gastrointestinal TB and HIV people.

Human to Animal: Transmission - Rare, Genito-urinary TB- urination in cowsheds / pasture.

Human to Human: Transmission- Rare, HIV infected are highly susceptible (Kalkidan *et al*., 2017; Anonymous, 2019) [9, 3] (Fig. 3).

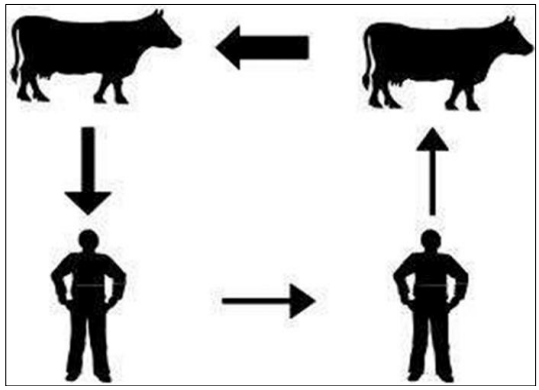

Fig 3: Transmission cycle of *M. bovis* between humans and cattle. The arrows thickness indicates the degree of probability (Anaelom *et al*., 2010) [2]

Population in risk

1. Veterinary professionals, lab techs, farmers, slaughterhouse employees, and anyone with close contact with animals are among the occupations that pose a risk.
2. Susceptible age group which includes infants, pregnant women and aged person
3. Medical conditions which include HIV positive humans and diabetics person.
4. Risk factors associated with consumption include those who are malnourished, drink unpasteurized milk, and eat raw or undercooked meat.
5. Developing nations are characterized by inadequate housing, overcrowding, poor sanitation, and a lack of health care.

Pathogenesis

M. bovis is shed via aerosol route, take up by inhalation and enters into pulmonary alveoli. Mycobacteria are engulfed by alveolar macrophages, followed by intracellular replication and spread to regional lymph nodes. After 30 to 50 days of infection tubercle formation and delayed type hypersensitivity or cell mediated immune response develop. In effective cell mediated immunity most of the mycobacteria are engulfed by macrophages and are killed. Few surviving mycobacteria develop localized arrested pulmonary lesion while shedding does not occur. In ineffective cell mediated immunity there is extension of lesions that leads to development of either active pulmonary tuberculosis which include necrosis and erosion of bronchial wall along with shedding of *M. bovis* by aerosol and faecal route, or it may spread via lymphatics or blood stream to lymphoid tissues, serosal surfaces and parenchymatous organs and develop generalized tuberculosis and this is open case of shedding *M. bovis* in mucus, urine, faeces and milk (Queen *et al.*, 2011) [15].

Clinical Symptoms

Clinical symptoms in animals can be asymptomatic in the early stages and progressive emaciation, low-grade fluctuating fever, moist cough, dyspnoea, enlarged draining lymph nodes, and udder induration in the late stages; in humans, the symptoms include coughing lasting longer than three weeks, blood in the sputum, chest pain, weight loss, chills, and night sweats (Müller *et al.*, 2013).

Diagnosis

The history, clinical symptoms, and postmortem lesions are used to diagnose bTB. Milk, aspirates from cavities, lymph nodes, and biopsies are specimens that should be obtained from living animals; lung and tubercle nodules should be obtained from dead animals. Post-mortem lesions seen are granulomas (tubercles) in the lung, lymph nodes, spleen, liver and the surfaces of body cavities (Kalkidan *et al.*, 2017) [9]. Laboratory diagnosis is carried out using direct smear examination, cell mediated immune response, serological test, molecular test and histopathology.

The Intradermal tuberculin test is recommended by the World Organization for Animal Health, whereas cultural isolation and biochemical characterisation are considered the "Gold standard" tests among all of them. *M. bovis* belongs to the risk/hazard group III of zoonotic organisms. For handling of TB infected material in laboratory 2 with biosafety level-3 (BSL-3) is required (Queen *et al.*, 2011) [15].

Direct microscopy-smear examination by acid fast stain

1. Conventional Microscopy with sensitivity of 32 to 94% -hot AFB staining (Ziehl-Neelsen) and cold AFB staining (Kynon staining).
2. Fluorescent Microscopy with sensitivity of 52% to 97%- Auramine / rhodamine stain.

Animal inoculation

Guinea-pigs are extremely susceptible to *M. bovis* and *M. tuberculosis* infections. *M. bovis* and *M. avium* infections are very common in rabbits (Queen *et al.*, 2011) [15].

Serological test

The infection known as bovine tuberculosis primarily induces a cell-mediated immunity (CMI) in the early stages of the disease. As the illness advances, a transition from Th1 to Th2 is linked to a reduction in CMI and the emergence of serological (humoral) responses (McNair *et al*., 2007; Rapid immunochromatographic Assay, ELISA Assay; Schiller *et al*., 2010) [11, 17].

Cultural isolation

The ground-up specimens need to be treated with decontaminating agents, such as 4% sodium hydroxide or 5% oxalic acid, and then the acid or alkali needs to be neutralized. After that, the specimens can be cultivated in Stone Brink or Lowenstein Jensen medium. Glycerol is necessary for the growth of *M. tuberculosis*, *M. avium*, and several atypical mycobacteria (eugonic). For *M. bovis* (dygonic), glycerol is a blocker, whereas sodium pyruvate promotes growth (Queen *et al*., 2011) [15] (Table. 1). BACTEC 460 TB is radioactive technique regards to this problem Becton and Dickinson developed a unique system called Mycobacteria Growth Indicator Tube (MGIT™) (non-radiometric and similar to BACTEC 460 TB System) and also used in drug susceptibility testing (Hines *et al*., 2006) [8]. According to the MGIT tube principle, the degree of oxygen depletion is closely correlated with the fluorescence intensity (Fig. 4).

Fig 4: BACTEC (MGIT™) 460

Table 1: Cultural medium for mycobacteria

Types of media	Examples	Time taken
Solid medium	Lowenstein -Jensen (LJ), Stone brink's, Dorset egg medium, Coletsos medium	6-8 weeks
Liquid medium	liquid Middlebrooks (7H9) broth media	14-15 days
Automated liquid systems	Bactec MGIT 960	15.8±0.8 days
	Bactec 460TB	28.2±1.0 days

Molecular diagnosis

Tissue, blood, milk and nasal exudates used for PCR based detection of Mycobacteria (Figueiredo *et al*., 2010) [7]. The most popular technique in *M. bovis* investigations is spoligotyping. Variable number tandem repeat (VNTR) typing in conjunction with spoligotyping is currently considered to be the most effective method for epidemiological analysis of *M. bovis* strains.

Test of cell mediated (CMI) immunity

1. Intradermal tuberculin tests.
2. Interferon-gamma (IFN-γ) assay.
3. Lymphocyte proliferation assays.

Recent Techniques

1. MALDI TOF (Matrix Assisted Laser Desorption Ionization Time of Flight MS) Very rapid technique (< 1 hour).
2. GeneXpert (Cepheid) System Rapid, simple and Gives species identification and drug susceptibility.

Public health significance

1. *M. bovis* is responsible for 5-10% of human tuberculosis cases
2. Has an effect on the respiratory system, meninges, genitourinary system, skin, bones, joints, and lymph nodes
3. Ingestion of contaminated milk causing scrofula, or cervical lymphadenopathy, in children
4. **Butcher's warts:** A localized, benign, self-limiting skin condition
5. **Lupus vulgaris:** A persistent skin disease

Prevention & Control

For prevention and control of bTB test and slaughter, test and segregation and good hygiene and management practices, tuberculin testing, slaughter/abattoir, surveillance, chemotherapy and vaccination and control wildlife reservoir hosts are recommended (Brooks-Pollock *et al*., 2014) [4]. Bacilli Calmette Guerin (BCG) vaccine was introduced in 1921. The only vaccine against tuberculosis that is currently available. When applied intradermally, 0.1 ml provides 15 to 20 years of protection. BCG (human cytopenia gene therapy) uses an attenuated wild type *M. bovis* isolate from cattle. However, BCG vaccination is less effective against bovine tuberculosis, hence it is not recommended.

Conclusion

Bovine tuberculosis, caused by Mycobacterium bovis, remains a global zoonotic concern. With complex transmission routes and varied hosts, including humans and wildlife, accurate diagnosis through clinical evaluation and advanced laboratory techniques is crucial. Prevention strategies encompass tuberculin testing, vaccination, and surveillance to curb transmission risks. Collaborative efforts are essential for effective control, emphasizing the importance of public health interventions and community engagement in mitigating the impact of this persistent disease.

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