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Popular Article

Bovine Tuberculosis in India: Zoonotic Perspective and Available Diagnostics

Kushal Grakh

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PhD Scholar, Department of Veterinary Public Health and Epidemiology, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar-125004, Haryana, India

Introduction

Bovine tuberculosis (bTB) and is a chronic and contagious bacterial disease of bovines caused by *Mycobacterium bovis* (*M. bovis*). *M. bovis* constitutes an undeniable portion of human TB cases worldwide which line this disease as an important global threat to animal and public health.

Distribution in India

The situation of bovine TB in developing countries like India is more dreadful due to a huge susceptible livestock population (300 million), and that too in close existence with human population. In India, bTB has been reported from several states (Fig. 1) with a varying pooled prevalence. Overall, India has a prevalence of 7.3% for the bTB, meaning around 21.8 million bovine populations in India might have bTB.

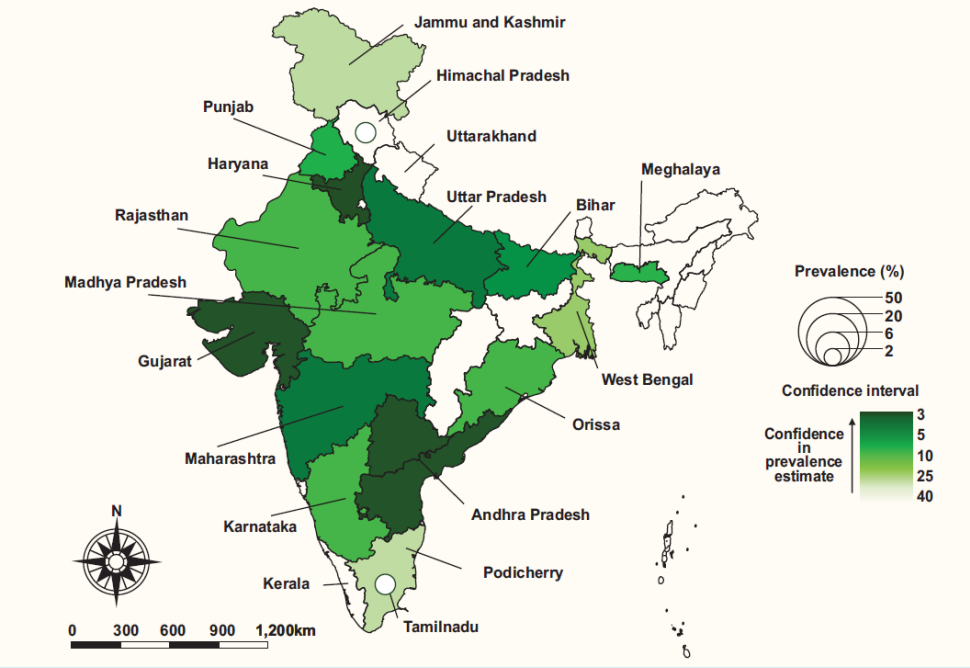

Fig. 1. Distribution and prevalence of bovine TB (bTB) in India (Srinivasan et al. 2018)

A survey conducted in central India with three different groups of population reported an incidence of 8.9 to 12.6% of bTB. However, the actual prevalence of bTB in humans seems to be under-estimated due to lack of surveillance of *M. bovis* in humans, ineffective differentiation between *M. bovis* and *M. tuberculosis* and lack of awareness among people, which results in under diagnosis and under-reporting of human cases due to *M. bovis.*

Transmission

As per the environmental conditions, *M. bovis* may survive for several months in soil, feed and faeces. However, direct exposure to sunlight, dry environment and high temperature may kill the bacteria within days or weeks.

***Transmission in animals*:** Infected animal can shed *M. bovis* organism intermittently in respiratory secretions, milk, feces and other bodily fluids (urine, vaginal secretions and semen) and act as source of infection to other susceptible animals. Inhalation of droplets containing *M. bovis* bacilli is a major route of infection in bovines. Furthermore, direct contact via mucous membranes and broken skin are other possible routes in disease transmission. Dogs with the renal signs for tuberculosis can transmit the infection through urine.

***Transmission in human*:** Ingestion, inhalation, entry through mucous membranes and breaks in the skin are major routes of disease transmission. Humans acquire *M. bovis* infection by drinking of raw/unpasteurized milk and dairy products and by eating of under-cooked meat products from *M. bovis* infected animals. Human to human transmission by aerosol route can also occur, if a person suffers from respiratory form of bTB. Farmers, dairy workers, slaughterhouse workers and animal handlers in close-contact with animals are at greater risk of acquiring infection.

Clinical signs

Animals: Bovine tuberculosis is a chronic and debilitating disease, but may have a quick onset. Infected cattle are asymptomatic during early stages of the infection but as the disease progress, loss of appetite, low grade intermittent fever, weakness and gradual weight loss are observed. If the respiratory tract is involved there is difficulty in breathing with moist, intermittent cough that aggravates in the morning and during cold weather seasons or exercise. Enlargement of lymph nodes such as retropharyngeal and superficial lymph nodes can be seen and sometimes these lymph nodes may rupture and drain. Intermittent diarrhoea or constipation may develop. Some other less common signs might include eye lesions, repeated abortions and infertility. Symptoms of bTB are generally similar in other species of animals as well, but the predominant syndrome or course of the disease may differ.

Human: Symptoms due to TB caused by *M. bovis* are more or less similar to the TB caused by *M. tuberculosis*, which may include fever, tooth abscess, chronic weight loss, chest pain and night sweats. If lungs are involved symptoms of persistent cough, shortness of breaths and blood spitting may be there. The involvement of gastrointestinal system can cause abdominal pain and diarrhoea. Lymph node enlargement is also a prominent clinical sign associated with *M. bovis.* In children consuming raw milk or improperly pasteurized milk from *M. bovis* infected animals may develop cervical lymphadenopathy of the tonsillar and preauricular lymph nodes and these nodes may suppurate and drain to the skin, which results into chronic skin lesions. Skin lesions have been given various names like scrofuloderma and lupus vulgaris. Cutaneous lesions can appear as papules, suppurative lesions, ulcers, soft gelatinous plaques with a central atrophy, reddish-brown to gradually enlarging subcutaneous nodules or as a vegetative lesion looks like a tumor. Central Nervous System (CNS) form of bTB causes chronic meningitis, has an insidious onset and is most often seen in immune suppressed young children and older adults. Meningoencephalitis is also reported in all age groups. Disseminated disease usually affects both pulmonary and extrapulmonary organs and occasionally includes widespread skin lesions.

Diagnostic tests available

In animals

Smear microscopy: Acid-fast staining (Ziehl–Neelsen) of smears directly prepared from the clinical samples and tissue samples to visualize the presence of acid fast bacilli of Mycobacterium species. Fluorescent acid-fast stain also can be used.

Culture and identification: For primary isolation, Lowenstein–Jensen, Coletsos base or Stonebrinks supplemented with either pyruvate or pyruvate and glycerol are in practice. An agarbased medium such as Middlebrook 7H10 or 7H11 supplemented with ODAC and egg yolk also can be used for isolation. A minimum incubation of 10–12 weeks (8 weeks optimum) at 37°C with or without CO2 is required to visualize the colonies. *M. bovis* shows dysgonic growth, is negative for nitrate reduction, niacin accumulation, nicotinamidase and pyrazinamidase. *M. bovis* is positive urease test. *M. tuberculosis* has eugonic growth, is positive for nitrate reduction and niacin accumulation. A major criterion for the differentiation of *M. bovis* is its intrinsic resistance to pyrazinamide.

Nucleic acid detection assays: Polymerase chain reaction (PCR) or Gen Probe TB complex DNA probe targeting 16S–23S rRNA, the insertion sequences (IS6110 and IS1081), MPB70 and the 38 kDa antigen genes have been used for rapid identification of *M. tuberculosis* complex. For specific identification of *M. bovis,* PCR targeting a nucleotide mutation at positions 285 (adenine residue for *M. bovis* and guanine residue in other MTBC) in the oxyR gene, 675/756/1311/1410 and 1450 of the

gyrB gene and 169 in the pncA gene and presence or absence of Regions of Difference (RD) are routinely employed. Spacer oligotyping (spoligotyping) is the most widely used DNA fingerprinting technique to differentiate *M. tuberculosis* complex, including *M. bovis* and also to distinguish *M. tuberculosis* from *M. bovis.*

Single intradermal tuberculin test: The test involves intradermal injection of bovine tuberculin (Purified Protein Derivative-PPD) of 2000-5000 IU (not more than 0.2 mL/injection) on the mid neck or caudal fold of the tail and measuring of the skin of swelling/thickness (delayed hypersensitivity) 72 hours after injection.

Comparative intradermal tuberculin test: The comparative intradermal tuberculin test need to be used for differentiation of animals infected with *M. bovis* and other mycobacteria. It involves the intradermal injection of bovine tuberculin and avian tuberculin into different sites on the same side of the neck and measuring delayed hypersensitivity 3 days later. In India, ICAR-IVRI provides PPD for *M. bovis* to be used for intradermal tuberculin test in bovines.

Gamma interferon (IFN- γ) assay: This test is based on the measuring of lymphokine gamma interferon (IFN- γ) released from the whole blood culture sensitized for 16–24 h with avian tuberculin and bovine PPD. Released bovine IFN- is detected with sandwich ELISA, which uses two monoclonal antibodies against the bovine gamma-interferon. The test is available commercially for bovine species and primates.

***In human*:** Tuberculin skin tests, direct microscopy for acid-fast bacilli, imaging techniques (chest X-rays, CT scans and MRIs), culture, IFN- γ release assays, PCR, Real Time PCR-based and/or other nucleic acid assays are used for the diagnosis of tuberculosis including *M. bovis.* Cartridges Based Nucleic Acid Amplification Test (CBNAAT) is nowadays used readily for quick and effective TB diagnosis. Another indigenously developed TruNAT test showed promising results for TB diagnosis and is being used for quick diagnosis and antimicrobial sensitivity of Mycobacterial species.

Treatment

Animals: Treatment of bovine TB is not recommended. The infected animal should be culled or removed from the herd.

Humans: Treatment regimen for new TB cases includes intensive phase of HRZE (Isoniazid (H), Rifampicin (R), Pyrazinamide (Z), and Ethambutol (E) for 8 weeks and a continuous phase of HRE, which lasts for 16 weeks. In cases of drug resistant TB such as MDR-TB, XDR-TB, different drugs are used in combination, details of which are available on official website (https://www.mohfw.gov.in/) of Ministry of Health and Family Welfare, Govt. of India.

Prevention

***Animals*:** The spread in animal population can be prevented by regular herd screening using intradermal tuberculin testing and segregation of the test positive animals. Before introducing new animals to herd, bTB test either by blood based or i/d skin test should be done. Animal handlers and other staff in close contact with animals must be tested for TB prior to handling animals using suitable tests. Currently there is no vaccine validated to be used in animals; however, BCG vaccine has been used in some countries.

***Humans*:** The raw/unpasteurized milk and milk products made from unpasteurized milk and under-cooked meat must not be consumed. The individuals in frequent contact with bovines (animal handlers, butchers, veterinarians, para-veterinary staffs) are being at the most risk should use protective clothing and equipment such as mask, gloves, etc. while dealing/handling animals. Open wounds, if any, should be covered properly before handling of bovines. Individual showing signs relevant to TB must get tested themselves as they can be the source of infection to other humans as well as animals. Vaccination in early childhood (up to 1 year of age) with BCG vaccine is recommended in India. The goal of WHO's End TB Strategy is to end the global TB epidemic by 2030. India also has set NTEP to eradicate TB by 2025.

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