Bovine Tuberculosis: A One Health Review of Diagnostic Limitations and Emerging Molecular Diagnostics

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***Abstract*—Bovine tuberculosis (bTB), caused predominantly by Mycobacterium bovis, remains a major constraint to livestock productivity, zoonotic tuberculosis control, and One Health surveillance efforts. Current diagnostic strategies for bTB rely primarily on immunological assays for ante-mortem screening and post-mortem confirmation through lesion detection, histopathology, or culture. While these approaches are well established, they are inherently limited by indirect detection, delayed confirmation, and reduced sensitivity during early or subclinical infection. Importantly, definitive confirmation of bTB in live animals is frequently not possible unless characteristic pathological lesions have developed, allowing prolonged undetected transmission within herds. Advances in molecular diagnostics and non-invasive sampling strategies in human tuberculosis have demonstrated that Mycobacterium tuberculosis DNA can be reliably detected from oral surfaces, including tongue swabs, using PCR-based methods. These findings challenge conventional assumptions regarding specimen selection and highlight the diagnostic potential of alternative sampling sites. Drawing on biological and epidemiological parallels between human and bovine tuberculosis, this review critically synthesizes current knowledge on bTB etiology, transmission, and diagnostic limitations, and evaluates the rationale for investigating tongue swab–based molecular screening in cattle. The integration of tongue swab PCR as an adjunct diagnostic approach may enhance early detection, reduce reliance on lesion-dependent confirmation, and strengthen herd-level surveillance within One Health–oriented control frameworks. Rigorous field validation studies are required to define its diagnostic performance and operational feasibility in bovine populations.**

***Index Terms*—Bovine tuberculosis; diagnostic limitations; early detection; molecular diagnostics; Mycobacterium bovis; non-invasive sampling; One Health; tongue swab PCR**

I. INTRODUCTION

Tuberculosis (TB) remains one of the most persistent infectious diseases affecting both human and animal populations worldwide. Despite advances in diagnostics, therapeutics, and public health interventions, TB continues to impose a substantial global burden, with millions of new infections reported annually. The disease is caused by members of the Mycobacterium tuberculosis complex (MTBC), a genetically conserved group of slow-growing acid-fast bacilli that exhibit distinct host adaptations while retaining shared pathogenic mechanisms [18].

At the cellular level, MTBC pathogens display a unique intracellular lifestyle characterized by survival and replication within host macrophages. Following inhalation, bacilli are phagocytosed but evade elimination by manipulating host immune signaling pathways, leading to granuloma formation. These granulomas function as both containment structures and niches that enable bacterial persistence, reflecting a dynamic host–pathogen equilibrium rather than complete immune clearance [9].

The immune response to MTBC infection involves coordinated interactions between innate and adaptive immunity, with $CD4^+$ T lymphocytes playing a central role in controlling bacterial replication through cytokine-mediated activation of macrophages. However, immune containment does not equate to sterilizing immunity, and many infected hosts enter a latent or subclinical state during which viable bacilli persist without overt disease. This latent reservoir represents a major obstacle to TB elimination efforts [48].

Tuberculosis is not exclusively a human disease, as several MTBC members are capable of infecting a wide range of mammalian hosts. Among these, Mycobacterium bovis is of particular importance due to its ability to infect cattle, wildlife, and humans. Genomic studies have demonstrated high similarity between M. bovis and M. tuberculosis, while also revealing host-adaptive traits that facilitate cross-species transmission and ecological persistence [6].

Bovine tuberculosis, primarily caused by M. bovis, follows a chronic disease course in cattle and is frequently characterized by prolonged asymptomatic infection. During this period, infected animals may contribute to transmission within herds without exhibiting clinical signs. Zoonotic transmission to humans can occur through occupational exposure, inhalation of aerosols, or consumption of unpasteurized dairy products, reinforcing the public health relevance of bovine TB [39].

A major challenge in TB control across species is the limitation of existing diagnostic strategies. Conventional diagnostic frameworks rely heavily on immune-based assays and culture-dependent confirmation, both of which exhibit reduced sensitivity during early infection and require substantial time or infrastructure. These constraints result in delayed diagnosis and underestimation of true disease burden in both human and animal populations [1].

Advances in molecular diagnostics and immunological profiling have reshaped contemporary understanding of tuberculosis detection. Improved insights into host–pathogen interactions and pathogen dissemination have demonstrated that MTBC DNA and antigens may be detected outside classical disease sites, challenging traditional assumptions regarding specimen selection for diagnosis [9].

In response to the shared epidemiology of tuberculosis across humans and animals, global control efforts have increasingly adopted a One Health perspective. This framework emphasizes that sustainable TB control requires integrated surveillance of human, animal, and environmental reservoirs, as failure to address zoonotic and animal sources undermines progress toward elimination goals [20].

Within this context, there is growing interest in diagnostic approaches that combine biological plausibility with operational feasibility. Early, non-invasive detection methods capable of identifying MTBC infection prior to lesion development or clinical manifestation are increasingly recognized as essential for improving surveillance effectiveness. Exploration of alternative sampling strategies therefore represents a critical research priority in modern tuberculosis control [20].

II. ETIOLOGY AND TRANSMISSION OF BOVINE TUBERCULOSIS

Bovine tuberculosis (bTB) is a chronic infectious disease caused predominantly by Mycobacterium bovis, a member of the Mycobacterium tuberculosis complex (MTBC). The pathogen is characterized by slow growth, acid-fast cell walls, and a capacity for long-term intracellular persistence within host macrophages. Unlike M. tuberculosis, which is primarily adapted to humans, M. bovis exhibits a broad host range, enabling sustained circulation across livestock, wildlife, and human populations [42].

The epidemiological success of M. bovis is partly attributable to its environmental resilience. Experimental and field studies have demonstrated that the organism can survive for extended periods in soil, water, and organic material under favorable conditions, creating opportunities for indirect transmission through contaminated environments. Such environmental persistence complicates eradication efforts, particularly in extensive grazing systems [19].

Transmission within cattle populations occurs mainly through inhalation of aerosolized bacilli expelled by infected animals. Close contact, overcrowding, and inadequate ventilation increase transmission risk, especially in intensive farming systems. Oral transmission via ingestion of contaminated feed, water, or milk has also been documented, particularly among calves, contributing to within-herd spread [38].

Silent transmission plays a critical role in bTB epidemiology. Longitudinal studies have shown that sub clinically infected cattle can shed M. bovis intermittently while remaining asymptomatic and lesion-negative. Such animals act as undetected reservoirs, sustaining infection within herds over prolonged periods and undermining control programs based solely on clinical detection [14].

Wildlife involvement further complicates transmission dynamics. Although no single dominant wildlife reservoir has been identified in India, multiple wild and feral species may contribute to pathogen circulation at the livestock–wildlife interface. Molecular epidemiological investigations indicate that wildlife can act as spillover or maintenance hosts depending on ecological context, increasing reinfection pressure on cattle populations [7].

Zoonotic transmission of M. bovis to humans represents an additional public health concern. Human infection has been associated with occupational exposure in farm and abattoir settings, inhalation of infectious aerosols, and consumption of unpasteurized dairy products. Genomic studies confirming overlap between bovine and human isolates underscore the bidirectional nature of transmission at the human–animal interface [43].

Socio-economic and structural factors significantly influence bTB transmission in endemic regions. Limited access to veterinary services, absence of systematic testing programs, and restrictions on animal culling contribute to the persistence of infected herds. These challenges are particularly pronounced in low- and middle-income countries, where bTB remains underdiagnosed despite its recognized economic and public health impact [53].

At a population level, bTB prevalence exhibits marked heterogeneity across regions and production systems. Molecular typing studies have revealed substantial genetic diversity among M. bovis strains, suggesting ongoing local transmission rather than sporadic introductions. These findings highlight the importance of region-specific surveillance and control strategies tailored to local epidemiological conditions [55].

The One Health framework provides a critical lens for understanding bTB etiology and transmission. Integrated analyses emphasize that failure to address animal reservoirs and environmental sources undermines TB control efforts in humans. Coordinated surveillance across livestock, wildlife, and human populations is therefore essential for interrupting MTBC transmission cycles [29].

Recent regional assessments continue to demonstrate the persistent burden of bovine tuberculosis in domestic livestock and wildlife. Underreporting due to diagnostic limitations remains a major concern, reinforcing the need for improved detection strategies capable of identifying infection earlier in the disease course [32].

III. CURRENT DIAGNOSTIC METHODS FOR BOVINE TUBERCULOSIS

3.1 Ante-mortem Diagnostic Tests

Ante-mortem diagnosis of bovine tuberculosis (bTB) primarily relies on immunological assays that detect host immune responses to mycobacterial antigens rather than direct identification of the pathogen. These tests are widely used due to their applicability in live animals and feasibility under field conditions; however, their indirect nature introduces inherent diagnostic uncertainty, particularly during early infection [12].

3.1.1 Tuberculin Skin Tests (SITT and CITT)

The single intradermal tuberculin test (SITT) and the comparative intradermal tuberculin test (CITT) assess delayed-type hypersensitivity reactions following intradermal injection of purified protein derivatives derived from Mycobacterium bovis and Mycobacterium avium. These reactions reflect prior immune sensitization rather than active bacterial replication, limiting their ability to distinguish between current infection and historical exposure [12]. Cross-reactivity with environmental mycobacteria remains a major limitation of tuberculin skin testing. Animals exposed to non-tuberculous mycobacteria may yield false-positive reactions, while animals in early stages of infection may fail to mount detectable responses, resulting in false negatives. Such variability reduces test specificity and sensitivity in endemic regions [25].

3.1.2 Interferon-Gamma Release Assays (IGRA)

Interferon-gamma release assays (IGRA) measure cell-mediated immune responses following ex vivo stimulation of whole blood with mycobacterial antigens. Compared with skin tests, IGRA offers improved sensitivity and earlier detection, as immune responses may be detected before measurable skin reactions develop [56].

Despite these advantages, IGRA implementation is limited by higher costs, strict sample handling requirements, and dependence on laboratory infrastructure. Variability in assay performance across epidemiological settings has also been reported, restricting its use primarily to confirmatory or parallel testing rather than routine herd-level screening [8].

3.1.3 Serological Assays

Serological assays detect antibodies directed against mycobacterial antigens and offer operational advantages such as rapid turnaround time and minimal animal handling. These assays are particularly useful in identifying animals with advanced disease, where humoral immune responses are more pronounced [57].

However, antibody-based tests exhibit limited sensitivity during early infection, as humoral responses typically arise later than cell-mediated immunity. Comparative evaluations indicate that while serology may detect cases missed by other tests, it cannot reliably function as a standalone diagnostic tool and is best used as an adjunct within multi-test strategies [54].

3.2 Post-mortem Diagnostic Methods

Post-mortem diagnosis remains a cornerstone of bTB confirmation and relies on pathological examination and direct detection of mycobacteria from tissues collected at slaughter. These methods provide higher specificity than ante-mortem tests but are inherently retrospective and unsuitable for early detection in live animals [13].

3.2.1 Slaughterhouse Inspection and Lesion Detection

Routine meat inspection focuses on identifying granulomatous lesions in organs and lymph nodes commonly affected by bTB. While this approach contributes substantially to surveillance, its sensitivity is highly variable and depends on lesion size, anatomical distribution, and inspector expertise [13].

Importantly, many infected animals do not exhibit visible lesions at slaughter, particularly during early or latent infection. Reliance on gross pathology alone therefore underestimates true prevalence and necessitates laboratory confirmation to support inspection findings [31].

3.2.2 Histopathology

Histopathological examination enables microscopic characterization of suspected lesions and supports differentiation between tuberculous and non-tuberculous granulomas. Features such as caseous necrosis, mineralization, epithelioid macrophages, and multinucleated giant cells strengthen diagnostic confidence [49].

Nevertheless, histopathological features are not pathognomonic for Mycobacterium bovis infection, as similar lesions may arise from other chronic inflammatory conditions. Consequently, histopathology serves as a supportive rather than confirmatory diagnostic method [49].

3.2.3 Mycobacterial Culture

Culture-based isolation of M. bovis is considered the reference standard for definitive diagnosis and enables strain characterization and epidemiological tracing. Advances in culture techniques have improved recovery rates from tissue samples [4]

However, prolonged incubation periods, sample contamination, and low bacterial load significantly limit culture utility for timely surveillance. These constraints reduce its practicality for rapid decision-making in control programs [22].

3.2.4 Ziehl–Neelsen Staining

Ziehl–Neelsen staining provides rapid visualization of acid-fast bacilli and is widely used due to its simplicity and low cost. Detection of acid-fast organisms offers presumptive evidence of mycobacterial infection [26]. The method exhibits low sensitivity in paucibacillary samples and cannot differentiate MTBC members from non-tuberculous mycobacteria, limiting its diagnostic specificity when used in isolation [11].

3.3 Molecular Diagnostic Approaches

Molecular diagnostic methods enable direct detection of mycobacterial DNA and offer distinct advantages over immune-based and culture-dependent approaches. These techniques are particularly valuable for early infection, extrapulmonary disease, and samples with low bacterial burden [28].

PCR-based assays targeting MTBC-specific genomic regions have demonstrated improved sensitivity and rapid turnaround times compared with conventional methods. Real-time PCR platforms further enhance diagnostic performance by enabling quantitative detection while minimizing contamination risk [23].

Automated and cartridge-based molecular systems have standardized PCR workflows and improved reproducibility across laboratories. Clinical evaluations support their utility as complementary tools within diagnostic algorithms rather than standalone replacements [10].

Recent methodological refinements have focused on enhancing analytical sensitivity through optimized amplification chemistries and sample preparation protocols. Such improvements are particularly relevant for veterinary applications, where bacterial load may be limited [24].

The diagnostic accuracy of PCR assays is strongly influenced by target gene selection. Comparative analyses highlight the trade-off between sensitivity and specificity when using multicopy insertion elements versus conserved single-copy genes, emphasizing the need for careful assay design [34].

To address these limitations, multiplex PCR strategies targeting multiple MTBC-specific genes have been proposed to improve robustness and reduce false results. This approach enhances diagnostic confidence in complex biological samples [16],[49].

IV. LIMITATIONS AND NON-RELIABILITY OF EXISTING DIAGNOSTIC TECHNIQUES

Despite widespread implementation, existing diagnostic approaches for tuberculosis suffer from fundamental limitations that restrict their reliability, particularly for early-stage detection. Immunological tests such as tuberculin skin testing and interferon-gamma release assays rely on host immune responses rather than direct pathogen detection, making them vulnerable to false-negative results in early infection and false-positive reactions due to prior sensitization or cross-reactivity [40].

Inter-test variability further complicates diagnostic interpretation. Comparative evaluations have demonstrated poor concordance between commonly used diagnostic modalities, with results influenced by antigen selection, assay cut-off thresholds, host immune status, and co-infection. Such inconsistencies reduce confidence in single-test decision-making and necessitate repeated or parallel testing, increasing cost and logistical complexity [47].

In bovine tuberculosis, a critical limitation is the inability to conclusively confirm infection in live animals. Definitive diagnosis frequently depends on post-mortem identification of characteristic tuberculous lesions or culture confirmation. Consequently, infection in farm animals often cannot be confirmed unless visible lesions have already developed, which typically occurs at advanced stages of disease [27].

This lesion-dependent diagnostic paradigm allows infected animals to remain undetected for prolonged periods while continuing to transmit Mycobacterium bovis within herds. Clinical and epidemiological analyses have highlighted that such silent transmission significantly undermines control programs, particularly in endemic regions where routine slaughter-based surveillance is limited [36].

Collectively, these limitations underscore a major diagnostic gap in tuberculosis control: the absence of reliable, early, and direct detection methods applicable to live animals. Addressing this gap is essential for improving surveillance sensitivity, reducing transmission, and enabling timely intervention, thereby justifying exploration of alternative non-invasive molecular diagnostic strategies.

V. MOLECULAR DIAGNOSTICS IN TUBERCULOSIS DETECTION

Molecular diagnostics have reshaped tuberculosis detection by enabling direct identification of mycobacterial nucleic acids from clinical samples, independent of host immune responses. Reviews of contemporary diagnostic platforms emphasize that nucleic acid amplification–based assays substantially reduce diagnostic delay and improve sensitivity in early-stage and paucibacillary disease compared with conventional methods [3].

The adaptability of molecular assays to diverse specimen types and testing environments represents a key advantage. Comparative evaluations indicate that optimized molecular workflows can be applied across respiratory and non-respiratory samples, supporting their role as complementary tools within existing diagnostic algorithms rather than standalone replacements [30].

Recent advances have focused on multiplexing and platform integration to enhance analytical robustness. By targeting multiple genomic regions simultaneously, modern molecular assays mitigate false results associated with single-target detection and improve performance in samples containing inhibitors or low DNA concentrations [51].

Despite these strengths, limitations remain that constrain widespread implementation. Clinical assessments highlight challenges related to cost, technical expertise, and interpretation of positive results in the context of non-viable organisms or environmental DNA. Consequently, molecular diagnostics are most effective when deployed within tiered diagnostic frameworks that integrate clinical, immunological, and epidemiological data [44].

VI. TONGUE SWAB SAMPLING IN HUMAN TUBERCULOSIS DIAGNOSIS

Conventional tuberculosis diagnosis relies heavily on sputum-based testing, which poses challenges in individuals who cannot expectorate adequate samples, including children, elderly patients, and those with early or paucibacillary disease. These limitations have driven interest in alternative, non-invasive sampling strategies that can capture mycobacterial material without reliance on sputum production [46].

Clinical investigations have demonstrated that Mycobacterium tuberculosis DNA can be recovered from the tongue dorsum, likely due to deposition of bacilli or bacillary DNA from respiratory secretions during coughing, speaking, or routine mucociliary clearance. Molecular analysis of tongue swabs has shown detectable MTBC DNA in individuals with confirmed pulmonary disease, supporting the biological plausibility of this sampling site [58].

Methodological optimization has been shown to substantially influence diagnostic performance. Studies assessing swab materials, sampling pressure, anatomical site, and DNA extraction workflows report that standardized collection protocols improve sensitivity and reproducibility. When combined with modern molecular platforms, tongue swab sampling has demonstrated utility even in cases where sputum-based assays yield negative or inconclusive results [33].

VII. RATIONALE FOR TONGUE SWAB–BASED SCREENING IN CATTLE

The effectiveness of bovine tuberculosis (bTB) control is limited by the absence of diagnostic tools capable of reliably identifying infection in live animals before the development of advanced pathology. Existing surveillance systems rely primarily on indirect immunological assays and post-mortem confirmation, which restrict timely intervention. Conceptual frameworks emphasizing early, non-invasive detection highlight the need for alternative sampling strategies that enable direct pathogen identification under field conditions [5].

Biological plausibility for tongue swab–based screening in cattle is supported by parallels with human tuberculosis pathogenesis and transmission. Infected animals intermittently shed mycobacterial material through respiratory secretions during breathing, coughing, and rumination, creating opportunities for deposition of mycobacterial DNA on oral surfaces, including the tongue. Within a One Health context, non-invasive oral sampling has been proposed as a practical approach that aligns animal welfare, biosecurity, and operational feasibility while complementing existing diagnostic frameworks [45].

VIII. POTENTIAL ADVANTAGES OF TONGUE SWAB PCR IN BOVINE TUBERCULOSIS

The application of tongue swab–based PCR for bovine tuberculosis (bTB) screening offers several practical and epidemiological advantages when compared with existing diagnostic approaches. One of the most significant benefits is the non-invasive nature of sample collection, which minimizes animal stress and reduces the need for prolonged restraint or specialized veterinary intervention. This feature is particularly advantageous in large herds and high-throughput settings, where repeated or large-scale testing is required [41].

In addition to improved animal welfare, tongue swab PCR has the potential to enhance early detection of infection. By enabling direct molecular identification of Mycobacterium bovis DNA, this approach may identify infected animals during stages when immunological responses are insufficient for detection by skin tests or interferon-gamma assays. Such early identification is critical for reducing silent transmission within herds and improving the overall effectiveness of control programs, especially in endemic regions [2].

Operationally, tongue swab PCR is compatible with rapid molecular workflows and can be integrated into existing surveillance frameworks without major infrastructural modifications. The simplicity of sampling, combined with the analytical sensitivity of PCR, supports its use as a screening or triage tool that complements established diagnostics rather than replacing them. Collectively, these advantages position tongue swab PCR as a promising adjunct approach for strengthening bovine tuberculosis surveillance and control strategies.

IX. TECHNICAL AND BIOLOGICAL CHALLENGES OF TONGUE SWAB–BASED DETECTION

Despite its promise as a non-invasive diagnostic approach, tongue swab–based molecular detection of tuberculosis presents several technical and biological challenges that must be addressed before routine application. One major limitation is the typically low concentration of mycobacterial DNA present on oral surfaces, particularly in early or subclinical infection. This paucibacillary nature increases the risk of false-negative results and places high demands on sampling consistency, DNA extraction efficiency, and assay sensitivity [37].

In addition to low bacterial load, biological variability within the oral cavity can influence diagnostic performance. Factors such as saliva composition, oral microbiota diversity, and presence of PCR inhibitors may interfere with nucleic acid amplification. Experimental evaluations have demonstrated that variations in swab material, sampling pressure, and anatomical site can significantly affect DNA recovery, underscoring the need for standardized collection and processing protocols [59].

Together, these challenges highlight that tongue swab PCR requires careful methodological optimization and validation before large-scale deployment. Addressing issues related to sensitivity, reproducibility, and biological variability will be critical for ensuring reliable interpretation of results, particularly when tongue swab–based testing is used as a screening or adjunct diagnostic tool.

X. SURVEILLANCE, CONTROL PROGRAMS, AND THE ONE HEALTH PERSPECTIVE

Effective control of bovine tuberculosis (bTB) depends on coordinated surveillance strategies that integrate animal health, human health, and environmental monitoring. Traditional control programs have largely focused on test-and-slaughter policies in cattle, supported by abattoir surveillance and movement control. However, evaluations of national and regional TB control initiatives indicate that fragmented surveillance systems and limited cross-sectoral data sharing reduce overall program effectiveness, particularly in endemic settings [15].

The One Health approach provides a comprehensive framework for addressing these shortcomings by recognizing tuberculosis as a shared problem at the human–animal–environment interface. Studies adopting One Health–oriented surveillance have demonstrated that neglecting animal reservoirs and environmental sources undermines progress toward TB elimination in humans. Integrated monitoring of livestock, wildlife, and exposed human populations is therefore essential for accurately assessing transmission dynamics and identifying intervention points [21].

In regions with high livestock density and close human–animal contact, including parts of South Asia and Africa, surveillance challenges are further compounded by socio-economic constraints and limited veterinary infrastructure. Analyses of TB control programs in such contexts emphasize the need for scalable, cost-effective diagnostic tools that can be deployed at the field level and incorporated into routine surveillance workflows. Non-invasive molecular screening approaches may offer added value within One Health frameworks by enhancing early detection and improving coverage across sectors [35].

XI. FUTURE PERSPECTIVES AND RESEARCH GAPS

Despite advances in tuberculosis diagnostics, significant gaps remain in the early and non-invasive detection of infection, particularly in animal populations. Recent forward-looking analyses emphasize that future diagnostic strategies must prioritize direct pathogen detection methods capable of identifying infection before clinical disease or lesion development. In this context, molecular approaches adaptable to alternative sample types are increasingly viewed as critical for strengthening surveillance and reducing transmission at the population level [17].

A major research gap lies in the limited validation of non-sputum and non-invasive sampling strategies across diverse epidemiological and host settings. While promising results have been demonstrated in controlled or human-centric studies, large-scale field evaluations in livestock are scarce. Reviews focusing on emerging diagnostic technologies highlight the need for standardized protocols, comparative performance studies, and integration of molecular tools with existing surveillance frameworks to ensure reproducibility and policy relevance [50].

From a translational perspective, future research must also address implementation challenges, including cost-effectiveness, scalability, and regulatory acceptance. Clinical and epidemiological commentaries stress that diagnostic innovation alone is insufficient without alignment to control programs and One Health strategies. Bridging laboratory advances with field applicability remains a central challenge, underscoring the importance of interdisciplinary research that connects molecular diagnostics, veterinary practice, and public health policy [60].

XII. CONCLUSION

Bovine tuberculosis continues to pose significant challenges to animal health, public health, and disease control programs, largely due to limitations in early and reliable diagnosis. Existing ante-mortem and post-mortem diagnostic approaches are constrained by indirect detection, delayed confirmation, and dependence on advanced disease pathology. These shortcomings highlight the need for complementary strategies that enable direct pathogen detection in live animals without imposing additional logistical or welfare burdens.

The evidence synthesized in this review supports the conceptual plausibility of tongue swab–based molecular screening as a non-invasive adjunct for bovine tuberculosis surveillance. Drawing from advances in human tuberculosis diagnostics and One Health–oriented research, tongue swab PCR offers potential advantages in terms of feasibility, animal welfare, and early detection. While this approach cannot replace established diagnostics, its integration into tiered surveillance frameworks may enhance case detection and reduce silent transmission within herds [31].

Future implementation of tongue swab–based screening will require rigorous field validation, optimization of molecular targets, and alignment with existing control policies. Nonetheless, the convergence of molecular diagnostics, non-invasive sampling, and One Health principles provides a promising pathway toward strengthening bovine tuberculosis control and advancing sustainable surveillance strategies across human–animal interfaces [52].

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