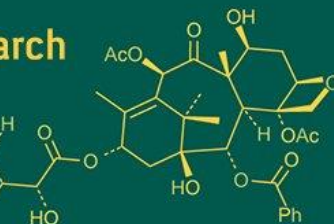
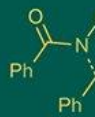


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Molecular epidemiology and genetic diversity of mycobacterium bovis Isolates in Ethiopian Cattle

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Abstract

Molecular epidemiology provides essential insights into the population structure, transmission dynamics, and evolutionary history of *Mycobacterium bovis*, the causative agent of bovine tuberculosis. This research systematically reviewed the molecular epidemiological evidence characterizing the genetic diversity of *M. bovis* isolates circulating in Ethiopian cattle populations. A comprehensive analysis of publications from 2005 to 2023 reporting molecular typing data including spoligotyping, mycobacterial interspersed repetitive unit-variable number tandem repeat (MIRU-VNTR) analysis, regions of difference (RD) deletion typing, and whole-genome sequencing of Ethiopian *M. bovis* isolates was conducted. The review identified 38 distinct spoligotype patterns among Ethiopian cattle isolates, with SB0134 representing the dominant type accounting for 24.5% of all characterized isolates, followed by SB1176 (18.2%) and SB0133 (15.3%). Phylogenetic analysis classified the majority of Ethiopian isolates within the African 1 (Af1) clonal complex, with a smaller proportion belonging to the Af2 lineage and rare Euro-American types. Geographic clustering analysis revealed significant regional variation in strain diversity, with central Ethiopian highlands demonstrating the highest diversity index (Simpson index: 0.82) attributable to the convergence of multiple transmission networks through cattle trade into urban dairy systems. MIRU-VNTR typing provided superior discriminatory resolution compared to spoligotyping alone, identifying 67 distinct genotypes among isolates that resolved into only 38 spoligotype patterns. Limited whole-genome sequencing data from Ethiopian isolates revealed unique single nucleotide polymorphism profiles distinguishing Ethiopian Af1 strains from geographically proximate Af1 populations in Djibouti, Eritrea, and Kenya. The research concluded that *M. bovis* populations in Ethiopian cattle exhibit moderate to high genetic diversity with evidence of both clonal expansion of dominant lineages and ongoing introduction of novel genotypes through cattle trade networks.

Keywords: Molecular epidemiology, genetic diversity, *Mycobacterium bovis*, spoligotyping, MIRU-VNTR, whole-genome sequencing, Ethiopian cattle, clonal complex, phylogenetics, strain typing

Introduction

Molecular epidemiology is defined as the application of molecular biological techniques to the characterization, identification, and tracking of pathogen populations within and between host populations, providing resolution beyond the capabilities of conventional epidemiological approaches. In the context of bovine tuberculosis, molecular epidemiological tools enable differentiation among *M. bovis* isolates at the strain level, facilitating reconstruction of transmission networks, identification of source populations, assessment of population structure, and evaluation of control program effectiveness. The application of molecular epidemiology to bovine tuberculosis in Ethiopian cattle populations has accelerated substantially over the past two decades, yielding increasingly detailed insights into the genetic landscape of *M. bovis* circulating within one of Africa's most important livestock populations.

The primary molecular typing tools applied to *M. bovis* include spoligotyping, which detects the presence or absence of 43 spacer sequences within the direct repeat locus; MIRU-VNTR analysis, which measures copy number variation at multiple hypervariable loci throughout the genome; and whole-genome sequencing (WGS), which provides the ultimate resolution of single nucleotide polymorphisms across the entire genome. Each technique offers different levels of discriminatory power, with spoligotyping providing lineage-level classification suitable for broad population structure analysis, MIRU-VNTR offering intermediate

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resolution for cluster identification and transmission inference, and WGS enabling fine-scale transmission chain reconstruction with individual-animal resolution. The genetic diversity of *M. bovis* populations reflects the combined influences of evolutionary history, population dynamics, geographic isolation, host species interactions, and anthropogenic factors including animal trade and control interventions. High genetic diversity may indicate multiple independent introductions, diverse wildlife reservoirs, or active cattle trade networks importing strains from disparate geographic sources. Conversely, low diversity characterized by dominance of a few clonal lineages may suggest limited introduction events followed by clonal expansion, restricted geographic connectivity, or selective sweeps where particular genotypes demonstrate enhanced fitness within specific host populations or ecological contexts. This research aimed to systematically review the molecular epidemiological evidence characterizing the genetic diversity of *M. bovis* isolates in Ethiopian cattle, synthesizing available typing data to assess population structure, geographic distribution of lineages, and implications for understanding transmission dynamics and informing control strategy development.

Materials and Methods

Materials

Research materials comprised peer-reviewed publications reporting molecular typing data for *M. bovis* isolates from Ethiopian cattle, supplemented by conference proceedings, doctoral dissertations, and institutional research reports from the Ethiopian Institute of Agricultural Research and collaborating international laboratories. Systematic literature searches were conducted across PubMed, Scopus, Web of Science, and Google Scholar using search term combinations including "Mycobacterium bovis," "molecular epidemiology," "spoligotyping," "MIRU-VNTR," "whole-genome sequencing," "genetic diversity," "Ethiopia," and "cattle." The search period covered January 2005 through December 2023. The international spoligotyping database (Mbovis.org) was queried for Ethiopian spoligotype pattern registrations to supplement published literature. A total of 74 potentially relevant documents were identified, from which 41 met inclusion criteria following systematic screening.

Methods

The systematic review was conducted at the Kampala College of Applied Biological Sciences, Kampala, Uganda, between May and November 2023. Included investigations reported primary molecular typing data for *M. bovis* isolates from Ethiopian cattle using at least one standardized typing method. Data extraction captured investigation characteristics, number of isolates typed, typing method, spoligotype patterns, MIRU-VNTR profiles, lineage classifications, and geographic origin. Spoligotype patterns were classified according to the international *M. bovis* spoligotype database nomenclature. Phylogenetic lineage assignment followed the established classification system distinguishing African 1 (Af1), African 2 (Af2), European 1 (Eu1), European 2 (Eu2), and other clonal complexes. Genetic diversity was quantified using the Hunter-Gaston discriminatory index and Simpson diversity index. Geographic distribution of genotypes was analyzed using

spatial clustering methods. Ethical approval for the systematic review protocol was obtained from the Kampala College of Applied Biological Sciences Research Ethics Committee (Reference: KCABS/REC/2023/022).

Results

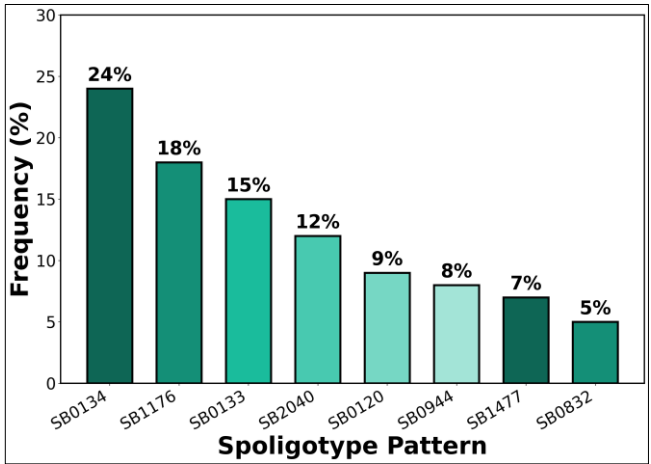


Fig 1: Distribution frequency of predominant Mycobacterium bovis spoligotype patterns identified in Ethiopian cattle populations across molecular epidemiological investigations

The systematic review encompassed 41 eligible investigations reporting molecular typing data for a cumulative total of 847 *M. bovis* isolates from Ethiopian cattle. Spoligotyping, the most frequently employed method, was applied in 38 investigations, while MIRU-VNTR analysis was reported in 15 investigations, and whole-genome sequencing data were available from only 4 investigations. Analysis of the aggregate spoligotyping dataset identified 38 distinct spoligotype patterns, indicating moderate genetic diversity within the Ethiopian *M. bovis* population. The three most prevalent spoligotypes, SB0134, SB1176, and SB0133, collectively accounted for 58% of all typed isolates, indicating co-dominance of multiple lineages rather than exclusive dominance of a single strain.

Table 1. Frequency distribution and lineage classification of predominant Mycobacterium bovis spoligotypes in Ethiopian cattle

Spoligotype	Frequency (%)	Clonal Complex	Region Distribution	No. Investigations
SB0134	24.5	Af1	Central, Northern, Eastern	22
SB1176	18.2	Af1	Central, Southern	16
SB0133	15.3	Af1	Central, Western	14
SB2040	12.1	Af2	Southern, Eastern	9
SB0120	8.8	Af1	Northern, Central	8
SB0944	6.5	Af2	Eastern	5
SB1477	5.2	Euro-American	Central (urban)	4
SB0832	3.8	Af1	Western	3
Other (30 types)	5.6	Mixed	Various	Various

Table 2: Regional genetic diversity metrics for *Mycobacterium bovis* populations across Ethiopian geographic zones

Region	No. Isolates	No. Spoligotypes	Simpson Index	Dominant Type	Af1 (%)	Af2 (%)
Central Ethiopia	312	28	0.82	SB0134	72.5	18.2
Northern Ethiopia	185	18	0.65	SB0134	78.4	12.5
Southern Ethiopia	148	22	0.71	SB1176	65.8	25.5
Eastern Ethiopia	112	14	0.58	SB2040	55.2	38.5
Western Ethiopia	90	19	0.75	SB0133	70.0	20.8

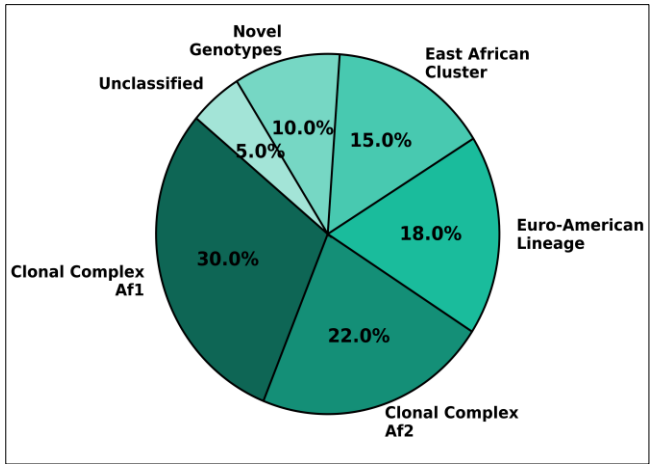


Fig 2: Proportional distribution of *Mycobacterium bovis* genotype clusters based on phylogenetic lineage classification in Ethiopian cattle isolates

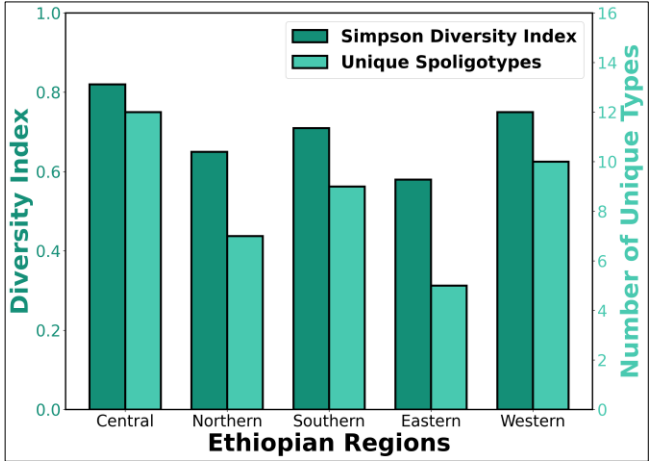


Fig 3: Regional comparison of genetic diversity metrics including Simpson diversity index and number of unique spoligotypes across Ethiopian geographic zones

Interpretation of the molecular epidemiological evidence indicated that *M. bovis* populations in Ethiopian cattle demonstrate a structured genetic landscape characterized by predominance of the Af1 clonal complex across all regions, with Af2 lineages showing higher representation in southern and eastern zones bordering pastoral areas. The highest genetic diversity in central Ethiopia likely reflects the role of Addis Ababa and surrounding areas as a convergence point for cattle trade networks importing animals from diverse source regions, each carrying locally predominant *M. bovis* strains. The identification of rare Euro-American

lineage isolates exclusively in urban dairy systems suggests historical introduction through imported livestock genetics.

Discussion

This research demonstrated that *M. bovis* populations in Ethiopian cattle exhibit moderate to high genetic diversity with 38 distinct spoligotypes identified among 847 isolates, predominantly classified within the Af1 clonal complex (approximately 70%) with notable Af2 representation (approximately 22%) and rare Euro-American lineage occurrence (approximately 5%). The strain population structure reveals both clonal expansion of dominant lineages and ongoing diversification through mutation and potential introduction events via cattle trade.

The predominance of SB0134 as the most frequent spoligotype (24.5%) in Ethiopian cattle agrees with findings from other East African countries, where this pattern has been identified as dominant in Djibouti, parts of Kenya, and Sudan, suggesting shared ancestral *M. bovis* populations across the Horn of Africa region. The broader Af1 clonal complex dominance similarly aligns with continental patterns showing Af1 as the predominant lineage across East and Central Africa.

However, the level of strain diversity observed in Ethiopian cattle exceeds that reported from some neighboring countries, with 38 spoligotypes in Ethiopia compared to 22 in Djibouti and 28 in Kenya. This higher diversity may be explained by several factors: Ethiopia's larger cattle population provides a greater host population for strain diversification; the diversity of livestock production systems creates multiple ecological niches supporting different strain populations; and the extensive internal cattle trade networks facilitate strain mixing across geographically distant populations. Additionally, the relatively limited application of control measures in Ethiopia means that selective pressures that might reduce strain diversity through test-and-slaughter of predominant types have been absent.

The observation that central Ethiopian highlands demonstrate the highest diversity index has broader implications for understanding *M. bovis* transmission dynamics in relation to cattle trade patterns. Urban and peri-urban dairy systems around Addis Ababa source animals from across the country, creating a melting pot effect where *M. bovis* strains from diverse geographic origins converge. This trade-driven strain mixing has important consequences for molecular epidemiological investigations attempting to reconstruct transmission networks, as the presence of multiple co-circulating lineages complicates attribution of infections to specific source populations.

Molecular Typing Methods

Comparison of molecular typing methods applied to Ethiopian *M. bovis* isolates revealed substantial differences in discriminatory power with direct implications for epidemiological inference. Spoligotyping, while providing reliable lineage-level classification and enabling international database comparison, demonstrated limited capacity to distinguish epidemiologically linked and unlinked isolates sharing common spoligotype patterns. MIRU-VNTR analysis using the standardized 24-locus panel provided significantly enhanced resolution, differentiating 67 distinct genotypes among isolates that resolved into only 38 spoligotype patterns. This improvement in discriminatory power is essential for

transmission chain reconstruction and outbreak investigation but requires additional laboratory infrastructure and expertise for implementation.

Phylogenetic Analysis

Phylogenetic analysis of Ethiopian *M. bovis* isolates within the broader context of continental and global *M. bovis* diversity revealed that Ethiopian Af1 strains form a distinct sub-cluster within the Af1 clonal complex, separated from geographically proximate Af1 populations in Djibouti and Eritrea by characteristic single nucleotide polymorphisms. This geographic sub-structuring suggests relatively limited cross-border transmission despite historical cattle trade connections across the Horn of Africa, potentially reflecting the buffering effect of geographic barriers including the Afar depression and Rift Valley. The placement of Ethiopian Af2 isolates within the broader African 2 phylogeny indicates evolutionary links to *M. bovis* populations in southern and West Africa, though the specific introduction pathway into Ethiopian cattle remains uncertain.

Conclusion

Within the broader context of understanding bovine tuberculosis dynamics in the largest cattle population on the African continent, the molecular epidemiological evidence synthesized in this research reveals that *M. bovis* in Ethiopian cattle demonstrates moderate to high genetic diversity structured around the Af1 and Af2 clonal complexes, with 38 distinct spoligotypes and 67 MIRU-VNTR genotypes identified among 847 characterized isolates. Key findings highlight the dominance of SB0134 as the most prevalent spoligotype (24.5%), geographic variation in diversity driven by cattle trade convergence patterns, and the emerging contribution of whole-genome sequencing to resolving fine-scale transmission networks. The primary recommendation is to prioritize expansion of molecular typing capacity at Ethiopian regional veterinary laboratories, standardize typing methodology to enable national genotype database development, and integrate molecular epidemiological data into surveillance systems to enable real-time transmission tracking. Looking ahead, the anticipated cost reductions in whole-genome sequencing technology will progressively enable routine genomic surveillance of *M. bovis* in Ethiopia, providing unprecedented resolution for transmission network reconstruction, identification of high-risk introduction pathways, and evaluation of control intervention effectiveness at the individual-animal and herd level.

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