

Inquisition of *Mycobacterium bovis* in Cattle and its Critiques

AUTHORS DETAIL

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Received: 14-10-2024

Revised: 2-12-2024

Accepted: 17-12-2024

Cite this Article as: Noor M, Tariq I, Raza R, Saeed S, Qamar U, Sharif H, Qurat-ul-ain, Manzoor I, Safdar A and Bibi M, 2024. Inquisition of *Mycobacterium bovis* in Cattle and its Critiques. In: Basit A, Khan SA, Muhyuddin S and Mughal MAS (eds), Anim Health Dis Management, Pioneer Page Publishers, Beijing, China, Vol. 2: 147-156. <https://doi.org/10.5281/zenodo.17072033>

Abstract

Mycobacterium bovis regularly kills cattle and wildlife all over the world. *M. bovis* infections can spread through several pathways but the respiratory and gastrointestinal systems are the main entry points. In certain nations, bovine TB is thought to be the cause of up to 10% of human TB cases. When cattle get bovine TB, they may have Swollen Lymph Nodes, Pneumonia, Otitis Media and Arthritis Infections. Usually, *M. bovis* has been diagnosed clearly through microbial culture, while other advanced techniques are; Polymerase Chain Reaction, ELISA, MALDI-TOF MS and Loop-mediated isothermal amplification. However, wear gloves and a breathing mask when working with infectious animals. This has thus come in handy through the control and eradication programs that have seen the *Mycobacterium bovis* disease almost nonexistent in developed nations or greatly reduced in agricultural animals to very low levels. Consequently, efforts must be made to produce effective vaccinations that will effectively prevent *M. bovis* infections.

Keywords: *Mycobacterium bovis*, Pneumonia, Swollen Lymph Nodes, ELISA, Polymerase Chain Reaction

Introduction to *Mycobacterium bovis*

The main cause of zoonotic tuberculosis in humans is *Mycobacterium bovis*, which regularly kills cattle and wildlife all over the world. The evolution and genetics of *M. bovis* have only been occasionally studied globally, and prior research revealed the existence of genetic groups of strains based on a small number of DNA markers (also known as clonal complexes) (Lombard et al., 2021). Approximately ten million new cases of tuberculosis (TB) are reported each year and it is the most prevalent infectious disease globally. Over ninety-five percent of the 1.5 million TB-related deaths that occurred in 2016 took place in nations with low incomes (Bespiatykh et al., 2021). Moreover, it is closely associated with malnourishment because the disease's prevalence varies by economic status both within and between nations. Furthermore, the transmission of bovine tuberculosis through animals directly to people is another significant source of burden to the global disease clientele. The World Health Organization (WHO) projected that zoonotic tuberculosis (TB) caused 12,600 fatalities and 143,000 new cases in 2018. These figures are probably understated because most countries do not routinely collect monitoring data (Kaur, 2019). The majority of strains of the causative agent are resistant to pyrazinamide, a key first-line drug in TB treatment. Additionally, a possible association with extra-pulmonary disease often delays diagnosis and the start of treatment, creating significant challenges for patients with zoonotic TB (Rito et al., 2023).

Furthermore, bovine TB causes significant financial losses for livestock producers worldwide by transcending national boundaries and frequently impeding efforts to conserve animals due to the creation of wildlife reservoirs or the spread of the disease from cattle to related animal groups (Revie et al., 2022). Twelve species or ecotypes of clonal bacteria with varying

host tropism and pathogenicity make up the MTBC. The primary cause of TB cases is *Mycobacterium tuberculosis*, which has evolved to live in humans. Nonetheless, *Mycobacterium bovis* is responsible for bovine tuberculosis, which is pathogenic in a broad host spectrum and can infect and produce disease in a range of host species, including humans, with distinct population stability (Bespiatykh et al., 2021). Lack of understanding of the biological characteristics of mycobacteria may contribute to the long-term epizootic development of tuberculosis in animals. Mycobacteria's ability to persist in animal species undoubtedly affects their biological characteristics, particularly the microbial cell's metabolism, the amount of lipids and other chemical components in the cell wall, and, mutations, modifications, and dissociative phenomena in one or more mycobacteria populations. As a result, diagnostic work quality declines and preventative actions become less effective. TB can be found anywhere in the world. Americas, most of the African continent, and parts of Asia have a higher prevalence of the disease. Bovine TB has been reduced or even eliminated from the cows in several developed countries of the world (Lombard et al., 2021). *M. bovis* has been well documented in many different domesticated and non-domesticated animal species, but cattle are believed to be the actual hosts. All the species of carnivoraria have been isolated and a list of some of them include; Buffaloes, cattle, sheep, goats, horses, camels, pigs, wild boars, deer, antelopes, dogs, cats, foxes, mink, badgers, ferrets, rats, primates, llamas, kudus, elands, tapirs, elks, elephants and sitat (Bespiatykh et al., 2021).

1. *Mycobacterium bovis* transmission and epidemiology

The respiratory and gastrointestinal systems are the main entry points, although *M. bovis* infections can spread through a number of pathways. Important features of pathogenesis, such as the infectious dose necessary to establish infection, are influenced by the route of infection. As a result, the route of infection affects an animal's vulnerability to *M. bovis* infection. Close communication with infected domestic and wild animals can also cause an infectious disease or other serious outbreaks. Self-inoculation by inhaling droplets contaminated with the virus which it expels through coughing, is the common mode of transmission (Krajewska-Wędzina et al., 2022).

Contamination by the bacteria may also occur from raw milk from infected cows, and the infection in the calves. Since the disease progresses slowly and may take months or years to kill an animal, an animal can pass on the disease to many others in the herd without having clinical signs of the disease. Adverse effects on animals by different routes, including intratracheal, oral, intravenous, and intraperitoneal routes in experimental studies, demonstrated that the type and degree of tuberculous lesions vary based on the animal exposure route. Among these are renal disease, gastrointestinal pathology, respiratory disorders, and abnormalities of the mammary glands (Fig. 1) (Lombard et al., 2021).

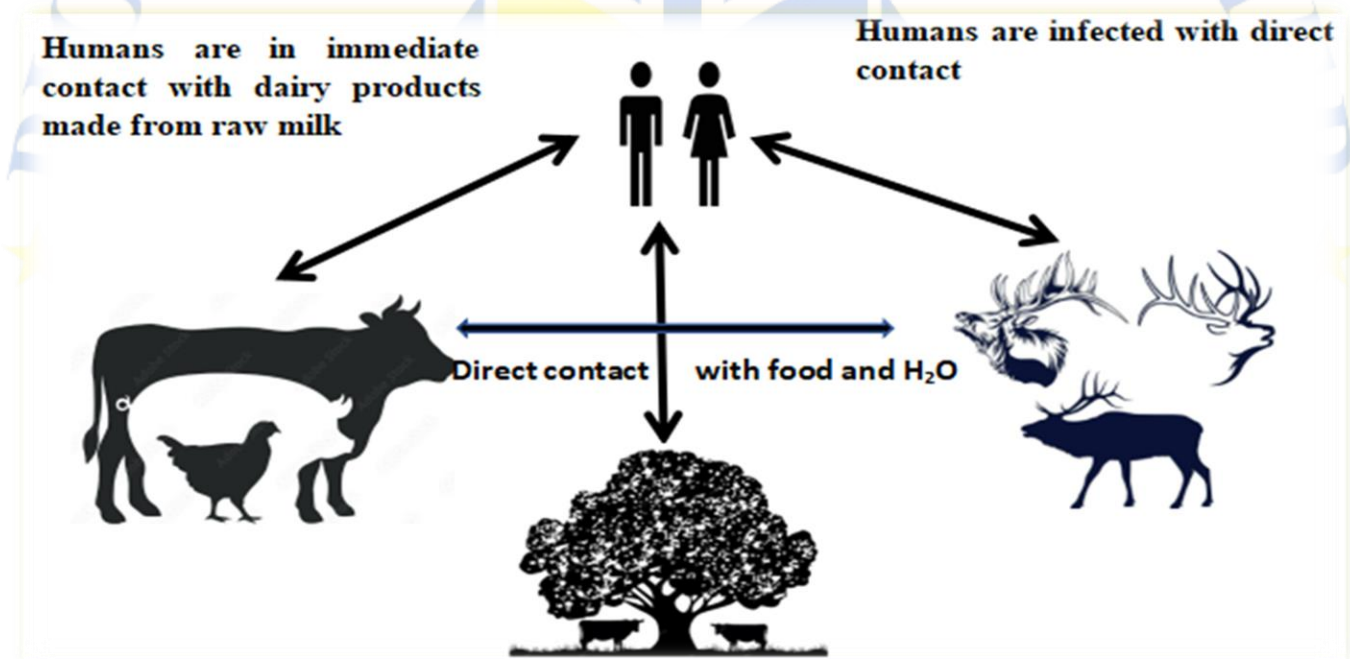


Fig. 1: Transmission Modes of *M. bovis*

M. Tuberculosis lesions do not isolate the mode of transmission of the disease, regardless of where they occur. However, the first-time exposure to the organism through ingestion or by inhaling it could cause lesions on the alimentary canal, as it has been found that naturally transmitted *M. bovis* commonly infects the lymph nodes of the respiratory and gastrointestinal tracts (Pokam et al., 2019). Despite most of the main nonaerosol sources of *M. bovis* infection in humans having been eradicated in industrialized countries, the number of new cases of *M. bovis* pulmonary infection is rising. Moreover, the post-primary *M. bovis* has been established to involve the lung consistent with a previous primary lesion, regardless of the region of the prior and cases of pulmonary *M. bovis* might be the effects of reactivation of prior non-clinical primary lesions. How *M. bovis* exits the infected host depends on the characteristics of the tuberculous lesions (Palmer & Waters, 2011).

M. bovis can be transmitted through ingestion, respiratory infection and lesion-associated pseudovertical transmission from the renal, gastrointestinal, respiratory and mammary tissues. For instance, geographical distribution proves relative throughout different regions of the world as much as this bacterium is discernible all over the world (Tables 1).

Table 1: *Mycobacterium bovis* transmission routes

Path	Transmission	Number of Organism	Collaborative Data
Breathing in	Direct Inhalation of air borne droplets	Single bacillus	Present in a variety of species
Ingestion	Milk from infected animals	Several million bacilli	Epidemiological evidence
Transcutaneous	Abrasions contamination	Few bacilli	Humans handling infected abrasions
Pseudovertical	Drinking milk of diseased mothers	Very few bacilli	Epidemiological evidence in cattle
Vertical	Vertically from the infected female to the offspring	Very few bacilli	Pathological changes of lesions in the liver in calves from infected female

Even though people are susceptible to bovine TB, it is *Mycobacterium bovis* and not the immediate source of human TB which is *Mycobacterium tuberculosis*. It is easily transmitted by people inhaling the affected droplets or through consumption of raw milk from infected cattle. In some countries, the disease is attributed to bovine TB and contributes to about ten percent of human TB (Yousif et al., 2024).

Clinical Signs

When cattle get bovine TB then they may have a chronic and painful disease. In the early stage, there are no symptoms of the illness. Later on, there is weakness, scalloped fever, progressive weakness, and poor appetite. A lung infection may result in trachypnea, wet cough, or shortness of breath. The respiratory system and its role in the disease's pathology is shown by the site of lesions, which are mostly seen in the tonsils, lungs, and upper respiratory tract in both infected humans and animals. Additionally, the mammary gland is typically painless, although it may be large (Dudek et al., 2020).

Swollen Lymph Nodes

In more extreme cases, enlarged lymph nodes can block the lungs, blood vessels, or digestive tract. At times, nodes may mimic those of an impacted node in the head and neck region with such features as collapse and spitting of fluids. Pressure on the

oesophagus mediastinal glands, which are enlarged and can cause inflation. Acute respiratory distress and severe fatigue are possible in the later stages of tuberculosis. The majority of the TB lesions are found in the lungs and the lymph nodes of the respiratory system (Ganchua et al., 2023).

Pneumonia

Any age group of cattle, especially the dairy/beef calves, adult cattle, and after the arrival of the feedlot beef cattle at the abattoir, are susceptible to *M. bovis*-associated pneumonia. Non-specific clinical symptoms include fever, tachypnea, breathing problems, and a lack of hunger with or without coughing and nasal discharge. *Mycoplasma pneumoniae* in the same animal or other animals in the herd may be complicated with otitis media, arthritis or both (Krajewska-Wędzina et al., 2022).

Otitis Media

M. bovis-induced otitis media is common in feedlot-born and can be an enzootic disease or outbreak in dairy or beef calves. Mild clinical signs of scours mean the calf remains bright, and the disease early stages, are characterized by ravenous appetite although the final stages will manifest poor feed intake and fevers. Head shaking and rubbing or scratching of the ears are signs of ear pain. Eyelid paresis can lead to exposure keratitis and epiphora. There may be swollen nasal discharge along with unilateral or bilateral clinical symptoms if the membrane that protects the ears breaks down (Lotfollahzadeh et al., 2024).

Arthritis Infections

Arthritis from *M. bovis* can infect cattle of different ages. Calves and dairy cows are the main ones showing the signs and symptoms of *M. bovis* arthritis but the outbreaks are generally sporadic and often accompany mastitis and pneumonia in many cases. Some people think that feedlot cattle have CPPS. Signs of septic arthritis are acute non-weight-bearing weakness, memorandum joint pain, and oedema; moreover, the joints get warm when touched (Fig. 2) (Bo et al., 2020).

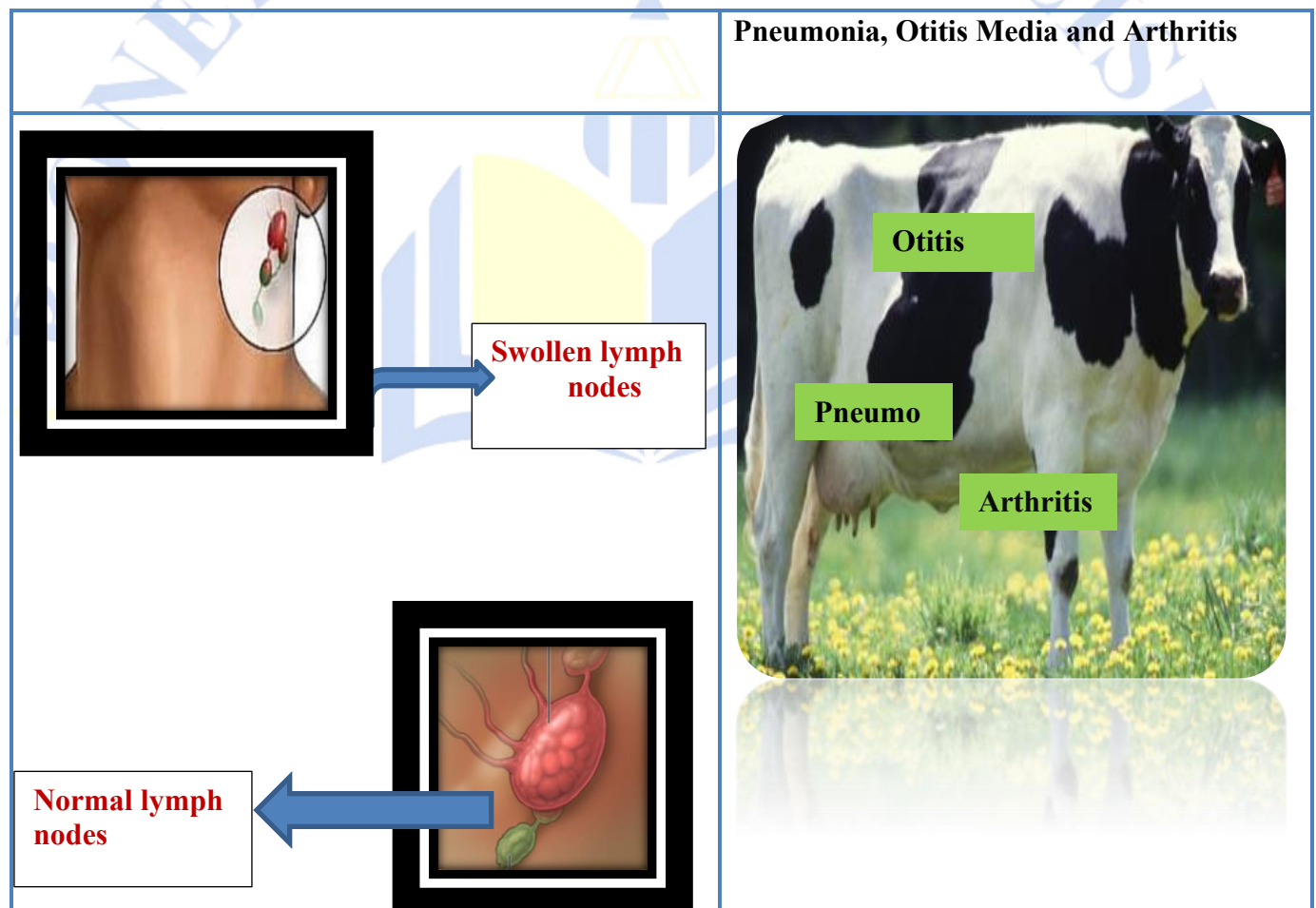


Fig. 2: Swollen Lymph Nodes

Diagnostic techniques and their challenges

Culture

M. bovis has been diagnosed clearly through microbial culture. Bronchoalveolar lavages and milk, deep nasal congestion swabs and joint fluids and semen are the most frequently employed specimen sources for identification of *M. bovis*. It showed that the lower limit of its quantification was above or equal to 272 CFU/mL, which implies that any number of cells at or below this mark may not be detected. Another issue is that culture methods cannot distinguish between different non-clinical *Mycoplasma* species, for instance, *Mycoplasma anatis*, which exhibits a ‘fried egg’ appearance and may contaminate or interfere with *M. bovis* samples. The typical procedure is to gather 10–40 mL of milk, centrifuge it, and then spread only 100–200 µL on the plates (Bernitz et al., 2021).

Polymerase Chain Reaction

PCR is so sensitive that it can identify as few as 10 CFUs in broth cultures when detecting *M. bovis*. The comparison established showed that PCR offered high specificity, sensitivity, and speed as compared to culture and enzyme-linked immunosorbent assay (ELISA). PCR can identify DNA from dead bacteria which has a number of impacts, but opposed to the culture approach that only finds living organisms. The primer sequences targeted UvrC gene of *M. bovis* using classical PCR and real-time PCR for the detection in lung and bulk tank milk samples. PCR has also been used to find *M. bovis* genes that are resistant to antibiotics (Palmer et al., 2024).

ELISA

ELISA determines anti-*M. bovis* antibodies in the host serum or milk resulting from previous or current *M. bovis* infections through the form of humoral immune responses. For identification of anti *M. bovis* antibodies in BTA and other epidemiological and historical investigations, ELISA has been utilized frequently. Given that cattle testing positive by ELISA sometimes show negative results on PCR when both tests are conducted simultaneously. ELISA could be an inaccurate marker of an active *M. bovis* infection (Souza et al., 2022).

MALDI-TOF MS

Another fast and reliable technique for identifying microorganisms is known as Matrix-Assisted Laser Desorption Ionisation, Time of Flight Mass Spectrometry or (MALDI-TOF MS). The basic concept of the method is to apply laser light to ions and form ions of healthy bacterial cells. Colonies from agar plates are prepared by mixing with matrix solution and then analyzed by placing it in front of an ion source in the mass spectrometer. The results are examined using colonies of recognized bacteria or against references that have been stored (Evangelista & Ferreira, 2022).

Loop-mediated isothermal amplification

The LAMP is an extremely sensitive method that uses a set of two to three primers to create many copies of the target DNA in less than an hour because it skips the denaturation stage. LAMP is simple to do in a heating block and does not require thermocyclers as compared to traditional PCR. After amplification by viscosity of the reaction mixture or SYBR green I staining, results can be read using 2% agarose gel electrophoresis. Specificity of UvrC-based LAMP was 74% while sensitivity was 10 times higher than PCR, with values of 100. Later, other scholars were able to bring close to 90.9% of specificity into question. To demonstrate that LAMP could selectively and accurately detect *M. bovis*, another gene of *M. bovis* was employed, and these genes include oppD (oligopeptide permease D), gltX (glutamate transfer RNA ligase), gyrB (component of gyrase B), and 16s rRNA.) (Gelgie et al., 2024).

Prevention and Control Strategies

Scotland and other nations that have declared themselves free of tuberculosis in cattle use surveillance to prove that they are infection-free (Group et al., 2024). The programs can be either active or passive with the former being initiated by the investigating agency and often involving required testing, and the latter depending on reports from an observer, such as hunters

reporting carcasses with suspicious lesions (Tomaselli, 2018). The prevention of new cases of *M. bovis* infection in cattle is dependent on monitoring systems, farm biosecurity, and testing of new stock before their introduction to the farm (Baraitareanu & Vidu, 2020). According to the disease risk in the area, the government of the United Kingdom currently pays for the tuberculin skin test to be performed on cow herds every one to four years (Romero et al., 2023). The gamma interferon assay can be used as a follow-up or supplement to the skin test as a new alternative test. As with the skin test, cell-mediated immunity is measured using the gamma interferon assay (Yousif et al., 2024). An enzyme-linked immunosorbent test (ELISA) is used to measure the amount of gamma interferon cytokine produced after a blood sample is treated with tuberculin in vitro (Steinbach et al., 2021).

In many nations, slaughterhouse carcass inspection, also known as inspection of meat, is utilized as an additional TB monitoring source. It serves as the mainstay of TB surveillance in nations where the disease is thought to be eradicated or where the prevalence is very low (Borham et al., 2022). Both the United States and Ireland have successfully used this strategy. For maximum effectiveness, the afflicted premises should not be restocked until the declining population and appropriate disinfection are finished and the premises have been free of all cattle for a minimum of 30 days (Pokludová, 2020).

As an alternative to totally depopulating herds, "test and remove" operations are based on herd testing and the removal of animals that test positive (Garwood et al., 2020). Regardless of the species, there is an increased *M. bovis* infection risk when cattle and buffalo herds are kept on the same land (Wielgus et al., 2021).

Establishing a comprehensive national surveillance program for *M. bovis* in wildlife that is financially and logistically sustainable could have a favorable economic impact on livestock health management by reducing transmission from livestock to wildlife through early identification (Miller & Sweeney, 2013). Among the solutions to minimize the possibility of shedding of *M. bovis* through raw milk or colostrum are pasteurization and heat treatment (Sedky et al., 2020). As previously reported, *Mycoplasma* spp. including *M. bovis*, were destroyed by a commercial on-farm pasteurizer when tested at 71.7 °C for 15 seconds. According to additional data, the concentration of total immunoglobulin in colostrum dropped by an average of 25% after 30 minutes of pasteurization from 22% at low temperatures (63.9–66.7 °C) to 27% at high temperatures (68.3–70.8 °C) (Dudek et al., 2020). However, colostrum's heat treatment might impact immunological response and cytokine absorption in neonatal calves (Silva et al., 2024).

Using efficient disinfectants to sanitize farms is an additional method of preventing or eliminating *M. bovis* infections. Few investigations have been conducted on the effectiveness of disinfectants in deactivating *M. bovis* (Buncic et al., 2014). The effectiveness of various sodium hypochlorite and citric acid dilutions against *M. bovis* was assessed in the most recent investigation (Blacksell et al., 2023). Five distinct classes of teat dips were evaluated for their ability to prevent *M. bovis* mastitis and maintain pre-milking hygiene and post-milking hygiene to *M. bovis* in a separate investigation (Haider et al., 2023). All of them showed germicidal efficacy against *M. bovis*, although in a different study, the iodine-based formulation was the most effective (Krupelnyskiy, 2023). Through control and eradication programs, BTB has been almost eliminated in developed nations or significantly reduced in agricultural animals to low levels. Many African nations have endemic animal tuberculosis because they are unable to implement skin test and slaughter control methods that have worked well in the industrialized world due to financial limitations (Aden & Tukue, 2021).

Because of a lack of funding, a lack of political will, and an underestimation of the importance of zoonotic tuberculosis in the animal and public health sectors by national governments and donor organizations can control measures are either not implemented at all or are implemented insufficiently in the majority of developing countries (Pokam et al., 2019). The absence of veterinary and transportation-related infrastructure significantly limits the extent of bovine TB testing and management in Africa. In domestic livestock, bovine TB is rarely treated except in rare cases involving valuable animals preserved as zoological exhibits (Borham et al., 2022). The most active anti-tuberculous first-line chemotherapies are streptomycin (SM), ethambutol (EMB), PZA, RIF, and INH. Capreomycin (CAP), cycloserine, ethionamide (ETH), and thioacetazone are examples of second-line medication. Furthermore, isolate infected animals within infected herds, kill diseased animals, restrict movement of the afflicted herds, and remove the animals (reactors) that tested positive for TB to control bTB. The primary strategies to test infected animals with TST and/or IGRA (Picasso-Risso, 2019).

New Zealand, where the illness was first discovered in 2017, has created the most ambitious and unique plan for complete *M. bovis* eradication. It was decided to cull infected and contact livestock when the number of affected farms was low (Dudek et al., 2020). However, the Technical Advisory Group in 2019 stated that this strategy is still feasible but is becoming more difficult due to the large number of infected farms that were later traced. Initially, the use of rather insensitive diagnostic procedures made it difficult to identify contaminated farms and these days, bulk tank milk is being tested using serological ELISA in conjunction with real-time PCRs (Fig. 03) (Fasogbon et al., 2024).

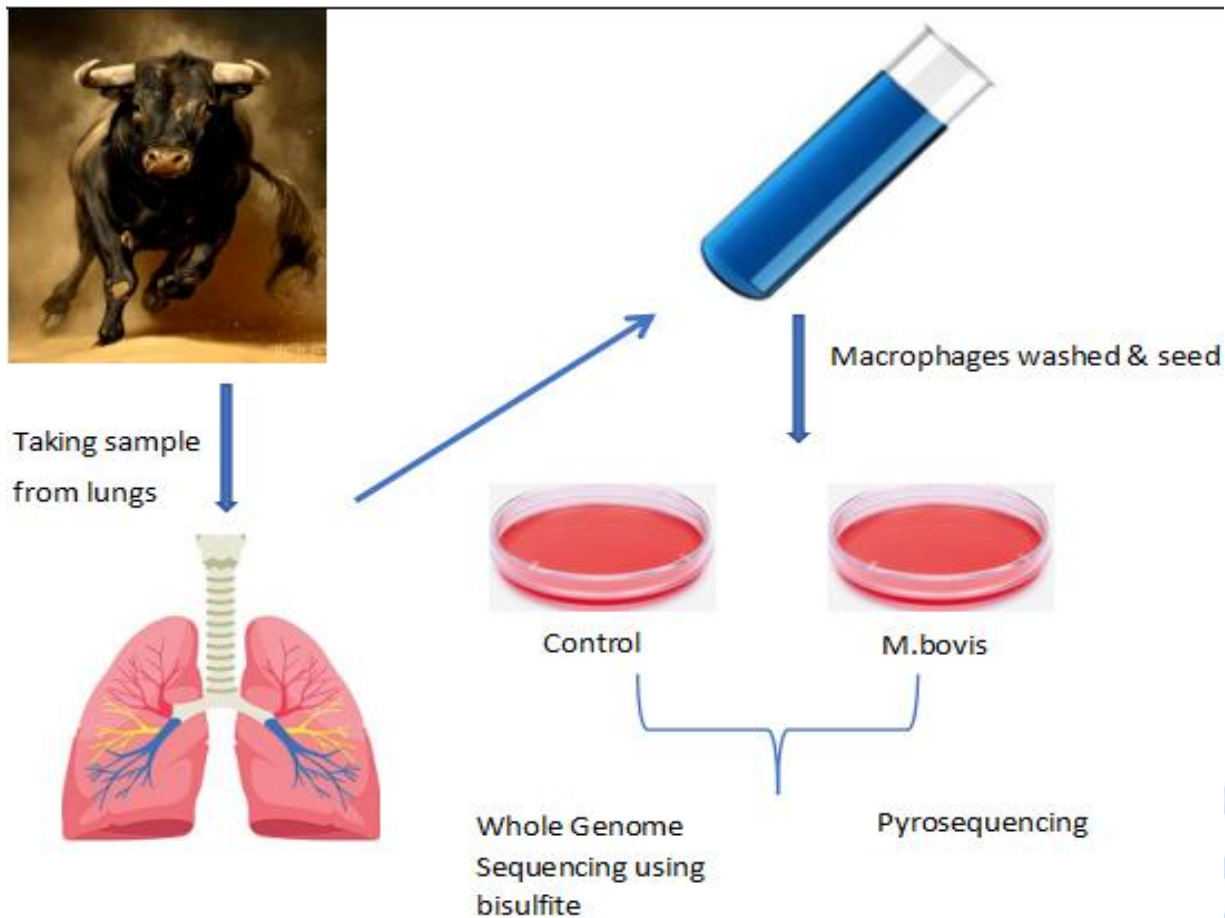


Fig. 3: DNA methylation analysis and *M. bovis* treatment by alveolar macrophage

Economic Impact of *Mycobacterium bovis* on the cattle industry

Three billion dollars was spent on TB surveillance in the United States between 1962 and 2017. By the year 1988, the Republic of Ireland's eradication effort, which had been started in 1954, had cost £1 billion (Zinsstag et al., 2006). Between 1981 and 1993, two areas in the province of Huesca in Spain had an eradication effort that cost around Pts 2.8 billion. The anticipated cost of Australia's 27-year (1970–1997) test and slaughter approach to eradicate bovine TB and brucellosis together was USD 1 billion (Tschopp et al., 2022).

Cattle and dairy cows in nations where BTB is either completely or partially controlled comprise almost 90% of Africa's human population (Dibaba & Kriek, 2019). Over half of the population in Africa has close contact with potentially infected animals, by increases their chance of catching *M. bovis*, compared to less than 5% of the people in the United States who routinely and directly interact with cattle (Caron et al., 2013).

Utilizing the comparative intradermal tuberculin skin test (CIDT) or intradermal tuberculin test to detect infected animals and followed by prompt isolation, slaughter, and necropsy to check for tuberculosis-related lesions has been nations examine their bovine tuberculosis control program in animals, particularly in cattle. This type of strategy is implemented in many low- and middle-income countries, but it is typically not well-executed or well-supported to support the program (Devi et al., 2021). Examining and separating TB-positive animals from healthy ones without killing them right away is a more practical approach (Kaur, 2019).

Economic significance of bovine tuberculosis

Dairy cattle are particularly vulnerable to tuberculosis because of its high morbidity and productivity loss, which causes infected animals to lose 10–25% of their productive efficiency (Borham et al., 2022). It is found in practically every country on the globe. Due to mortality, low productivity, carcass condemnation, and trade restrictions, bovine TB generates significant economic losses, particularly in urban and peri-urban cross-breed dairy cattle (Igbal et al., 2024). In countries with poor socioeconomic position and insufficient veterinary and public health services, the risk of infection to humans is the most important consideration, particularly for women and children who appear to be more susceptible to the disease (Zerfu et al., 2023). The data, which is now available, indicates that bovine tuberculosis has a significant economic impact on the world, even though estimates of the expenses of the disease and its management only apply to certain countries (Reis et al., 2021). These losses cover costs related to animal production, markets, and trade, as well as the implementation of surveillance and control systems. When it comes to endangered wildlife species, TB losses are also very significant (Fig. 3) (Clemmons et al., 2021).

Conclusion

Factors including species, herd size, and production system must be taken into account when creating a disease surveillance and control program. It is necessary to prohibit practices that encourage the mingling of buffalo and cattle. Restricted and raw imports of live cattle, national disease control, search for causative agents across the whole country, and control programs are the major attributes that favor a favorable cattle disease.

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