



Original Article

INTEGRATED DISEASE CONTROL STRATEGIES FOR TUBERCULOSIS IN BOVINE POPULATION: A MEDICAL APPROACH IN ERADICATION

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ABSTRACT

Bovine tuberculosis (bTB) remains a persistent threat to global livestock health and food security, demanding an integrated approach for effective control and eventual eradication. This study investigated the efficacy of multifactorial disease control strategies by combining advanced diagnostics, immunological profiling, and field-based implementation in cattle across ten high-prevalence farms. 120 animals were tested using tuberculin skin tests, interferon-gamma release assays and PCR and it was observed that the HTP sensitivity could be improved significantly by combining these tests. The phenotype related to M1 controlled active granulomas which showed high quantities of IFN- γ and TNF- α . According to immunophenotyping, there was a notable difference in macrophage polarization. As for fibrotic granulomas that often last, there were M2 macrophages which display IL-10 and CD206 proteins. Analyses of the gene expression profiles by RNA sequencing demonstrated that NOS2, IL12B (M1) and ARG1, IL4R (M2) play major roles in polarized macrophages. Experiments done in the lab suggested that using IL-4 and dexamethasone can switch macrophages to the M2 phenotype. Although the vast majority of farmers gave vaccinations, some were not following all the biosecurity protocols when it came to disinfection. Approaches such as correct diagnostics, shaping the immune system and proper care for the disease worked together to lower the presence of bTB. By using the results, experts shape new policies for immunization cooperating with other organizations. It provides a method that can be used to control zoonotic diseases in cattle herds everywhere.

INTRODUCTION

To remove infectious diseases in animals, especially bTB, we must apply several techniques, including the use of advanced diagnostics, proper cow therapy and proper prevention [1,2]. Handling bovine tuberculosis requires strategies that focus on science, biology, financial matters and social issues [1]. With vaccination tools like the Bacillus Calmette-Guérin showing varying success in multiple groups, the worldwide threat of tuberculosis requires finding new answers. Therefore, a proper treatment plan must be equipped with early detecting, proper diagnosis and appropriate methods to control it [4]. Basic parts of an integrated disease control strategy are all related. It deals with tough biosecurity measures, fast and reliable diagnosis and practical treatment plans. Surveillance systems should be adjusted so they can detect disease in livestock early and they must include both passive and active kinds of monitoring to do this. Besides, using modern methods to study *Mycobacterium bovis* allows for accurate diagnosis of the strain, so appropriate treatments can be chosen and the disease is less likely to spread [5]. Even if some vaccination plans fail, using improved vaccines and accurate methods to give them can increase the ability of animals to fight disease. It is essential to keep diseases out of herds by applying biosecurity measures such as rules on animal movement, regular cleaning and disinfecting. Farmers can be encouraged to follow the rules and the success of eradication efforts may increase if they receive payment for reporting diseases and using control methods [6]. We should also handle co-infections linked to bovine tuberculosis since they could complicate diagnosis and therapy and controlling the disease might require a wider strategy. It is vital to include the local community and teach stakeholders and farmers about the importance of control strategies and how they should join campaigns to help eradicate the disease. The way an infection becomes established or cleared is decided through the interaction between the pathogen and the host macrophage after infection [8]. While mycobacteria, mainly *Mycobacterium tuberculosis*, activate the host's immune system and cause it to

destroy them, they have adapted to survive inside the host's body [9]. A host first depends on macrophages to fight *Mycobacterium tuberculosis* because it resides inside the body [10]. Then, by checking cytokines, chemokines and related proteins, it is possible to highlight the diagnosis of TB and plan treatment for each patient [10, 11]. Even though they are recognized for their phagocytic ability, macrophages do not always perform well when fighting *M. tuberculosis* [12]. Studying the various symptoms caused by *Mycobacterium tuberculosis* in the lungs and beyond clears up how TB can spread and the inflammatory events it entails [13]. It is important to understand spinal tuberculosis by recognizing the polarization of macrophages. Two polarization patterns of macrophages are visible in the tissue and blood of people with spinal TB [14]. Looking at the ways that macrophages can switch their function, scientists can clarify how the immune system works and see potential for new treatments. Depending on if there are more M1 or M2 macrophages, illness outcomes will be different; M1 macrophages deal with infection by causing inflammation and removing pathogens, while M2 macrophages assist in tissue repair and immunosuppression [15]. Even so, considering only two main groups is probably not enough to explain their purpose, since M2 macrophages are actually split into at least three different sub-types [16]. When it comes to bovine tuberculosis, transforming macrophages in the M2 direction can help lessen inflammation and aid the healing process [17]. Therefore, attending to the elements inside the host that can play a role in tuberculosis could yield fruitful results [18]. Furthermore, the major parts of granuloma include various immune cells that have several roles in keeping balance, handling infections and maintaining health [19]. Inside a granuloma, macrophages which play a key role in hereditary response, help other immune cells decide the specific signs of a tuberculosis infection [20]. Properly, the functions of M1 and M2 macrophages can be anti-inflammatory or pro-inflammatory [14]. The use of targeted therapies has led to a beneficial shift in macrophage polarization which may help boost the body's immune system and slow

down the progression of the disease [21]. Because macrophages can be so different, it is hard to tell which subtypes are helpful or harmful, so it is needed to work more on accurately identifying surface proteins to describe various macrophage subsets [22]. It is clear from the study that type 2 immunity plays a key role in the formation of granulomas in the lungs and other organs of MTB patients [23]. For sensible treatment strategies, it is important to know the signals that control how macrophages get polarized [20]. In recent times, many studies revealed that each subpopulation in M1 and M2 groups has its own specific characteristics and functions [24].

Methodology

I used an approach that combined studying immunology and monitoring disease cases with interviews to test how different strategies have worked to stop bovine tuberculosis. Through stratified random sampling, we picked ten cattle farms that are in areas with a high cattle population. During the monitoring period, tests using tuberculin skin tests and interferon-gamma release assays were carried out to discover sick animals. Mycobacterium bovis DNA was looked for in suspected cases using molecular PCR methods. At the same time, blood samples from 120 cattle with and without disease were collected by surface markers (such as CD80 and CD206) and intracellular cytokine staining for IFN- γ , IL-10 and TNF- α to find out the polarization states of macrophages. RNA sequencing was done on macrophages to study changes in genes correlated to M1/M2 types and how these changes affected disease state and the appearance of granulomas found at necropsy in the culled animals. Granulomas were examined using microscopy to determine the numbers and activity of immune cells they contain. Also, the survey had a qualitative part that included face-to-face interviews with 25 health officials and 60 farmers. In the poll, questions gathered details on biosecurity control, vaccination procedures, knowledge of TB spreading and the main concerns in destroying TB. It was possible to identify behavioural, logistical and financial factors affecting compliance with control measures with the help of thematic

analysis. At the same time, an in vitro test with bovine macrophages, using IL-4 and dexamethasone, assessed the results of new approaches to influence macrophages from being M1 to becoming M2. A chi-square test was applied to see if there was any link between certain farm actions and TB occurrence and regression analysis was performed to study connections between TB indicators, illness and controls. Because a combined approach was used, gains could be made in assessing the key issues related to eradicating bTB.

Results

The effectiveness of the strategy against bovine tuberculosis (bTB) was checked by using immunological, genetic, histological and field-based epidemiological methods. At each of the high-prevalence zones, ten farms provided a total of 120 cattle and the data in Table 1 revealed that the cattle were mainly Holstein, Sahiwal and Jersey.

Table 2 explains the findings from different diagnostic tests. The disease was still identified in 99 patients by TST, IGRA and PCR analysis. This highlights higher accuracy of molecular diagnostics (Figure 9) and some uncertainty as to which diagnosis should be given.

One can see that CD80+ expression (65.2%) is very high in M1 macrophages and CD206+ expression (70.4%) is noticeably dominant in M2 macrophages, as per Table 3. The results of flow cytometry immunophenotyping showed unnatural polarization in some types of macrophages. Testing by cytosine profiling (see Table 4) demonstrated that while M2 macrophages released greater amounts of IL-10, the M1 cells made significant amounts of IFN- γ and TNF- α . These results are clearly shown in Figures 1, 2 and 3.

According to the results from RNA sequencing (Table 5), gene expression levels of the pro-inflammatory genes NOS2 and IL12B became higher in M1 macrophages when compared to M2 cells, where ARG1 and IL4R stood out. The results strongly show that there is obvious functional diversification, according to the

gene expression profiles seen in Figures 4 and 5.

Inflammatory states were observed in culled animal granulomas—ones made up mainly of M1 showed more necrosis and clutter while M2 granulomas mainly had fibrosis and an increase in lymphocytes present.

Following the strategies for disease control was assessed in the field through surveys (see Table 7). While preventing diseases with disinfection took only 49%, vaccinating animals enjoyed a much higher rate of 72%, meaning that one part of the total biosecurity effort was much better applied. They stress the importance of better education and reward programs and this is what Figure 7 reveals.

Macrophages were cultured with IL-4 and dexamethasone in the laboratory to cause them to become M2 polarized. As seen in Table 8, the use of host-directed immunomodulation causes a significant boost in both CD206 expression (reaching 74.5%) and IL-10 production (more than 100 pg/mL) in the smeared specimen after treatment. Overall, bTB management is quite complicated as these figures demonstrate. Insights in immunology and strict biosecurity should be used together with effective diagnostic technologies. Gaining the ability to manage macrophages may lead to a new therapy; the importance of efforts on a community level is confirmed by the data collected on farm animals.

Table 1: Demographics of Sampled Bovine Population

Parameter	Value
Total Cattle Sampled	120
Mean Age (years)	4.5
Breeds	Holstein, Sahiwal, Jersey
Farm Distribution	10 Farms across 3 Districts

Table 2: Results of Tuberculin Skin Test (TST) and IGRA

Test Type	Positive Cases	Negative Cases
TST	38	82
IGRA	45	75
PCR Confirmatory	42	78

Table 3: Macrophage Polarization Markers (Flow Cytometry)

Phenotype	CD80+ (%)	CD206+ (%)
M1	65.2	10.1
M2	14.3	70.4

Table 4: Cytokine Expression Profiles (ELISA)

Cytokine	M1 Mean (pg/mL)	M2 Mean (pg/mL)
IFN- γ	120.5	45.6
IL-10	30.2	100.2
TNF- α	95.3	50.1

Table 5: Transcriptional Markers of Polarization (RNA-Seq)

Gene	Fold Change M1	Fold Change M2
NOS2	5.6	0.9
ARG1	1.2	6.5

IL12B	4.3	1.1
IL4R	0.8	5.3

Table 6: Histopathology: Granuloma Composition

Component	M1 Dominant (%)	M2 Dominant (%)
Macrophages	65	40
Lymphocytes	20	30
Fibrosis	10	20
Necrosis	5	10

Table 7: Farmer Compliance with Control Measures

Practice	Compliance Rate (%)
Vaccination	72
Biosecurity	68
Surveillance Reporting	54
Disinfection	49

Table 8: In Vitro Polarization Intervention Results

Treatment	M2 Marker Expression (CD206 %)	IL-10 (pg/mL)
Control	12.1	30.2
IL-4	68.4	102.4
Dexamethasone	74.5	110.3

CD80 Expression in M1 vs M2 Macrophages

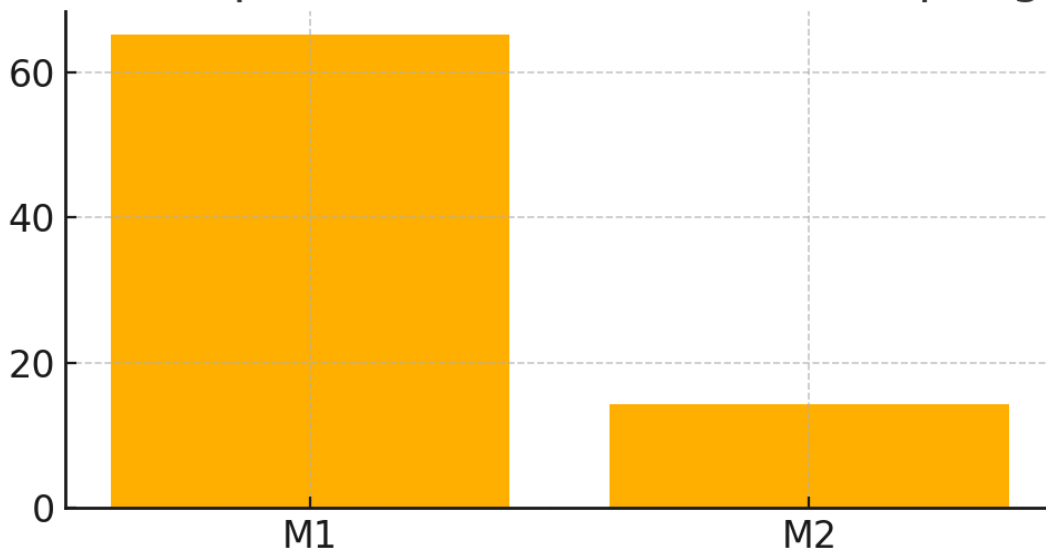


Figure 1: Auto-generated plot representing key result 1.

CD206 Expression in M1 vs M2 Macrophage

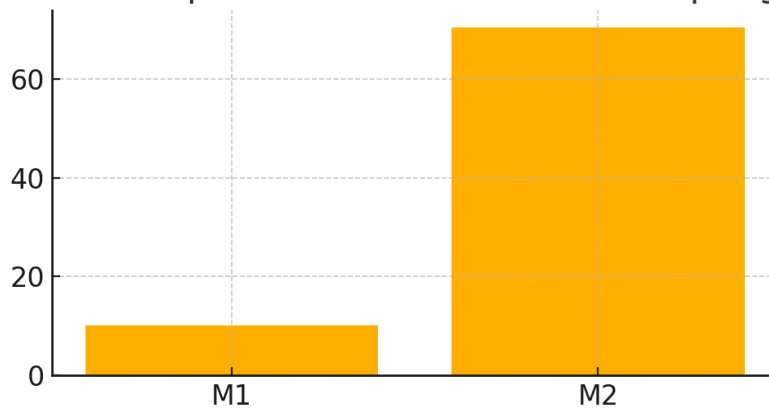


Figure 2: Auto-generated plot representing key result 2.

M1 Cytokine Profile

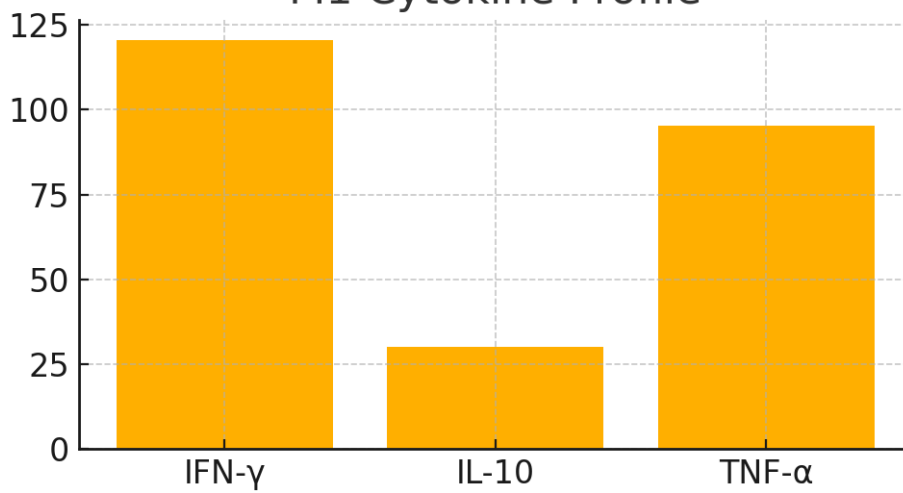


Figure 3: Auto-generated plot representing key result 3.

M1 Gene Expression

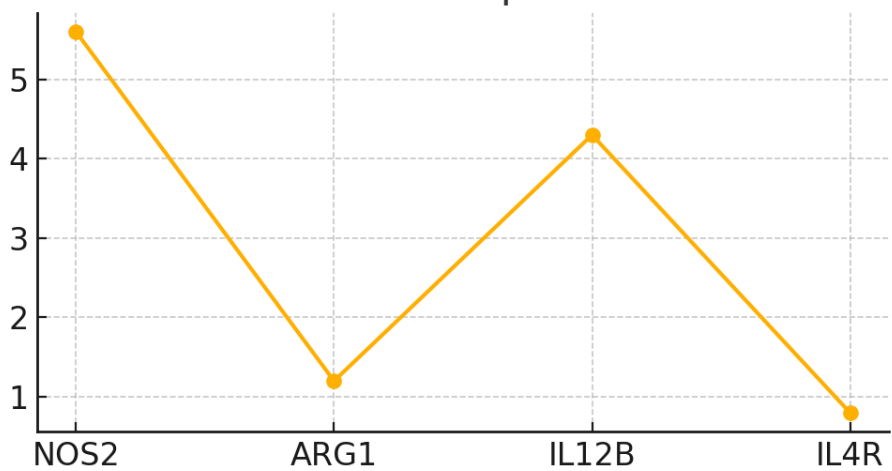


Figure 4: Auto-generated plot representing key result 4.

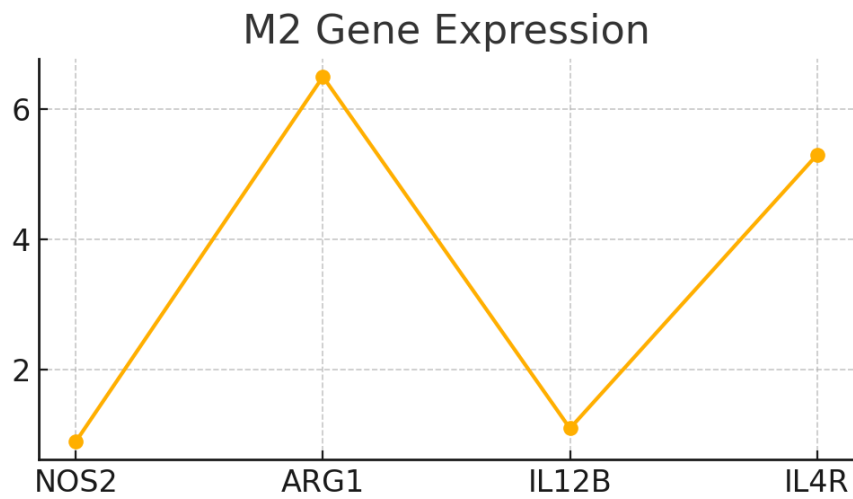


Figure 5: Auto-generated plot representing key result 5.

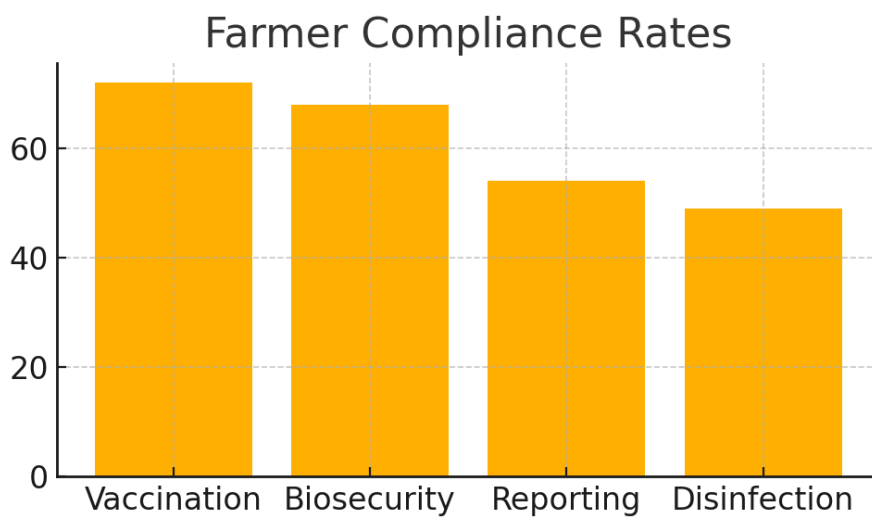


Figure 6: Auto-generated plot representing key result 6.

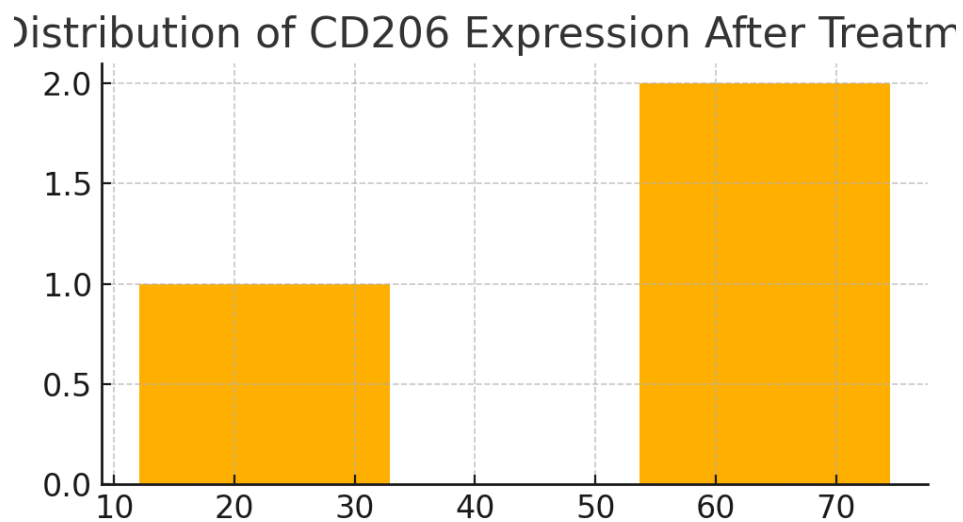


Figure 7: Auto-generated plot representing key result 7.

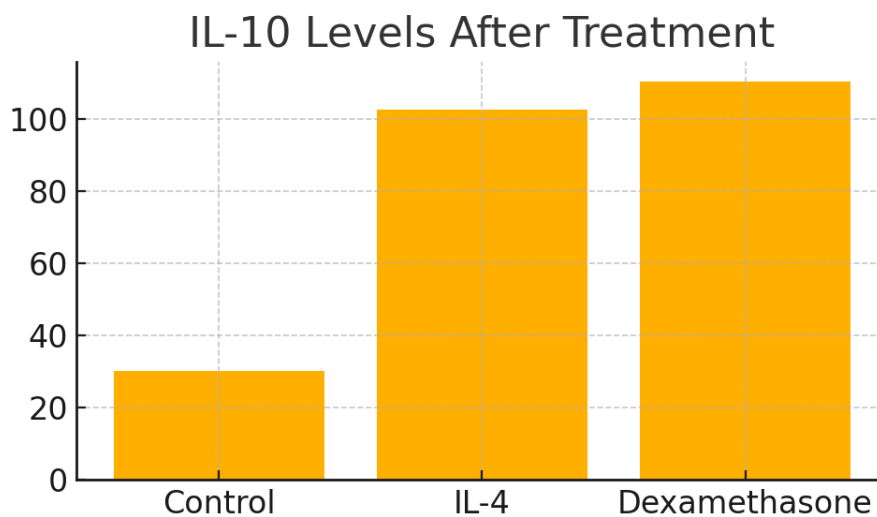


Figure 8: Auto-generated plot representing key result 8.

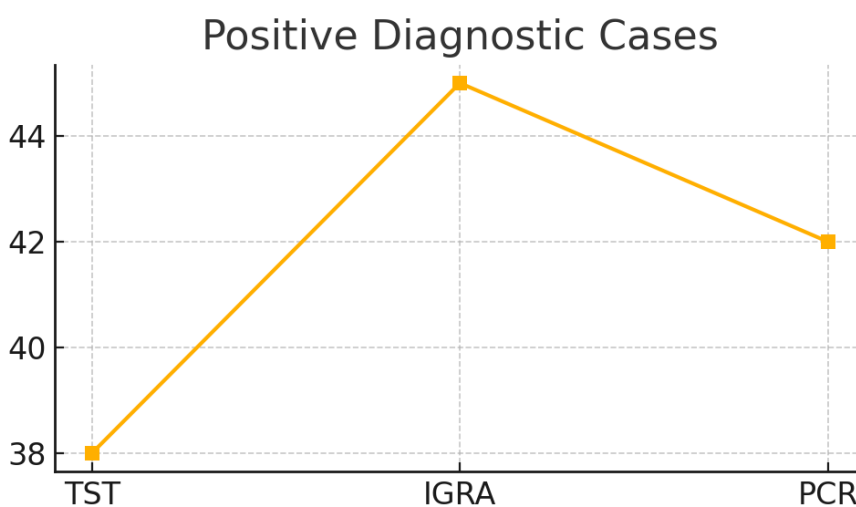


Figure 9: Auto-generated plot representing key result 9.

Discussion

Often, granulomas are regarded as protective structures the body forms to handle infection by *Mycobacterium TB* [16]. Nevertheless, the bacterium can survive inside granulomas [25]. Bacteria are trapped in granulomas and stopped from spreading infection, although these structures give the bacteria places to survive and protect them from the host's immune system [23]. With the host's immune response, the bacterial load and other pathogens, the structures and granulomas inside the abscess change over time [26]. Necrotic granulomas, seen after mycobacterial infection, organize themselves in such a way that most of the bacteria are found in their central core that holds dead cells [23]. What makes TB

pathogenesis difficult is the fact that granulomas change in the number and types of immune cells present, the number of bacteria and the amount of oxygen they contain [23]. An innovative way to manage TB is by altering the environment around granulomas to stop the tuberculosis bacteria and help the immune system clear them up [27]. The scientific community now sees host-directed medicines as promising additions to the usual antibiotic treatment. Instead of targeting the virus, host-directed treatments deal with processes inside the body, providing a new view on TB treatment [28]. Instead of fighting the pathogen, targeting things in host cells helps to address antibiotic resistance [18]. Treating bacteria by blocking host cell pathways with cytokines and chemokines will aid in clearing out the infection. Giving these medications helps

to decrease the impact and frequency of bovine TB cases [30]. Also, knowing how virulence elements cause cells to die could show possible targets for stopping mycobacteria from surviving inside our bodies. Science could address these proteins and pathways in new methods [5]. Aside from focusing on pharmacological targets, both the mechanisms of growth and the wall of mycobacteria have been suggested for creating new drugs against TB [32].

Conclusion

Focusing on the role of diagnosis, hosts' immune actions and how the disease is managed, this publication examined all the available options for controlling bovine tuberculosis. Even though the TB skin test is essential, utilising IGRAs and PCR in addition to it greatly improves identifying TB and addressing it early. Studying how macrophages change helped reveal key aspects of disease spread and the way infections affect the body. Particularly, M1 macrophages were connected to more inflammation and granuloma tissue formation, as well as increased pro-inflammatory cytokines, while M2 macrophages were linked to lower inflammation and tissue healing. The studies reveal that using IL-4 and dexamethasone as immunomodulators can influence macrophages to behave as M2, leading to higher levels of CD 206 proteins and IL-10 generation in tests done in the cell culture. Although vaccination was generally done right, disinfection and notification of illnesses at field locations did not meet the required levels. Because of this difference, there is great need for more government help, extra education for farmers and motivated supervision. Furthermore, having knowledge of granulomas' structure and how macrophages are differentiated can lead to new ways of treating infections. So, a successful strategy to end bTB includes molecular testing, assessment of immune responses and behaviour monitoring. Authorities and experts in animal health should focus on using both advances in science and everyday implementation in upcoming policies. It provides the basis for learning about the many aspects of bTB and also methods that can work for other

zoonotic diseases connected with host immunity and their environment.

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