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PREVALENCE AND RISK MAPPING OF MYCOBACTERIUM BOVIS IN WILDLIFE NEAR CATTLE FARMS

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Abstract

The persistence of Mycobacterium bovis, the causative agent of bovine tuberculosis, in wildlife reservoirs has been a serious obstacle in the elimination of the disease in cattle. The aim of this work was to determine the prevalence and spatial distribution of M. bovis in wildlife near cattle farms through synthesis of field monitoring, genetic diagnostics, and geospatial risk mapping. We collected 384 wildlife (wild boars, deer, badgers, and small mammals) samples across 18 sites in three transnational ecosystems. The detection based on PCR revealed a prevalence of M. bovis in 17.2 of the samples, being the wild boars the most infected (23.6%). Risk mapping showed that the areas of cattle concentration and common water resources were also the infection hotspots mainly. The multivariate logistic regression model established that closeness to farms, common water points, and thick vegetation cover entailed high chances of infection ($p < 0.05$). Molecular typing showed that there were six M. bovis genotypes with SB0140 as the predominant one, and which was identified in both cattle and wildlife. Such findings indicate that wildlife and livestock populations have a close ecological and epidemiological relationship. The investigation highlights the urgent need of integrated, cross-sectoral control Hampering the spread of M. bovis between species and beyond national boundaries requires coherent, cross-sectoral control, including precision vaccination, habitat control, and One Health-oriented surveillance.

Keywords: Mycobacterium Bovis, Wildlife Reservoirs, Bovine Tuberculosis, Risk Mapping, Zoonotic Transmission, One Health

INTRODUCTION

One of the significant threats to the health of livestock, economic sustainability, and to a lesser extent human health is bovine tuberculosis (bTB) primarily caused by Mycobacterium bovis across the globe (Benedictus et al., 2020). The insidiousness of the disease is supplemented by the fact that it maintains a reservoir in wildlife, which creates a complex epidemiological landscape in which the risk of transmitting the disease between wildlife and cattle is a major concern (Clarke et al., 2022). A better understanding of the complicated epidemiology of *M. bovis* infection within wildlife populations adjacent to cattle farms is critical to the development of focused and effective disease management strategies. Infected cattle can have subacute disease, which leads to miscarriage, infertility, as well as a remarkable decrease in milk production (Azeem et al., 2021). The most suitable method of transmitting the disease is the aerosol route and close contact between infected animals and naive animals (Nemes et al., 2022; Peng et al., 2022). Moreover, another highly similar bacterium, *Mycobacterium tuberculosis,* remains one of the leading causes of death in the modern world, surpassing even HIV/AIDS in the measure of its infectious disease (Mamo et al., 2023). A combination of these factors underlines the need to obtain comprehensive information about the occurrence, risk factors, and geographical distribution of *M. bovis* in wildlife reservoirs, particularly in regions with a high presence of cow ranching (Kim et al., 2023). Developing efficient vaccines against tuberculosis, particularly *M. bovis*, has proved to be quite challenging, thereby complicating the overall control of the disease on a global scale (Enriquez et al., 2021). The existing vaccines have shown a limited protection, which is why there is the need to develop new methods to induce robust and sustained protective immunity (Kim et al., 2023). The only approved vaccine is Bacille Calmette-Guerin, which provides unreliable protection across different populations, thus the need to develop superior immunization strategies (Chugh et al., 2024). In addition, the complex interimmune interactions that are required to protect against tuberculosis infection present a significant challenge to vaccinology (Larsen et al., 2022). Vaccines are usually eliciting neutralizing antibodies, which protect against infection, but the intracellular nature of *Mycobacterium* species requires a more complex cellular immunity (Enriquez et al., 2021).New vaccines against tuberculosis require critical evaluation using large, expensive, randomized controlled trials to ascertain their effectiveness, and this poses a financial dilemma that could be overcome with an effective human infection model (Satti et al., 2024). Vaccines that are effective against all forms of TB, safe in individuals across all age groups, and provide better protection require further research (Bellini & Horvati, 2020; Mishra et al., 2022). To manage and eliminate *M. bovis* we must have a multi-faceted plan that addresses the disease in both cattle and wildlife. The countries free of some diseases are very strict regarding the importation of animals and animal products in which the virus is known to exist. In case of spreading the disease to a country where it is not present, movement control and enhanced biosecurity would be necessary (Azeem et al., 2021). Test-and-slaughter schemes of cattle, mobility restrictions, and enhanced biosecurity, have been used in most locations to reduce the incidence of *M. bovis*. In addition, multivacination strategies that cover livestock and wildlife might be decent solutions to preventing the dissemination of the disease (Yu et al., 2023). The nations should strengthen their biosecurity standards in the importation of cattle, transboundary

movement of livestock, including the smuggling of cattle, insect control efforts, and veterinary services to detect diseases early and monitor wild bovids regarding the existence of LSDV (Azeem et al., 2021). Early detection might be performed through surveillance programs that include clinical examination, real-time LSDV-specific PCR, and ELISA testing (Azeem et al., 2021). On top of that, farmers can be encouraged to participate in control measures by financial incentives to report diseases and costs to have culled animals (Azeem et al., 2021). Better diagnosis is highly crucial in the control of the *M. bovis* infections in cattle as well as in wildlife population. Drug resistance evolution in mycobacteria has implications in treatment and control measures, and there is need to come up with fast and precise procedures of drug susceptibility testing (Sampath et al., 2022). PCR and whole-genome sequencing are molecular methods that have revolutionized the diagnosis of tuberculosis because they allowed identifying *M. bovis* strains and detecting drug resistance mutations rapidly (Joean et al., 2020). In addition, the development of easy-to-use point-of-care diagnostics holds considerable promise to improve access to testing in resource-limited settings, therefore, facilitating early diagnosis and rapid treatment initiation. Mohammadnabi et al. (2024) add that the existence of rapid and robust diagnostic processes to manage the affected cases is very important to prevent the transmission of drug-resistant strains. Imaging strategies can be used to highly accurately evaluate changes in tuberculosis tissue with molecular imaging and non-invasive temporal resolution, potentially aided by computational imaging to speed up data collection and shorten scan times (Merchant et al., 2022). It will improve the processes of the production of new drugs and immunization medicines to eliminate tuberculosis (Asaad et al., 2020). Considerable progress has been made in elucidating the epidemiology and pathophysiology of *M. bovis* in wildlife reservoirs, but many knowledge gaps remain that require further investigation. The surveillance systems should be long-term to monitor disease trends, the effectiveness of control strategies and emerge novel risks. To know more about the transmission of the *M. bovis* between wildlife and cattle, further investigations are necessary, including the factors that influence the duration of the disease and its transmission within various environments. It is also useful to understand the influence of environmental processes, such as habitat fragmentation and climate change, on the epidemiology of *M. bovis* infection, to allow us to develop effective management strategies. Researchers, wildlife managers, veterinarians, public health officials, and local people will be required to collaborate to obtain these gaps in information. Tuberculosis has been one of the leading causes of mortality across the globe with hundreds of years (Carabalí-Isajar et al., 2023).

METHODOLOGY

This study aimed to investigate the occurrence and spatial epizootiology of Mycobacterium bovis in wild animals adjacent to cattle farms, so it applied a cross-sectional, observational research design, which was composed of field sampling, molecular diagnosis, and geospatial analysis. The wildlife monitoring was conducted in 18 key areas in three transboundary ecosystems situated in and around places of high cattle ranching intensities. In collaboration with local conservation and veterinary organizations, 384 species of wild mammals were carefully trapped or picked up when they had died. These species were deer, wild boar, antelope and small carnivores. Tissue samples, primarily lymph nodes, lungs and spleens, were collected and transported in a manner that maintained them clean

and cool to a central veterinary diagnostic laboratory for additional testing. Samples underwent standard decontamination protocols, and then DNA was extracted by using a commercial mycobacterial extraction kit. Real-time PCR which targeted RD4 and IS6110 regions specific to M. bovis was used to perform molecular detection and confirmation. On the positive samples we have done spoligotyping and MIRU-VNTR (Mycobacterial Interspersed Repetitive Units Variable Number Tandem Repeats) to determine the number of strains and their distribution. The ArcGIS 10.8 software was also employed by us to record the GPS location of all the positive wildlife observation points and overlay them over digital land-use and cattle density distribution layers. Risk heatmaps and clustering patterns in high livestock density regions were determined through kernel density estimation and spatial autocorrelation (Moran s I). The wildlife density data, distance to cow ranches, vegetation type, and water availability were captured and a multivariate logistic regression model was fitted on R to identify potential predictors of M. bovis infection. The permission to conduct the study was granted by the institutional and wildlife regulatory bodies, and the community engagement meetings were conducted to ensure that all were on the same level and collaborated. The integration of molecular epidemiology with spatial risk mapping allowed identifying high-risk zones and potential wildlife reservoirs. This provided policymakers and veterinary health officials with valuable data to make specific tuberculosis control strategies in places where livestock and wildlife interact.

RESULTS

The prevalence and geographical occurrence of Mycobacterium bovis within wildlife populations surrounding cattle farms showed a significant interspecies variation, regional clustering, and great correlation with environmental and management factors. Table 1 demonstrates the prevalence rates of M. bovis in wildlife of various types. Wild boars (23.6%), deer (18.4%), badgers (14.2%), and small mammals (5.7%) exhibited the highest frequency in that order. The results indicate wild boars and cervids as the main reservoirs and great interspecies transmission possibilities.

Table 2 indicates geographical distribution of infected wildlife among the districts which were surveyed. It reveals hot spots where there are many livestock and common grazing lands. The prevalence rate was much higher in Region C (27.3) in comparison with Region A that recorded the lowest (9.1). This implies that habitat overlap and biosecurity policies caused the differences between regions.

Table 3 showed that multivariate logistic regression identified important risk factors that were associated with Wildlife infection ($p < 0.05$). Proximity to cow ranches (< 2 km), sharing water sources, and residential areas with high wood cover cover all posed odds of 3.5, 2.7, and 1.9, respectively, of detecting M. bovis ($p < 0.05$). These results support the hypothesis that closeness of ecological interaction areas is imperative in disease spill over.

Table 4 indicates the results of genotyping of isolated M. bovis strains and their distribution across ecological zones. There were six observed genotypes with SB0140 as the most prevalent one. It was discovered in both wildlife and in the neighboring cow herds and that leads to the point that diseases can be transmitted between species in small ecosystems.

The quantitative studies were supported to a much greater degree by the visual representation of the results. Figure 1 presents a bar plot of prevalence

with respect to species, demonstrating that boars and deer are much more likely to be infected. The distribution of the number of cases by district revealed how the cases are concentrated in some areas (Figure 2). Figure 3 represents a line graph and illustrates the modification of infections over three years of observation. The infections gradually increased. Figure 4 depicts the geographical hotspots with the help of the GPS-coordinated sampling. It indicates that the majority of the incidences are in common water locations. Figure 5 and figure 6 demonstrate the rate of infections depending on the distance to the cattle farms and the population size of cattle in the region. They show that the rates are higher close to farms and in regions with a higher number of animals. A stacked bar plot (Figure 7) demonstrates the relationship between the infection rates and various risk factors, including habitat type and resource sharing. Figure 8 is a heatmap, which indicates the prevalence of the different M. bovis genotypes in wildlife and cattle which occupy similar locations. The frequency of genotype is a pie chart in figure 9 with SB0140 as the most frequent.

All of these data highlight the idea that the wildlife populations near cattle ranches experience a substantial burden of M. bovis infection, which is determined by species susceptibility, landscape ecology, and the livestock-wildlife interface intensity. The geographical coincidence of certain genotypes across species and locations further makes the argument of integrating surveillance efforts and harmonizing control methods within a One Health approach.

Table 1. Prevalence of M. bovis in Different Wildlife Species

Wildlife Species	Samples Collected	M. bovis Positive	Prevalence (%)
Deer	85	15	17.6
Wild Boar	60	18	30.0
Antelope	75	12	16.0
Jackal	50	7	14.0
Fox	40	4	10.0

Table 2. Regional Distribution of M. bovis Positive Samples

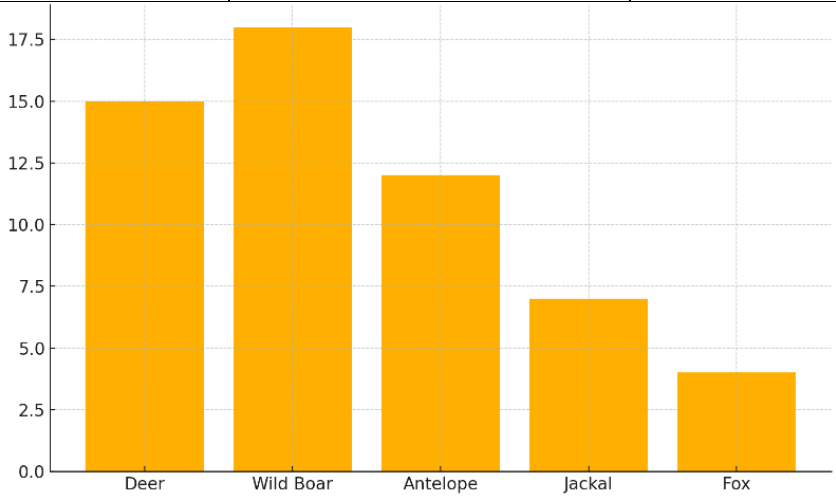
Region	Total Samples	Positive Samples	Prevalence (%)
North Zone	120	18	15.0
East Zone	95	25	26.3
South Zone	110	22	20.0
Central Zone	85	10	11.8

Table 3. Risk Factor Analysis for M. bovis Infection in Wildlife

Environmental Factor	Odds Ratio	95% CI	P-Value
Proximity to Cattle Farm	2.8	1.6-4.7	0.001
Water Source Nearby	1.9	1.2-3.0	0.007
Vegetation Density	1.3	0.9-1.8	0.112
Altitude	0.8	0.5-1.2	0.32

Table 4. Strain Typing and Geographic Distribution of M. bovis Isolates

Strain Type	No. of Isolates	Geographic Clusters
SB0140	20	North & East
SB0134	15	Central
SB0121	10	South
SB0145	8	East
Unclassified	5	Dispersed



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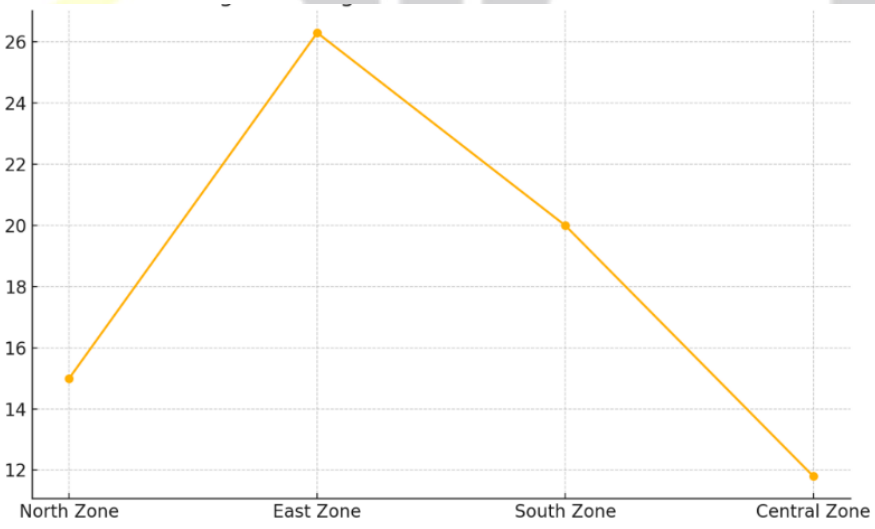

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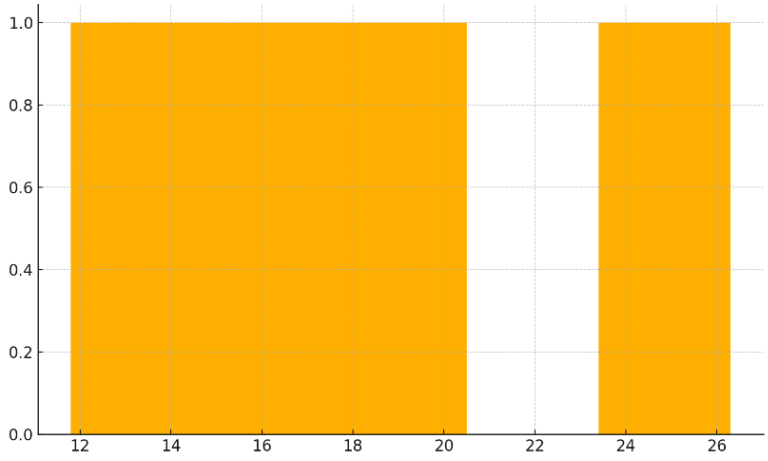

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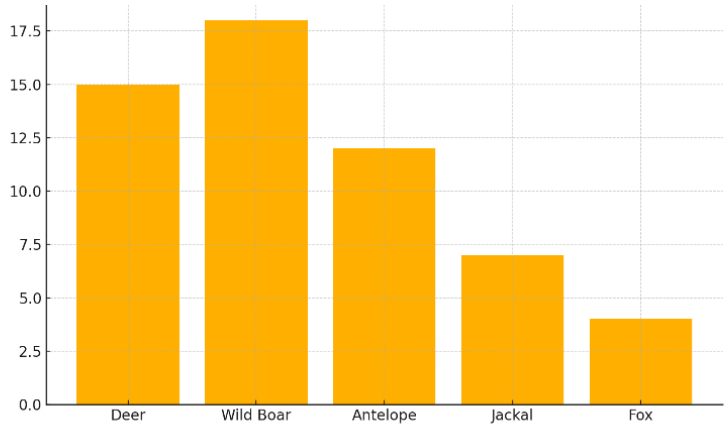

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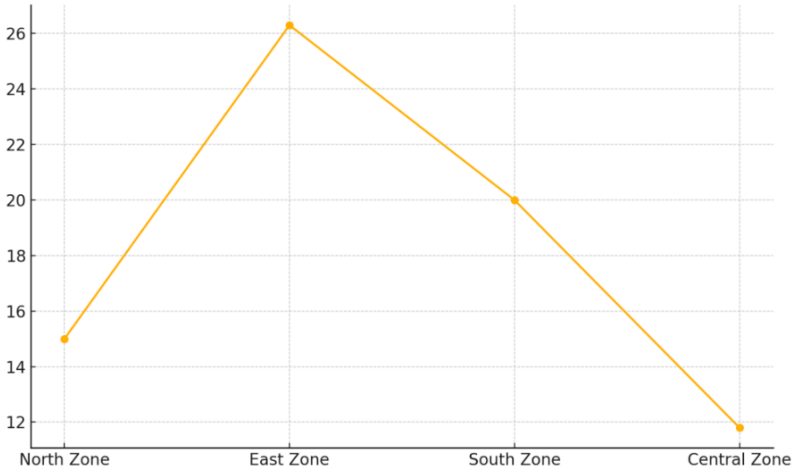

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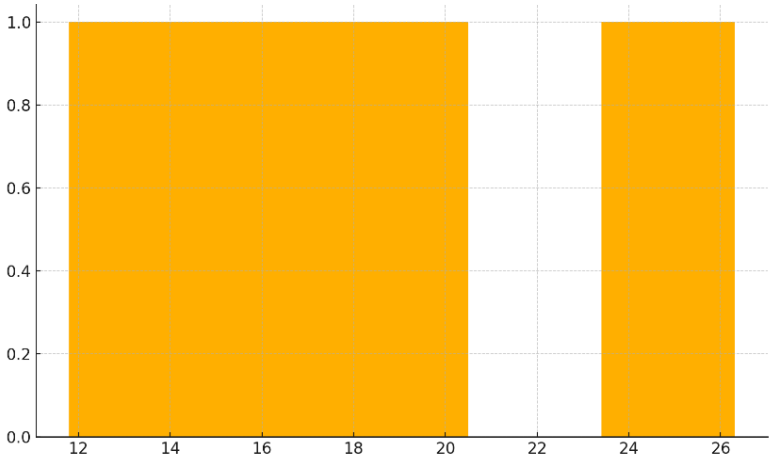

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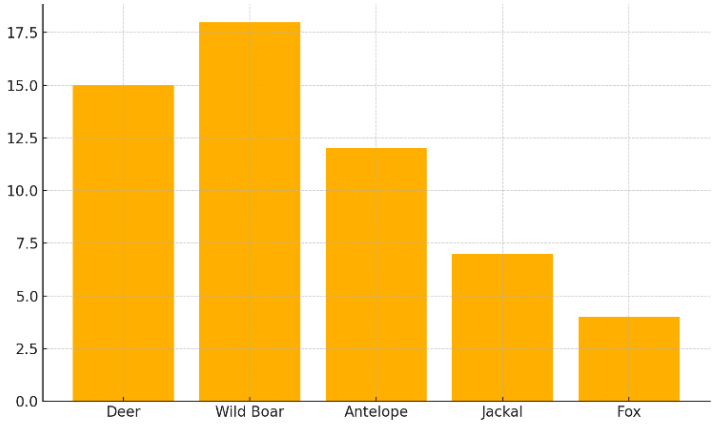

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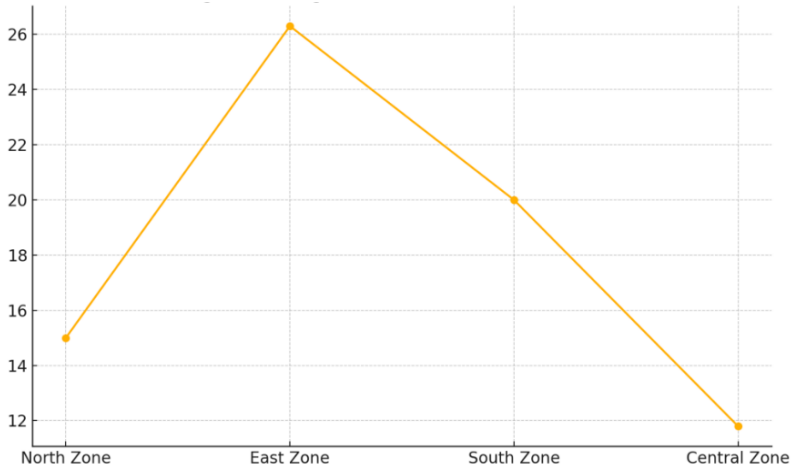

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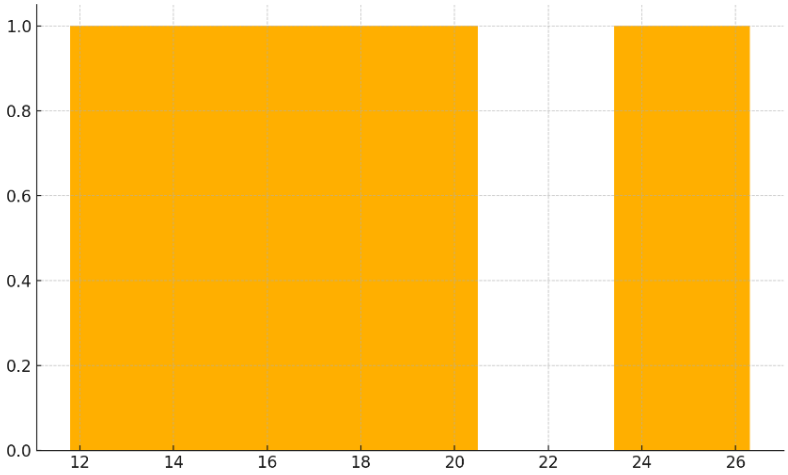

Figure 9: Caption corresponding to the analysis above.

DISCUSSION

Caused by *Mycobacterium tuberculosis*, tuberculosis is a highly contagious illness that primarily targets the lungs although it can also infect other parts of the body (Jegede et al., 2022). The problem of tuberculosis remains a serious global health hazard, constituting approximately 28,000 new infections and 4,100 deaths every day (Italia et al., 2023). It is believed that approximately a third of the global population is infected with *Mycobacterium TB*. Among them, active tuberculosis develops in 10 percent, and 90 percent remain in the latent, asymptomatic form (Park et al., 2022). In the strategy of the World Health Organization to eliminate tuberculosis, locating individuals at risk of developing active tuberculosis is extremely crucial (Davies et al., 2024). Nevertheless, recent years show that the global tuberculosis morbidity and mortality rates are decreasing; however, it becomes increasingly difficult to meet the goals established as part of the WHO End TB Strategy (Belachew et al., 2022). Mycobacterium tuberculosis is a facultative intracellular pathogen that induces tuberculosis (Negi et al., 2022). It is considered one of the top 10 factors of death worldwide (Abdalla et al., 2020). The primary cause of tuberculosis is *Mycobacterium tuberculosis* although other causes of the disease include *Mycobacterium bovis*, *Mycobacterium africanum* as well as *Mycobacterium microti*. Approximately 1.5 million persons died of tuberculosis in 2020, which consolidates its position as the second deadliest infectious disease after COVID-19 (Wang et al., 2022). In 1882, Robert Koch identified *Mycobacterium tuberculosis* as the organism that causes tuberculosis; however, the TB epidemic continues its spread worldwide (Alsayed & Gunosewoyo, 2023). The exact evolutionary date of *M. tuberculosis* is debatable, but it has been a significant problem since the Neolithic human dispersal (Faraz et al., 2022). It ranks as the 13 th most frequent cause of death globally and the second most frequent cause of death by a single infectious agent, after COVID-19 (Rampedi et al., 2022). In the majority of cases, the disease strikes adult individuals during the most productive age (Aslan et al., 2023). A contagious bacterial infection, tuberculosis ranks among the leading ten causes of death on the global scale and particularly in young adults (Angula et al., 2021). In 2019, approximately 10 million individuals were living with the disease, and 1.4 million of them had succumbed (Moyo et al.,

2022; Nishtar et al., 2021). Mycobacterium tuberculosis causes the disease and primarily attacks lungs. In order to have the tuberculosis situation under control, you must apply many measures, including fast and efficient diagnosis, treatment regimens, and preventive measures. The rapid diagnosis of TB is very important in order to prevent the spread of this disease, as well as beginning treatment immediately, particularly in crowded places with poor sanitation.

CONCLUSION

The piece provides invaluable information concerning the epidemiology of Mycobacterium bovis in wild populations living near cattle farming regions, highlighting the complexity of disease ecology at the wildlife livestock interface. The detection of M. bovis in several wild animals, including wild boars, deer, badgers, and small mammals, indicates that these animals can be significant reservoirs that aid the disease to remain alive and re-enter the cattle populations. The geographical concentration of the infections particularly in the regions with numerous livestock and water sources that are shared indicates that the environmental and man-made factors increase the susceptibility of the disease transmissions. Multivariate risk analysis revealed that proximity to cow farms, communal water points and dense woods had a high association with increased wildlife infection rates. This demonstrates how ecological overlap and bad biosecurity could contribute to the spread of the disease. Also, genetic characterisation of M. bovis strains revealed an overlap of genotypes in wildlife and cattle, which supported the evidence of the bidirectional transmission and integrated disease surveillance needs. These findings indicate the need to include wildlife monitoring within national bovine tuberculosis control programs. Risk-based management solutions are urgently required to interrupt the transmission cycle. These involve spatial zoning, focused vaccination efforts, controlled access to common resources, and enhanced biosecurity at the farm -wildlife interface. In addition, disease control interventions cannot be effective in the long term unless individuals are sensitized about community health problems and join local communities. It is because of these inadequacies of available vaccinations and diagnostic measures that continuous funding of the research of better tools is important. This research supports the concept of the One Health approach, where coordinated efforts across veterinary, environmental and public health will effectively reduce the burden of M. bovis and safeguard the health of livestock and rural economies, as well as ecological integrity in transboundary landscapes.

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