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Advancements in Molecular Diagnostics for Bovine Tuberculosis: Implications for Wildlife Disease Surveillance and Homeopathic Treatment Approaches

Avances en el diagnóstico molecular de la tuberculosis bovina: implicaciones para la vigilancia de enfermedades en fauna silvestre y enfoques de tratamiento homeopático

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ABSTRACT

Bovine tuberculosis (bTB) is a chronic, infectious, and zoonotic disease caused mainly by Mycobacterium bovis, a member of the Mycobacterium tuberculosis complex. It affects cattle, other domestic animals, and wildlife, generating serious challenges for animal health, public health, and livestock productivity. Human infection occurs primarily through the consumption of unpasteurized dairy products or direct contact with infected animals, making agricultural, dairy, and veterinary workers particularly vulnerable. Globally, bTB remains a persistent issue. According to the World Organisation for Animal Health (WOAH), 29 of 82 surveyed countries (35.4%) reported cases in both cattle and wildlife, highlighting its widespread nature and the complexity of cross-species transmission. The coexistence of infection in livestock and wildlife complicates eradication and requires a One Health perspective to achieve sustainable control. In India, the estimated prevalence of bTB in cattle is about 7.3%, meaning nearly 21.8 million animals could be infected. This high rate reduces productivity and elevates the risk of zoonotic transmission. Addressing bTB demands coordinated, multisectoral strategies, including regular testing and culling, milk pasteurization, stronger biosecurity, and awareness programs. Enhancing veterinary capacity and integrating animal, human, and environmental health approaches are essential to mitigate the impact of bTB.

Keywords: Bovine tuberculosis; Homeopathy; Mycobacterium tuberculosis complex; Real time Polymerase chain reaction; Tuberculin skin test

RESUMEN

La tuberculosis bovina (bTB) es una enfermedad crónica, infecciosa y zoonótica causada principalmente por Mycobacterium bovis, miembro del complejo Mycobacterium tuberculosis. Afecta al ganado, a otros animales domésticos y a la fauna silvestre, generando serios retos para la salud animal, la salud pública y la productividad pecuaria. La infección humana ocurre sobre todo por el consumo de productos lácteos no pasteurizados o el contacto directo con animales infectados, lo que convierte a los trabajadores agrícolas, ganaderos y veterinarios en poblaciones especialmente vulnerables. A nivel global, la bTB sigue siendo un problema persistente. Según la Organización Mundial de Sanidad Animal (WOAH), 29 de 82 países encuestados (35,4%) notificaron la presencia de casos tanto en ganado como en fauna silvestre, lo que refleja su amplia distribución y la complejidad de la transmisión entre especies. La coexistencia de la enfermedad en animales domésticos y silvestres dificulta los esfuerzos de erradicación y exige un enfoque integral de Una Sola Salud (One Health). En la India, la prevalencia estimada en el ganado es de aproximadamente 7,3%, lo que equivale a unos 21,8 millones de animales infectados. Esta elevada tasa reduce la productividad pecuaria y aumenta el riesgo de transmisión zoonótica. Para su control se requieren estrategias coordinadas como pruebas periódicas y sacrificio de animales positivos, pasteurización de la leche, prácticas de bioseguridad más estrictas y campañas de sensibilización, junto con el fortalecimiento de las capacidades veterinarias y la integración de la salud animal, humana y ambiental.

Palabras clave: Tuberculosis bovina; Homeopatía; Complejo Mycobacterium tuberculosis; reacción en cadena de la polimerasa en tiempo real; prueba cutánea de tuberculina

1. INTRODUCTION

Bovine tuberculosis is a chronic bacterial disease caused by members of the MTBC, particularly M. *bovis*. Wildlife, domesticated animals, and livestock are all susceptible to this serious zoonotic disease (1). In 2019, WOAH stated that 29 (35.4%) of the 82 nations have bTB in both cattle and wildlife (2). With a high number of cases and fatalities, the Asia-Pacific area is a significant TB hotspot. In 2021, there were an anticipated 10.6 million TB cases worldwide, with over 63% of those infections occurring in these regions (3-4). The incidence of bTB in cattle in India is estimated to be 7.3%, which means that around 21.8 million cattle are infected with bovine tuberculosis (5-6).

Taxonomy

Bacterial Morphology – rod shaped (14), sometimes branching (15), mold like colony surface (11).
Cell Wall - waxy lipid-rich, presence of mycolic acid (12).
Classification – Gram-positive, aerobic (14).
Diagnosis – acid-fast staining (13).

The primary cause of bovine tuberculosis is *Mycobacterium bovis*, while additional MTBC species, including M. *caprae* and M. *tuberculosis*, have been identified in diseased cattle (7-8). Virtually identical 16S rRNA sequences and a 99.9% nucleotide level similarity characterize the MTBC (9-10). Clinical diagnoses of BTB include progressive emaciation, low-grade fluctuating temperature, weakness, inappetence, and dyspnea. Infections may remain inactive for years before resurfacing in old age or stressful situations. BTB lesions in slain cattle are part of pathologic diagnosis. Granulomas, encapsulated, yellowish, and calcified, are characteristic of BTB. Cattle have tubercles in lymph nodes and other body cavities (69-73).

Medical Context

Infected animals may cough or exhale invisible droplets (aerosols) that carry TB bacteria, which vulnerable animals or people may subsequently inhale. In confined spaces, exposure risk is highest.16 Direct contact with aerosols from infected animals is the primary method of transmission (17). However, direct contact with an infected animal's milk, placenta, saliva, or nasal discharge may transmit bTB18 and indirectly in unhygienic settings or by consuming contaminated food or drink (19), excreta of an infected animal (20-21).

Rate and modes of transmission

The pathogenicity of M. *bovis* is high for both humans and animals (22). Globally, it accounts for 10–15% of human tuberculosis cases in developing nations (22) and fourth most significant livestock disease with serious risks to humans, other livestock and wildlife (23). Although infectious aerosols are the main way that M. *bovis* infections are transmitted, but they can also be transmitted by consuming contaminated grass or feed or drinking contaminated milk (24). Incubation periods can range from months to years (25). This chronic disease can rapidly progress and worsen (26-27). Animals usually exhibit clinical symptoms as they age or during periods of elevated stress (28).

Interventions for Public Health

Since M. *bovis* may cross the species barrier and infect humans and other mammals with TB-like disease, treating bovine tuberculosis is currently not advised owing to its contagious nature (29). An animal should be removed from the herd if it exhibits symptoms (30) making sure there is no direct or indirect interaction with humans, wildlife, or healthy livestock (16).

Prohibition of hunting and feeding infected animals, (28) drug therapies (31), culling of infected animals, improving sanitary and hygienic standards in dairy farms, (32) surveillance and regular screening are some control measures of BTB infection. Various TB control and eradication programs are being implemented globally and nationally to control the spread of BTB.

Calmette and Guerin developed the M. *bovis* vaccine in the 1920s. It does not stop infection, but it lessens the severity of the disease by letting the bacteria infect a small number of lymph nodes. An upgraded vaccination that can provide protection against the disease should be developed and administered to wildlife to avoid the transmission of the disease to or from livestock (16, 28). Wildlife vaccination poses a number of distinct difficulties, particularly in terms of delivery (33). The dissemination of oral baits is the most feasible delivery strategy (34).

Transmission in wildlife

Wildlife diagnosis is (i) a key component of disease control and management (35), but it is also crucial for (ii) assessing monitoring tactics, (iii) etiology, epidemiology, and transmission investigations, and (iv) determining the effectiveness of vaccine trials (35–37). But diagnosing animals can be difficult (35). Nonetheless, tuberculosis in animals remains an important topic of research (38).

Risk factor for transmission

Sharing grazing and watering locations between herds and with wildlife, eating undercooked meat, drinking raw or soured milk, and failing to take precautions when slaughtering cattle are examples of social and cultural practices that have been found to be risky for the spread of M. *bovis* infection (39).

Wildlife Species affected or Hosts

The following table captures essential information about tuberculosis (TB) prevalence across different species, locations, years, and pathogens studied in various research papers. Below are the details of each column.

Table 1. Case studies of bTB prevalence in Wildlife

S. No	Species affected	Scientific name	Location	Year	Pathogen	Remarks	Ref.
1	Bengal tiger	*Panthera tigris*	Gwangju Uchi Park Zoo, Gwangju, Republic of Korea	2006	*Mycobacterium avium* subsp. *avium*	feeding of tigers with culled chickens infected with *M.avium.*	(40)
2	Siberian tiger	*Panthera tigris altaica*	Budapest Zoological and Botanical Garden	October 2001	*M.bovis* subsp. *caprae*	Infection alimentary tract by ingesting infected meat	(41)
3	Leopard	*Panthera pardus*	*Stellenbosch University*	2020-23	*M.bovis*	ingestion of infected prey	(42)
4	Snow leopard	*Panthera uncia*		1998	*M.bovis*	ingestion of infected prey	(43)
5	Sloth bear	*Melursus ursinus*	Maharaj Bag Zoo, Nagpur	2016	*Mycobacterium tuberculosis*		(44)
			Jaipur zoo	1999		*Mixed infection of Mycobacteria and Cornebacterium pygens*	(45)

6	African Wild Dog	*Lycaon pictus*	Kruger National Park, South Africa	2019	*Mycobacterium bovis*	Direct contect with infected pack members and intake of infected prey	(46)
7	Gaur	*Bison bison*		1988	*M. bovis*		(47)
			Bandhavgarh National Park, Madhya Pradesh, central India	2016-2020	*Mycobacterium orygis*		(48)
8	Spotted dear	*Cervus Axis*	Nepal	2015	*M.orygis,*		(49)
			India	2010	*M.avium*		(50)
			Wildlife Sanctuary, Gujarat, western India	2016-2020	*M.tuberculosis, M.bovis, M.orygis*	Feces in pastures, contaminated water	(51-55)
9	Sambar deer	*Cervus unicolor*	Safari park, Rio Grande do Sul, Brazil	2021	*Mycobacterium bovis*		(56-57)
			Assam State Zoo	1984-1993		*Mycobacterium* of human and bovine	(58)
			Gujarat			Captive, *Mycobacterium* of human and bovine	(59)
10	White spotted deer (chital)	*Axis axis*	Ranthambore National park,India	1985		Contact with lifestock and sharing same water source	60)
			Kamla Nehru Park, Indore	1990		Transmission from human and other zoo animals	(61)
11	Sika deer	*Cervus nippon*	Maltro Garden Zoo, Bhillai Stell Plant, Bhillai	1993	*M.tuberculosis*		(62)
12	Langur	*Presbytis obscurus*	Metro park zoo,Ohio	1978	*Mycobacterium bovis*		(63)
13	Chimpan-zee	*Pan troglogytes*	Sri Chamarajendra Zoological Gardens, Mysore	1985	*Mycobacterium bovis M.hominis, M.avium*		(64)
14	Orangut-an	*Pongo pygmaeus*		1984			
15	Monkey	*Cercocebus torquatus*	Nehru Zoo Park, Hyderabad	1979	*M.tuberculosis*		(65)
16	Rhesus macaque	*Macaca Mulatta*	Central Drug Research Institue Lucknow	1987 -1989		*Mycobacterium* of human and bovine	(66)
17	Black rhinoce-ros	*Diceros bicornis*	Mysore zoo	1992-1994	*M.tuberculosis, M.bovis*		(67)
18	Asian Elephant	*Elephantas maximumus*	Kerela	1995	*Mycobacterium elephantis*		(68)

A selection of case studies of various wildlife infected with bovine tuberculosis are summarised in the above table according to the pathogen causing bTB, the species' scientific name, the case study's location, and the year it was conducted. These case studies offer important new information about how bovine TB affects various animal species across different geographical areas. Developing efficient management plans to safeguard livestock and wildlife populations requires an understanding of the disease's effects and transmission.

2. DIAGNOSTIC METHODS

2.1. Radiography

In 1930, pulmonary TB was described in the first radiographic description of feline mycobacteriosis (74). Standard nomenclature provides a clear description of lung anomalies together with a pattern diagnosis (75–76). It has been suggested that radiography might be a helpful diagnostic technique for mycobacterial infections. It has been reported that thoracic changes caused by M *bovis*, M *microti*, or M *avium infections* are highly variable and can include pleural effusion, tracheobronchial lymphadenopathy, bronchial or mixed-pattern lung infiltration, unstructured interstitial, miliary, or nodular interstitial changes (77–83). There have been very few reports of axillary lymph node involvement or pulmonary and/or lymph node calcification (84–85).

2.2. Tuberculin Skin test (TST)

The typical ante mortem test for tuberculin in cattle is based on adelayed type hypersensitivity to mycobacteria (16). It is the "gold standard" for diagnostic screening MTC infection and is both practical and economical (86). As recommended by the OIE, the tuberculin skin test remains the preferred method for diagnosing BTB in cattle, with the IFN-γassay serving as a backup test (87). In many nations around the world, the skin test has been employed as an individual animal test to help eradicate BTB from contaminated herds (88). The in vivo tuberculin skin test (TST), which gauges the delayed hypersensitivity reaction to mycobacterial Purified Protein Derivative (PPD) antigens, has been the most widely used test for diagnosing tuberculosis in animals for the past century in the majority of species (89–93).

2.3. Nasal discharge

Cattle that respond to tuberculin can have mycobacteria isolated from their nasal secretions. Cattle pulmonary exudates are often ingested and travel through the feces to pollute feed and the ground (94). According to reports, 70% of reactive cattle had tuberculous lung lesions when TB-positive animals were detected; in only 19% of verified cases was M. *bovis* isolated from nasal or tracheal swabs (95–96). Consequently, a more precise diagnosis of BTB may be possible with the integration of information from the pathology and bacterial culture (97–98).

2.4. Genetics Diagnosis

The proliferation of antigen-specific T cells is a component of in vitro CMI responses, and this may be measured by utilizing enzyme-linked immunosorbent assays (ELISA) to measure cytokine production or real-time quantitative polymerase chain reaction (real-time qPCR) to measure cytokine gene expression. (99–101). In many species, these assays are thought to provide a quicker and more accurate way to detect infected individuals than tests that rely on humoral reactions (102–103).

Recommended Methods of Diagnosis of Bovine Tuberculosis

ELISA: Antibody-based ELISA has superior sensitivity for identifying anergic animals and is practical, cost-effective, and simple to collect. However, TST enhances antibody response, so ELISA without skin testing may lower sensitivity. ELISA tests are used to supplement TST in recent years (105-110).

PCR: Real-time PCR assays targeted the RD4 region for M. *bovis* in cattle feces, using a universal bacterial assay to check DNA quality. Although PCR detection provides some evidence of M. *bovis* shedding in cattle feces, it doesn't definitively indicate viable organisms. The universal bacterial assay proved reliable for amplifiable DNA acquisition. More PCR-based techniques could increase detection limits and make it more effective for detecting M. *bovis* in cattle fecal samples (111, 116)

Homeopathic Approach: Homeopathy effectively controls bovine tuberculosis, overcoming side effects and expensive costs. Methodical approach, repertorization, and symptomatology recording aid remedy selection. Homeopathy improves immunomodulation, lowers disease burden, and offers a long-term, comprehensive treatment option in veterinary medicine.

For the diagnosis of bovine tuberculosis, the following steps should be adapted:

- Discharges, respiratory patterns, and other systemic symptoms should be noted.
- Underlying symptoms such as weakness, emaciation, chronic cough, and swollen glands should be identified.
- General behaviour and food preferences should be observed.
- A standard repertory such as Boenninghausen's Therapeutic Pocket Book, Kent's Repertory, or Boger's General Analysis Synthesis Repertory should be referred to.
- Assign numerical values to each symptom based on intensity and frequency; the case should be tabulated and cross-referenced with remedies listed in the repertory.

Homeopathy Grading System (3-Pont Scale) in Remedy Selection

The homeopathy grading system assesses the severity and applicability of symptoms for specific treatments. Grades are assigned based on symptom similarity in the repertory. Grade 1 indicates weak indication, meaning the medicine is less commonly recommended. Grade 2 indicates moderate signal, but not the strongest match. Grade 3 indicates strong indication, suggesting the therapy is precise and often prescribed for the symptom.

Tuberculinum Bovinum is recommended for chronic TB patients with emaciation, cough, sputum, and fevers, assigned a grade of 3. Phosphorus is grade 2 and associated with hemorrhagic expectoration, weakness, and sensitive lung diseases. Silicea is grade 1 and classified as persistent cough, glandular swellings, suppuration propensity, and failure to heal. Calcarea carbonica is grade 2 and mildly advised for cold-related cough, sweat, and weakness.

The following table 2 represents grading for ready selection in Bovine tuberculosis.

Table 2. Comparison of homeopathic remedies according to symptoms associated with tuberculosis

Symptoms	Tuberculinum Bovinum	Phosphorus	Silicea	Calcarea Carbonica	Sulphur
Chronic cough with expectoration	3	2	1	2	1
Glandular swelling	2	1	3	2	2
Emaciation with weakness	3	3	2	2	2
Low-grade fever	2	3	2	1	1
Difficulty in breathing	2	2	3	1	2
Loss of appetite	2	3	2	3	2
Total Score	14	14	13	11	10

The most likely remedies for bovine tuberculosis cases are TuberculinumBovinum and Phosphorus, with Silicea as a secondary remedy for suppuration and glandular involvement, CalcareaCarbonica for slow-developing cases with weakness and sweating, and Sulphur for deep-seated chronic conditions with skin symptoms and weakness.

3. DISCUSSION

Bovine tuberculosis (bTB), which is largely caused by Mycobacterium bovis, is a major zoonotic concern due to its chronic progression and substantial interspecies transmission potential. Diagnosis in free-ranging wildlife involves logistical obstacles, necessitating the use of molecular diagnostics like ELISA and RT-PCR for accurate detection. Traditional clinical diagnostics are ineffective in non-captive conditions. Homeopathic intervention has immunomodulatory advantages, is cost-effective, and complements traditional control techniques. Integrative techniques that combine advanced molecular technologies with alternative medicines could improve disease surveillance, management, and long-term eradication efforts across species. Expanding on this, bTB continues to pose a threat to cattle and wildlife populations due to its capacity to spread across species, including humans. A wide range of transmission mechanisms, such as direct contact, environmental contamination, and aerosol dissemination, makes disease prevention difficult. In free-ranging wildlife, where traditional diagnostic procedures are problematic, molecular technologies such as ELISA (which detects antibodies) and RT-PCR (which recognizes genetic material from M. bovis) have emerged as critical tools for accurate identification. These procedures offer early detection, which allows for prompt action.

Beyond conventional procedures, homeopathic intervention has gained popularity as an alternative strategy, with possible immunomodulatory advantages that may boost natural disease resistance in affected populations. While homeopathy is not a standalone cure in itself, its low cost and consistency with existing control methods make it an excellent supplemental treatment option. Integrating homeopathy with molecular diagnostics and established veterinary methods could improve disease surveillance and management.

A comprehensive eradication strategy must balance contemporary molecular technologies with traditional remedies. Surveillance programs must address persistent wildlife reservoirs that constantly disrupt eradication efforts. Coordinating efforts across fields, such as veterinary science, epidemiology, and conservation biology, can improve disease control while reducing environmental impacts.

CONCLUSION

Bovine Tuberculosis (bTB) is a chronic infectious disease primarily caused by *Mycobacterium bovis*. It poses a serious threat to animals, wildlife, and public health. The disease spreads through direct contact, aerosol inhalation, and the consumption of contaminated food or water, making timely diagnosis and effective control strategies essential. In domestic animals, bTB can be diagnosed using methods such as X-ray and skin tests by observing their symptoms. However, in wildlife or free-ranging animals, where the disease has been observed, it is not feasible to diagnose using X-ray or skin tests. This research paper, after reviewing various articles, concludes that in free-ranging animals, bTB can be detected using molecular diagnostic techniques like ELISA and RT-PCR on fecal samples. For its treatment, alternative medicine like homeopathy has been found to be effective in controlling the disease. Homeopathic remedies are easy to administer to animals, cost-effective, and efficient. This approach can certainly be highly effective in wildlife management. Integrating conventional and alternative treatments requires strengthening research and policy frameworks, which can provide a more holistic and sustainable approach to bTB management.

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CONFLICT OF INTEREST

There is no conflict of interest related to the subject matter of the work.

AUTHOR CONTRIBUTIONS

Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Supervision, Writing – review & editing: Sandeep Fellows, Kajal Jadhav, Himanshu Joshi, Shailendra Singh Yadav, and Dilshad Masih.

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