

MYCOBACTERIA AND TUBERCULOSIS IN FORENSIC ANTHROPOLOGY AND BIOARCHAEOLOGY: PALEOPATHOLOGICAL, BIOMOLECULAR EVIDENCE AND THEORETICAL-METHODOLOGICAL IMPLICATIONS

MICOBACTERIAS Y TUBERCULOSIS EN ANTROPOLOGÍA FORENSE Y BIOARQUEOLOGÍA: EVIDENCIAS PALEOPATOLÓGICAS, BIOMOLECULARES E IMPLICACIONES TEÓRICO-METODOLÓGICAS

MICOBACTÉRIAS E TUBERCULOSE EM ANTROPOLOGIA FORENSE E BIOARQUEOLOGIA: EVIDÊNCIAS PALEOPATOLÓGICAS, BIOMOLECULARES E IMPLICAÇÕES TEÓRICO-METODOLÓGICAS

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ABSTRACT: This article presents a theoretical review on mycobacteria and tuberculosis (TB) in forensic anthropology and bioarchaeology, emphasizing paleopathological and biomolecular evidence. The aim was to systematize knowledge about the *Mycobacterium tuberculosis* complex (MTBC), skeletal diagnostic criteria, contributions of paleomicrobiology (aDNA and lipid biomarkers), and implications for forensic practice and the historical reconstruction of the disease. The methodology consisted of a narrative review of international and regional scientific literature, prioritizing studies in paleopathology, paleogenomics, and identified skeletal collections. Main findings indicate that classic lesions (Pott's disease) and non-specific indicators (visceral rib periostitis, endocranial changes) should be interpreted together with molecular data; that the true prevalence of past infection was underestimated by the skeletal record; and that recent evolutionary scenarios point to an ancient human origin of the MTBC and complex zoonotic routes, including in pre-contact Americas. It is concluded that integrating morphological and biomolecular methods strengthens diagnosis and contextual interpretation of TB in archaeological and forensic human remains.

Keywords: Tuberculosis. Paleopathology. Forensic anthropology. Ancient DNA. *Mycobacterium tuberculosis*.

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RESUMEN: Este artículo presenta una revisión teórica sobre micobacterias y tuberculosis (TB) en el ámbito de la antropología forense y la bioarqueología, con énfasis en evidencias paleopatológicas y biomoleculares. El objetivo fue sistematizar el conocimiento acerca del Complejo *Mycobacterium tuberculosis* (MTBC), de los criterios de diagnóstico óseo, de las contribuciones de la paleomicrobiología (aDNA y biomarcadores lipídicos) y de las implicaciones para la práctica forense y la reconstrucción histórica de la enfermedad. La metodología consistió en una revisión narrativa de la literatura científica internacional y regional, priorizando estudios de paleopatología, paleogenómica y colecciones osteológicas identificadas. Los principales resultados indican que las lesiones clásicas (enfermedad de Pott) y los indicadores no específicos (periostitis visceral de costillas, alteraciones endocraneales) deben interpretarse junto con datos moleculares; que la prevalencia real de infección en el pasado fue subestimada por el registro óseo; y que escenarios evolutivos recientes apuntan a un origen humano antiguo del MTBC y rutas zoonóticas complejas, inclusive en las Américas precontacto. Se concluye que la integración de métodos morfológicos y biomoleculares fortalece el diagnóstico y la interpretación contextual de la TB en restos humanos arqueológicos y forenses.

Palabras clave: Tuberculosis. Paleopatología. Antropología forense. ADN antiguo. *Mycobacterium tuberculosis*.

RESUMO: Este artigo apresenta uma revisão teórica sobre micobactérias e tuberculose (TB) no âmbito da antropologia forense e da bioarqueologia, com ênfase em evidências paleopatológicas e biomoleculares. O objetivo foi sistematizar o conhecimento acerca do Complexo *Mycobacterium tuberculosis* (MTBC), dos critérios de diagnóstico ósseo, das contribuições da paleomicrobiologia (aDNA e biomarcadores lipídicos) e das implicações para a prática forense e a reconstrução histórica da doença. A metodologia consistiu em revisão narrativa da literatura científica internacional e regional, priorizando estudos de paleopatologia, bioarqueologia, paleogenômica e coleções osteológicas identificadas. Os principais resultados indicam que lesões clássicas (doença de Pott) e indicadores não específicos (periostite visceral de costelas, alterações endocranianas) devem ser interpretados em conjunto com dados moleculares; que a prevalência real de infecção no passado foi subestimada pelo registro ósseo; e que cenários evolutivos recentes apontam para origem humana antiga do MTBC e rotas zoonóticas complexas, inclusive nas Américas pré-contato. Conclui-se que a integração de métodos morfológicos e biomoleculares fortalece o diagnóstico e a interpretação contextual da TB em remanescentes humanos bioarqueológicos e forenses.

Palavras-chave: Tuberculose. Paleopatologia. Antropologia forense. DNA antigo. *Mycobacterium tuberculosis*.

INTRODUCTION

Tuberculosis (TB), caused by bacteria belonging to the *Mycobacterium tuberculosis* complex (MTBC), remains one of the infectious diseases with the greatest impact on human populations, both in the present and throughout the past. Its ability to produce skeletal lesions in a subset of affected individuals, combined with the possibility of recovering ancient DNA (aDNA) and lipid biomarkers from human remains, has made TB a central subject in paleopathology, bioarchaeology, and, increasingly, forensic anthropology (ROBERTS; BUIKSTRA, 2003; DONOGHUE, 2011). The investigation of TB in bioarchaeological contexts enables the reconstruction of pathogen evolutionary trajectories, patterns of host-pathogen coevolution, population density, human mobility, and zoonotic interactions. Within forensic

anthropology, these approaches contribute to biological profiling, the reconstruction of past living conditions, and, in some cases, the development of hypotheses regarding the cause of death in human remains that have undergone advanced skeletonization.

Traditionally, paleopathological diagnosis of TB has relied on the identification of tuberculous spondylitis (Pott's disease) and other osteoarticular lesions. Studies conducted on identified skeletal collections have expanded the range of diagnostic indicators, including periosteal reactions on the visceral surface of ribs and endocranial alterations (SANTOS; SUBY, 2012; MARIOTTI et al., 2015). In parallel, paleomicrobiological investigations have demonstrated that infection can be detected even in the absence of visible skeletal lesions, indicating that the true prevalence of TB in past populations has likely been underestimated (MÜLLER et al., 2014; DONOGHUE, 2016). Evolutionary scenarios based on ancient genomes have challenged the classical hypothesis of an exclusively Neolithic zoonotic origin of tuberculosis and have revealed complex transmission pathways, including those involving marine mammals in pre-contact American populations (BOS et al., 2014; VÅGENE et al., 2022).

Despite these advances, significant gaps remain regarding the systematic integration of morphological and molecular criteria, as well as the translation of this knowledge into forensic practice. This theoretical review aims to synthesize paleopathological and biomolecular evidence regarding mycobacteria and tuberculosis, discuss diagnostic criteria and their theoretical and methodological implications for forensic anthropology and bioarchaeology, and highlight current limitations and future research perspectives. The relevance of this topic lies in the dual role of TB as a marker of health status and social vulnerability in both past and contemporary populations, as well as in the need for robust diagnostic frameworks applicable to human remains from diverse temporal and contextual backgrounds.

METHODS

This study consists of a narrative review of the scientific literature, with a theoretical and interdisciplinary approach. Scientific databases and repositories were consulted, including journals in physical anthropology, paleopathology, microbiology, and bioarchaeology, focusing on publications addressing tuberculosis (TB) in human skeletal remains, MTBC paleogenomics, identified osteological collections, and forensic applications. Priority was given to review articles, original studies supported by molecular confirmation or validated through documented skeletal series, and contributions addressing diagnostic criteria and pathogen

evolution. No strict chronological timeframe was established, allowing the inclusion of both foundational studies and recent advances in ancient genomics and lipid biomarker analyses.

The selection process emphasized theoretical and methodological relevance rather than quantitative exhaustiveness. The retrieved information was organized into thematic axes: MTBC etiology and evolution; paleopathological diagnostic criteria; contributions of paleomicrobiology; spatiotemporal evidence; and applications and limitations within forensic anthropology and bioarchaeology. Ethical considerations and contamination issues related to ancient DNA (aDNA) analyses were incorporated into the methodological discussion. As this study involved a review of previously published literature, no primary data collection involving human or animal subjects was performed; therefore, submission to a research ethics committee was not required, in accordance with standard practices for theoretical literature reviews.

RESULTS AND DISCUSSION

Etiology and Evolutionary Scenarios of the *Mycobacterium tuberculosis* Complex

The *Mycobacterium tuberculosis* complex (MTBC) comprises closely related species, including *Mycobacterium tuberculosis* (primarily adapted to humans), *M. bovis*, *M. africanum*, *M. pinnipedii*, among others (ZINK et al., 2005; DONOGHUE, 2011). Classical human transmission occurs mainly through the airborne route; however, zoonotic transmission pathways also occur. Only approximately 3–5% of untreated active infections result in diagnostically identifiable skeletal lesions, leading to an underestimation of tuberculosis prevalence in archaeological records (ROBERTS; BUIKSTRA, 2003; HOLLOWAY et al., 2011). The hypothesis of an exclusively Neolithic origin of tuberculosis from *M. bovis* has been revised by genomic and paleogenomic studies, which suggest an older human-associated ancestry and previous divergence from animal-adapted lineages (HERSHKOVITZ et al., 2008; BOS et al., 2014).

Estimates of the most recent common ancestor (MRCA) of the MTBC vary considerably. Models based on modern genomes indicate an African origin associated with *Homo sapiens* migrations, whereas calibrations incorporating ancient genomes suggest an emergence during the Holocene, on the timescale of only a few thousand years (SABIN et al., 2020). In the Americas, pre-Columbian genomes closely related to *M. pinnipedii* indicate that seals and sea lions may have acted as reservoirs and transmission vectors before European contact (BOS et al., 2014; VÂGENE et al., 2022). These findings add complexity to the

evolutionary history of tuberculosis and have direct implications for the interpretation of skeletal lesions and molecular results across different geographic and temporal contexts.

Paleopathological Diagnostic Criteria

Classical paleopathological diagnosis is primarily based on tuberculous spondylitis (Pott's disease), characterized by vertebral body destruction, possible angular kyphosis, and cold abscess formation, as well as arthritis affecting large joints (ROBERTS; BUIKSTRA, 2003; DONOGHUE, 2016). Studies of identified skeletal collections have demonstrated a statistical association between visceral rib periostitis and death from pulmonary tuberculosis, as well as the relevance of endocranial alterations and hypertrophic osteoarthropathy as indicators of chronic respiratory disease processes (SANTOS; SUBY, 2012; MARIOTTI et al., 2015). However, these findings are not individually pathognomonic and require differential diagnosis with conditions such as brucellosis, osteomyelitis, and other infectious or inflammatory disorders.

Meta-analytical studies indicate a reduction in the relative frequency of skeletal lesions over time and a shift from predominantly spinal involvement toward a greater contribution of extraspinal lesions in more recent periods (HOLLOWAY et al., 2011). Macroscopic examination should be complemented, whenever possible, by radiographic imaging or micro-computed tomography (micro-CT), which may reveal internal cavities in vertebrae without evident external lesions (MARIOTTI et al., 2015). In forensic anthropology, these criteria contribute to the interpretation of skeletal remains lacking clinical documentation, provided that the limitations regarding sensitivity, specificity, population variability, and preservation conditions are carefully considered.

Paleomicrobiology: aDNA and Lipid Biomarkers

The detection of MTBC ancient DNA (aDNA) through PCR-based approaches (including the IS6110 insertion sequence) and, more recently, high-throughput sequencing, has enabled the confirmation of infection in skeletal remains exhibiting typical, atypical, or even absent pathological lesions (SPIGELMAN; LEMMA, 1993; MÜLLER et al., 2014). Studies involving mummified remains, such as those from Vác (Hungary), have revealed high rates of molecular positivity (ZINK et al., 2023). Cell wall lipid biomarkers, including mycolic acids and mycocerosic acids, provide independent evidence and, in some cases, may exhibit greater preservation stability than degraded DNA molecules (DONOGHUE et al., 2017). The

combined analysis of aDNA and lipid biomarkers confirmed tuberculosis infection at Atlit-Yam (approximately 9250–8160 BP) and in other emblematic archaeological contexts (HERSHKOVITZ et al., 2008).

Among the main methodological challenges are aDNA fragmentation, low endogenous DNA content, and the risk of contamination by environmental mycobacteria or modern DNA. These limitations require dedicated clean-room facilities, negative controls, and authentication through multiple loci analyses or whole-genome approaches (MÜLLER et al., 2014; WITAS et al., 2015). Ribs exhibiting visceral bone formation and dental tissues have emerged as promising substrates for molecular investigations. The integration of morphological, molecular, and lipid-based approaches represents the most robust diagnostic strategy for both bioarchaeological studies and long-term forensic investigations.

Bioarchaeological Evidence and Forensic Applications

The paleopathological record of tuberculosis extends from the Neolithic period to historical contexts, with a greater concentration of evidence from Europe and the Near East and an increasing body of data from the Americas (ROBERTS, 2015; HOLLOWAY et al., 2011). Pre-contact and post-contact cases in the Americas, including molecular evidence from Brazilian Indigenous populations, have expanded our understanding of the geographic dissemination and evolutionary history of the disease (JAEGER et al., 2018). In forensic anthropology, the identification of TB-compatible lesions and, when feasible, molecular biomarkers contributes to health profiling, hypotheses regarding living conditions, and, in unidentified skeletal cases, the reconstruction of life histories shaped by deprivation, overcrowding, and social vulnerability (SANTOS, 2020).

The transfer of knowledge between paleopathology and forensic anthropology is bidirectional: identified skeletal collections provide calibration for morphological criteria, whereas contemporary forensic cases expand the spectrum of observed pathological variation. Limitations include the absence of skeletal lesions in many infected individuals, the need for rigorous differential diagnosis, and biosafety and aDNA preservation challenges in more recent contexts. Therefore, tuberculosis functions as a proxy for social vulnerability, demographic processes, and epidemiological dynamics, both in past populations and in contemporary investigations.

Limitations and Future Perspectives

The underrepresentation of skeletal lesions, differential biomolecular degradation, contamination risks, and the unequal distribution of osteological collections and molecular expertise constrain broad interpretations and generalizations (DONOGHUE, 2011; MÜLLER et al., 2014). Diagnoses based exclusively on morphological evidence often remain at the level of compatibility rather than confirmation, requiring caution in epidemiological interpretations. Future perspectives include the expansion of ancient genome datasets with improved chronological calibration, validation of diagnostic criteria across collections from diverse geographic regions (including the Global South), advances in paleoproteomics, and integration with historical and social datasets. Ethical and curatorial considerations, particularly regarding the partial destruction of human skeletal remains for molecular analyses, must be explicitly addressed (ZINK et al., 2023).

CONCLUSION

This review demonstrates that tuberculosis and MTBC mycobacteria occupy a central position at the interface between forensic anthropology, bioarchaeology, and paleopathology. Classical and non-classical skeletal lesions, ancient DNA (aDNA), and lipid biomarkers enable the reconstruction of the antiquity, dissemination, and evolutionary history of tuberculosis while also supporting diagnostic frameworks applicable to human remains of archaeological and forensic interest. Current evolutionary scenarios reject the simplicity of an exclusively Neolithic zoonotic origin hypothesis and incorporate complex transmission pathways, including zoonotic events mediated by marine mammals in pre-contact American populations.

The true prevalence of tuberculosis infection in past populations was likely considerably higher than that inferred solely from skeletal lesions. The integration of morphological and biomolecular approaches, combined with contextual interpretation encompassing environmental, demographic, and social factors, strengthens diagnostic assessments and improves the understanding of TB as a historical and social process. In forensic anthropology, tuberculosis represents a marker of health status and vulnerability, as well as a potential indicator for reconstructing life histories. Continued interdisciplinary research is recommended, with standardized protocols, the inclusion of underrepresented geographic regions, and careful ethical considerations to consolidate knowledge regarding the long-term relationship between humans and pathogenic mycobacteria.

Beyond the aspects discussed above, the role of nutrition and physiological stress in tuberculosis progression and skeletal lesion formation should be further considered. Protein and iron availability, as well as nutritional deficiencies, influence immune responses and the dissemination of bacilli to skeletal tissues, with direct implications for paleopathological interpretations in populations exhibiting evidence of nutritional stress, as previously demonstrated by Wilbur and colleagues (2008). Therefore, the association between stress indicators, such as porotic hyperostosis and enamel hypoplasia, and TB-related skeletal lesions should be investigated through an integrated approach rather than interpreted in isolation.

From the perspective of forensic anthropology applied to contemporary contexts of social vulnerability, tuberculosis remains a relevant comorbidity among homeless populations, incarcerated individuals, and migrant communities (CONTELLI, 2025). The ability to recognize skeletal patterns compatible with tuberculosis in skeletonized remains may assist both identification processes and the reconstruction of circumstances surrounding life and death, particularly when previous medical records or information provided by relatives are available. Biosafety procedures and preservation protocols for potential molecular analyses should be maintained even in recent forensic contexts (CONTELLI, 2025).

The standardization of diagnostic criteria among different research groups remains a significant challenge. Variations in the definition of lesions considered “suggestive” or “compatible” with tuberculosis, as well as differences in the recognition and recording of non-classical indicators, hinder interpopulation comparisons and meta-analytical approaches. The implementation of explicit recording protocols, systematic photographic and radiological documentation, and, whenever possible, molecular validation of subsamples is recommended to improve data comparability and reliability in paleopathological and forensic investigations.

Finally, the integration of paleopathology, ancient genomics, and the history of medicine provides new perspectives for investigating the evolution of virulence, the emergence of antimicrobial resistance in historical populations, and the demographic impacts of tuberculosis epidemics under different social and economic systems. As an interdisciplinary research subject, tuberculosis continues to provide valuable insights into the complex interactions among biology, society, and environment throughout human history and within contemporary practices of bioarchaeology and forensic anthropology.

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