Contribution of paleopathology to the knowledge of the origin and spread of tuberculosis: evidence from Portugal

Contribuição da paleopatologia para o conhecimento da origem e dispersão da tuberculose: evidências de Portugal

Ana Luisa Santos[1a], Vítor M. J. Matos[1b*]

Abstract Paleopathology contributes to the knowledge of health and disease in past populations. In the case of tuberculosis, paleopathological research contributes to a better understanding of the antiquity and spread of the disease around the globe, as well as in Portugal. These aspects are the objectives of this work. Genomic research on the Koch bacillus indicates a co-evolution with African *Homo sapiens*. However, macroscopic, microscopic, imaging and biomolecular analyzes of human skeletal remains suggest that tuberculosis (TB) began to affect humans during the Neolithic period. For several decades the paleopathological diagnosis of tuberculosis was essentially based on the identification of Pott's disease. More recently, the study of identified skeletal collections has revealed a statistically significant association between both new

Resumo A paleopatologia contribui para o conhecimento da saúde e da doença em populações do passado. No caso particular da tuberculose auxilia na pesquisa que pretende determinar a antiguidade e a dispersão da doença pelo mundo, bem como as evidências existentes em Portugal. Estes aspetos constituem os objetivos deste trabalho. As pesquisas genómicas ao bacilo de Koch indicam uma coevolução com o *Homo sapiens* a partir de África. No entanto, análises macroscópicas, microscópicas, imagiológicas e biomoleculares dos vestígios osteológicos humanos apontam para que a tuberculose tenha começado a afetar a humanidade no período Neolítico. Durante várias décadas o diagnóstico paleopatológico da tuberculose fez-se, essencialmente, pela identificação do Mal de Pott. Mais recentemente, fruto de estudos em coleções

[1] CIAS — Centro de Investigação em Antropologia e Saúde, University of Coimbra, Portugal.
[a] orcid.org/0000-0001- 6073-1532, [b] orcid.org/0000-0003-2620-7352
* Autor correspondente/Corresponding author: vmatos@antrop.uc.pt

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bone formation on the visceral surface of the ribs and hypertrophic osteoarthropathy and cause of death by pulmonary tuberculosis. Therefore, these skeletal signs have been used to assist in the differential diagnosis of pulmonary TB. Portugal, as in many other countries, notably European countries, was greatly affected by tuberculosis. However, the paleopathological record in the national territory can be considered quite scarce. Amongst the 8000 individuals studied from archaeological excavations, only 81 have bone changes compatible with the disease. Continued research on human and animal osteological remains will certainly bring new developments concerning the antiquity, evolution and spread of tuberculosis across populations and continents.

Keywords: Paleotuberculosis; Pott's disease; biological anthropology; bioarchaeology; paleopathology.

osteológicas identificadas, verificou-se uma associação estatisticamente significativa entre a formação de osso novo na superfície visceral das costelas e a osteoartropatia hipertrófica nos indivíduos que tiveram tuberculose registada como causa de morte e, portanto, estas lesões começaram a ser usadas no diagnóstico diferencial desta doença. Portugal, tal como muitos outros países, maioritariamente europeus, foi bastante afetado pela tuberculose. No entanto, o registo paleopatológico em território nacional pode ser considerado escasso. Dos mais de 8000 indivíduos estudados provenientes de escavações arqueológicas em território português apenas 81 apresentam alterações ósseas compatíveis com a doença. A continuação das pesquisas em vestígios osteológicos humanos e de animais irá, certamente, trazer novos desenvolvimentos acerca da antiguidade, evolução e dispersão da tuberculose pelas populações e continentes.

Palavras-chave: Paleotuberculose; mal de Pott; antropologia biológica; bioarqueologia; paleopatologia.

Introduction

Paleopathology is the study (*logos*) of ancient (*paleo*) suffering or disease (*páthos*). Its first documented use was in 1892 by the physician and ornithologist Robert Wilson Shufeldt (1850–1934) who proposed this term, that "[…] described all diseased or pathological conditions found fossilized in the remains of extinct or fossil animals" (1893: 679). Today, it is considered as a subdiscipline of Biological Anthropology (Jurmain et al., 2017) and focuses on the study of the history of diseases following the biocultural approach, i.e. biological data are interpreted within appropriate cultural contexts which include relevant details of the historical development, local geography, and material culture of the people whose remains are under examination (Roberts and Manchester, 1995; Suby, 2015; Grauer, 2018).

Paleopathologists examine both primary evidence (bones, calcified tissues, preserved bodies such as mummies, and coprolites) and secondary sources, such as contemporary documents (medical and historic records) and iconographic representations, such as artefacts and works of art (Roberts and Manchester, 1995; Santos, 1999/2000; Santos and Suby, 2012).

In Portugal, to the best of our knowledge, the first published work referring to human paleopathology (trepanations in Neolithic skulls) dates back to the end of the 19th century (Delgado, 1884). Later, by the 1950s, the word paleopathology started to be used by Sueiro and Frazão (1956; 1957/1959). More recently, starting in the 1990s, the discipline witnessed an increasing attention by many scholars and students and since then human remains excavated in the Portuguese territory, with chronologies from the Mesolithic period to the Modern Era, and individuals from documented collections (19th-early 20th century), have been object of study. A short history on the developments of paleopathology in Portugal can be consulted in Santos and Cunha (2012).

The aims of this work are twofold: summarize the contributions of paleopathology to the study of tuberculosis (TB) spread in the past and to reveal the existing evidence in Portugal.

Short history of tuberculosis

In humans, TB may be a chronic or acute infection of bone and/or soft tissues (Ponnuswamy, 2014). For this reason, TB was classified in the past under many different names, reflecting specific anatomical locations and lesions, each kind being considered a different disease. For example, *lupus vulgaris* was the designation of a tuberculous infection of the skin (Benedek, 2008).

The word "tuberculosis" was presented by Johann Lukas Schoenlein in 1834 to ascertain the pathological development of tubercles as defined by Sylvius (Ferlinz, 1999). It is a Latin noun with a Greek ending (Magyar, 1999) to refer to disease caused by a bacterium of the genus *Mycobacterium*. The word "tuber" comes from the Latin word "tumesco", which refers to all kinds of degenerative protuberances or tubercles (Georges, 1880 *in* Magyar, 1999) and "osis" is a Greek suffix denoting a disease condition.

The term "tuberculum" was probably used for the first time "in a medical context and meaning" by the Roman physician Celsus in the first century CE (Magyar, 1999). However, Greek was the language used for medical writings in the Classical world, so "phthisis" (pulmonary illness), from the Greek word "phthio" (Magyar, 1999) which means "emaciated" (Almeida, 1995) was used in the Hippocratic writings to describe this disease (Almeida, 1995; Magyar, 1999). For several centuries, "phthisis" was used interchangeably with "consumption", the term for any and all diseases causing wasting away of tissues but especially for pulmonary tuberculosis (Ott, 1996). From the Medieval period to the 18th century tuberculous abscessing of the lymph nodes was known as "scrofula" (Daniel et al., 1994). Depending on the precise anatomical locations, several subdivisions emerged such as "scrofula vulgaris", "scrofula mesenterica", "scrofula americana" and "scrofula fugax" for the old term "struma" or goitre identified already by the Classical physicians (French, 1993). During the 19th century, "scrofula" survived as an adjective, e.g., "scrofulous tumours", but after Koch discovered the *M. tuberculosis* bacillus in 1882 the word ceased to be used in current medical contexts and was relegated to medical history (French, 1993).

Between the 17th and 19th centuries, TB spread throughout almost all of Western Europe (Daniel et al., 1994) stimulated much medical research. The disease was so deadly that it was named the White Plague, an obvious reference to the Black Plague that devastated Europe in the late Middle Ages (Daniel et al., 1994). The post-medieval villages and cities suffered an increase in population due to the exodus of people from the countryside. The overcrowded houses and famine, due to wars and environmental problems that caused bad crops, weakened the immune system of the population who shared their living accommodations with animals, such as tuberculous cattle (Roberts et al., 1998).

Until the 18th century, tuberculous patients were not accepted in hospitals because they were considered as incurable. Tuberculosis was not considered as an infectious disease, which has contributed to the spread of the disease (Santos, 2000). It was estimated that during the 19th century one seventh of the world population died from tuberculosis (Oliveira, 1954; Koch, 1982 [1882]).

In recent years, many works have been published about clinical aspects of TB and about TB through time. It is now accepted that the disease may attack any part of the human body and the infection is mainly caused by *M. tuberculosis*, *M. africanum* and *M. canetti*, but at least seven other species, that are part of the *Mycobacterium tuberculosis* complex (MTBC), affect a wide range of domesticated and wild species with potential transmissibility to humans (Smith et al., 2009), including *M. bovis* (bovids), *M. caprae* (sheep and goats), *M. microti* (field mice, voles and llama) and *M. pinnipedii* (whales and sea lions).

The knowledge of the origin and evolution of the disease may be gained through the study of ancient human remains (see Roberts and Buikstra, 2003). Regrettably, tuberculosis may leave marks on skeletal elements which are not strictly pathognomonic of this disease. Depending on the affected area of the body and whether the infection is acute or chronic, tuberculosis may or may not leave skeletal signatures (Santos, 2000). Thus, there is a huge discrepancy between frequencies of tuberculosis recorded in documents from the past and the prevalence of evidence from both archaeological and modern skeletons.

For several decades the paleopathological diagnosis of tuberculosis was made essentially by the identification of Pott's disease, named in honor of Sir Percival Pott (1713–1788), a surgeon at St. Bartholomew's Hospital, London, who in 1779 described the condition of tuberculosis affecting the spine (Roberts and Manchester, 1995).

The skeleton may be affected in different forms, such as arthritis of joints (shoulders, elbows, hip and knees), and as isolated osteomyelitis (Roberts and Buikstra, 2019). These situations are difficult to identify macroscopically and/ or radiologically. Since the 1990s, both hosts and pathogenic agents started to be studied, namely the mycobacterial ancient DNA by polymerase chain reaction (PCR) technique (Donoghue, 2017; Sparacello et al., 2017) and the mycolic acids from mycobacterial cell envelope analyzed by high-performance liquid chromatography (HPLC) (e.g. Redman et al., 2009; Baker et al., 2015).

Medical knowledge, particularly on the manifestations of tuberculosis in the pre-antibiotic era, is essential for the paleopathological diagnosis. This includes hospital and sanatorium archives, sources of nosological, therapeutic and epidemiological information (Santos, 1999; 2000; 2015; Matos and Santos, 2013; 2015; Silva and Marques, 2019).

In the last decades of the 20th century the presence of new bone formation on the visceral surface of the ribs started to be associated to pulmonary infection (Kelley and Micozzi, 1984; Roberts et al., 1994). However, this hypothesis was not unanimously accepted because pulmonary tuberculosis affects the soft tissues (see Santos, 2000) and because it was expected that people would die quickly and thus before bones being affected. Interestingly, there is grounded evidence that many pulmonary TB patients from the pre-antibiotic era survived for many years (Santos, 1999; 2000; Matos and Santos, 2013; 2015, Silva and Marques, 2019). Later, the study of Portuguese documented skeletons (with known data such as the cause of death, sex and age at death) who died after the identification of the bacillus by Robert Koch and before the development of antibiotics brought new data to this discussion. Skeletons from persons who lived between 1819 and 1941, and had pulmonary tuberculosis recorded as cause of death, presented significantly higher frequencies of new bone formation on the visceral surface of their ribs when compared with those who died from non-tuberculous causes of death, in both identified collections in Coimbra (Santos, 2000; Santos and Roberts, 2001;

2006) and in Lisbon (Matos, 2003, Matos and Santos, 2006). More recently, this trend was also confirmed in the Bologna collection (Mariotti et al., 2015). These authors also found that foramina (of various shapes <3 mm) on the vertebral bodies are significantly more frequent in the individuals from the TB group of cause of death compared with other groups.

Statistically significant is also the presence of hypertrophic osteoarthropathy in the long bones of individuals from the Coimbra identified collection. This condition is characterized by symmetrical new bone deposition on the long and short tubular bones, and clinical studies have established a possible association between hypertrophic osteoarthropathy and pulmonary conditions (Assis et al., 2011).

Although technical and therapeutic advances, TB continues to be poorly understood despite the identification of its causative pathological agent in 1882 by Robert Koch. TB is an infectious-contagious disease that has affected humans since prehistory, and its prevalence has increased with sedentarization and urbanization (Roberts and Buikstra, 2003).

It is hypothesized by Galagan (2014), based on genome sequencing, that human infected by *M. tuberculosis* may go back to 2.8 million years ago and, as such, has co-evolved with the African *Homo sapiens*. Other studies suggest a common ancestor for the *M. tuberculosis* complex dating around 6000 years ago, supporting a Holocene dispersal of the disease (Bos et al., 2014).

So far, macroscopic, microscopic, imaging and biomolecular analyses of human osteological remains indicate that TB has begun to affect humans in the Neolithic period. Older evidence is limited to the study of Baker et al. (2015) who announced pre-domestication TB in ancient Syria (8800–8300 cal BCE).

In individuals from the Neolithic, evidence were found in Italy (e.g. Formicola, 1987; Canci et al., 1996; Sparacello et al., 2017), Hungary (Spekker et al., 2012; Masson et al., 2015), Poland (Borowska-Strugiń et al., 2014) and Eastern Mediterranean (Hershkovitz et al., 2008).

The sedentarization of populations facilitated the transmission of the disease both by the aerial form and through the consumption of meat and milk of contaminated animals. Since then TB has never left Europe, having increased its prevalence during the Medieval period as a result of the environmental and housing conditions of the largest population clusters (Santos and Suby, 2012).

In Asia, evidence of this disease begins to accumulate. In China, the earliest case dates from 2500–2000 BCE (Pechenkina et al., 2007) and in Thailand the oldest identified individual affected with TB lived ca. 300 BCE to ca. 500 CE (Tayles and Buckley, 2004). In Japan, it has been suggested that TB first appeared during the Aneolithic "Yayoi" period (from 454 BCE to 124 CE) (Suzuki and Inoue, 2007) and in Korea in the first century BCE (Suzuki et al., 2008). In Siberia, evidence are from 400 BCE to 400 CE (Murphy et al., 2009).

For many years it was believed that, like leprosy, TB arrived at the Americas after the 15th century. However, in the last three decades, evidence preceding Columbus arrival has accumulated. In Mesoamerica, the presence of TB is suggested by Maya terra-cotta anthropomorphic figures with kyphotic upper back (10th–16th centuries CE), resembling the physical appearance of Pott's disease patients (see Mackowiak et al., 2005). Similar spine deformations are visible in figurines from North to South America, dating from the second half of the first millennium CE (Mackowiak et al., 2005). Like these authors mentioned, visual representations are helpful documents but "they provide no meaningful inferences about either the incidence or prevalence" (2005: 515).

So far, the earliest primary evidence of TB comes from mummified individuals found in the Atacama Desert (acid-fast bacilli identified in the lungs and in the healed Ghon's complex) dating from the first millennium CE (Allison et al., 1981 *in* Mackowiak et al., 2005). Other skeletal evidence of TB has been found (see Roberts and Buikstra, 2003; Mackowiak et al., 2005; Arrieta et al., 2014), in some cases confirmed with DNA and mycolates analyses (e.g. Luna et al., 2018). However, open questions persist such as: Which strain of *M. tuberculosis* firstly arrived? When were humans firstly affected by TB in the Americas? Recent analyses on mycobacterial genomes suggested seals and sea lions as the source of TB in the New World (Bos et al., 2014).

On the African continent and in Oceania the paleopathological evidence dates back to the nineteenth century CE. This recent presence may be justified by the nomadic way of life of past populations in these regions. Also, in some areas soils and environmental conditions are not prone to bone preservation. Moreover, excavation and paleopathological analyses are less frequent than in other regions of the globe.

Paleotuberculosis in the Portuguese territory

In Portugal, as in other countries, TB has been at the center of paleopathological research. However, until now, the skeletal record of TB in national territory can be considered rather scarce, contrasting with documentary sources that refer to high values of this infection in the past (Almeida, 1995; Santos, 2000; Matos and Santos, 2013). This disparity between historical written sources and osteological evidence of TB, which is also reported in other geographic contexts (Pálfi et al., 1999; Roberts and Buikstra, 2003; Roberts and Cox, 2003), may be partially explained by the fact that soft tissues, rather than the skeletal system, are the most common location of tuberculous lesions (Ormerod, 2014; Ponnuswamy, 2014). Also, in some clinical forms of TB, patients' survival is confined to a short period, precluding the development of skeletal lesions (Cohen and Dye, 2014).

A systematic search of skeletal evidence of TB was conducted in a database belonging to CIAS (Research Centre for Anthropology and Health, University of Coimbra) which contains bioarchaeological data from a minimal number of 8886 individuals (3333 from primary burials) derived from 196 Portuguese archaeological sites — excavated between 1990 and 2017 and covering a timespan of around 7000 years. We found that 23 (11.7%) of these sites have at least one individual showing bone lesions compatible with TB.

Overall, 81 suspected cases of TB (2.4% of 3333 primary burials) were found, with the ribs (48.1%; n=39) being the most affected skeletal element, followed by endocranial lesions (39.5%; n=32), vertebrae (14.8%; n=12) and joints (3.7%; n=3). Besides these well circumscribed lesions, a group of five individuals (6.2%) presented symmetrical periosteal new bone, affecting several long bones, attributed to hypertrophic osteoarthropathy (HOA). Although not pathognomonic, when compared with other causes of death, a significant higher frequency of HOA in individuals who died from pulmonary TB has been reported by Assis et al. (2011) in Portuguese identified collections from the 20th century. The coexistence of different types of lesions was reported only in eight (9.9%) of the 81 individuals.

It is important to emphasize that with the exception of the typical tuberculous vertebral destruction, also known as Pott's disease, the remaining lesions are compatible with a TB diagnosis but are not exclusive of this infection and, as such, their differential diagnosis is mandatory and, if possible, complemented by ancient DNA and/or mycolic acids analysis. The paleopathological diagnosis of TB is always a step-by-step procedure considering parameters such as the topography and typology of lesions, their radiological appearance and clinical significance, the biological profile of the individual and the archaeological and funerary contexts. Unfortunately, a definitive diagnosis is not always possible.

The temporal distribution of suspected cases (Figure 1) ranges from the Roman to the Modern periods and no convincing evidence of TB cases was found in Portuguese pre-historic sites. The highest prevalence was found in the high/late Medieval and Modern periods corresponding to periods presenting an historically documented increase in population density and urban development (Rodrigues, 2008). Moreover, these periods are those presenting a larger number of excavated and analyzed skeletons.

The suspected cases of TB identified so far are located all over the country (Figure 2) and the two confirmed cases are the following:

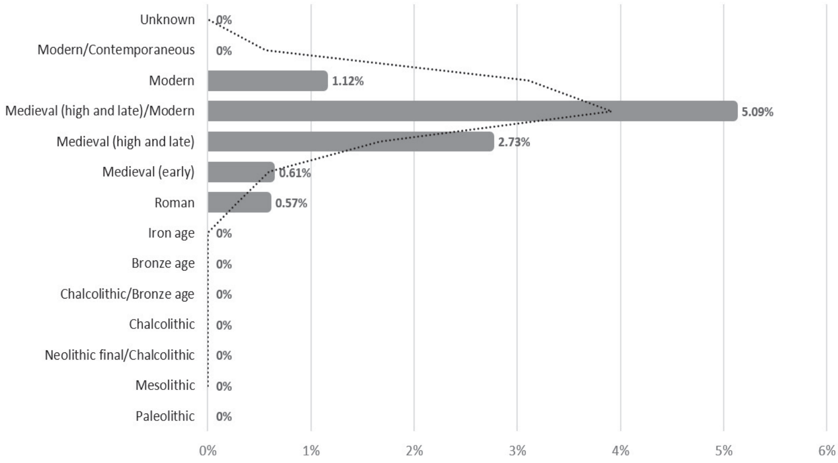

Figure 1. Distribution of possible and probable cases (n=81; 2.43%) of TB by chronological period. Data derived from 3333 primary burials unearthed from 196 Portuguese archaeological sites compiled in CIAS' database.

1) A 12-year-old child (skeleton no. 4) exhumed from São Miguel Cathedral in Castelo Branco. The pathological changes were noticed during the fieldwork and, later, the laboratorial macroscopic inspection and radiological study, revealed a severe kyphosis and correlated thoracic deformities. The spine presented the most striking lesions, namely a gibbus deformity — which is characterized by a sharply angular kyphosis — as the result of vertebral collapse and fusion at multiple levels (Matos et al., 2011). Bone samples of this individual were PCR screened for part of the genomic element IS6110, which, according to Donoghue (2008) can be present in up to 27 copies in organisms of the *Mycobacterium tuberculosis* complex (MTBC) that cause TB. The results were negative due to poor bone preservation. However, this does not mean that this individual was not infected with TB. Hopefully, whole genome sequencing techniques will provide positive results soon. A recent radiocarbon analysis (BETA-524725) revealed a dating for this individual between 1426 and 1516 cal CE.

2) An adult woman from the Monastery of Flor da Rosa, in Crato, dated from 14^{th}–19^{th} centuries, showing pleural calcifications which, according to

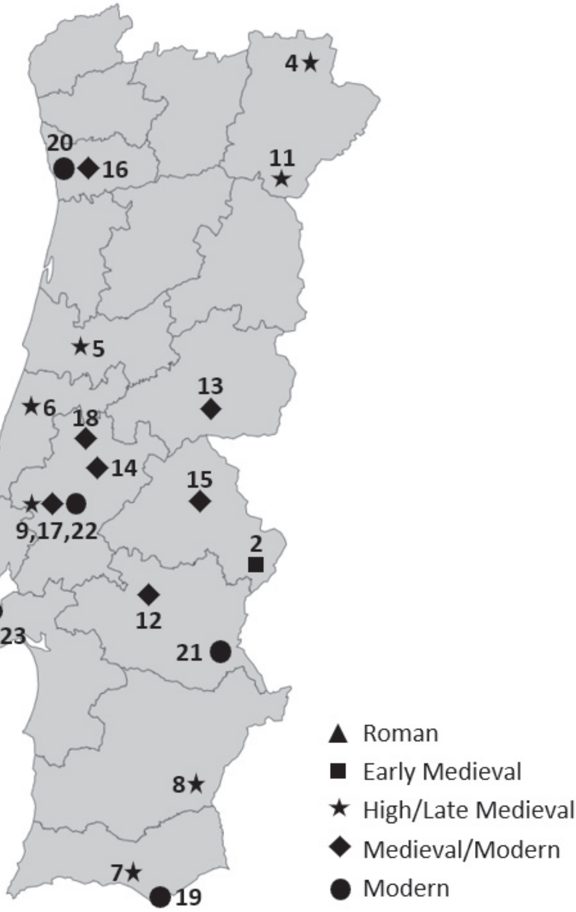

Figure 2. Geographic distribution of the archaeological sites with suspected TB cases over the last 2000 years in the Portuguese territory. Legend: **ROMAN**: 1) Cascais (Macedo, 2002). **EARLY MEDIEVAL**: 2) Elvas (Silva, 1999); 3) Sintra (Santos and Cunha, 1997; Santos, 2000). **HIGH/LATE MEDIEVAL:** 4) Bragança (Marquez-Grant, 2009); 5) Coimbra (Cunha, 1994); 6) Leiria (Garcia, 2007); 7) Loulé (Cunha et al. 2000); 8) Mértola (Silvério, 2008; Umbelino et al., 2008); 9) Santarém (Rodrigues, 2013); 10) Seixal (Lopes, 1998; Lopes, 2000; Codinha et al., 2004); 11) Torre de Moncorvo (Marques, 2000). **MEDIEVAL/MODERN:** 12) Arraiolos (Tavares, 2003); 13) Castelo Branco (Matos et al., 2011); 14) Constância (Assis, 2007); 15) Crato (Fernandes et al., 2014); 16) Paredes (Nogueira, 2013); 17) Santarém (Gomes, 2005; Faria, 2006; Antunes, 2006; Pombal, 2006; Januário, 2006; Gonçalves, 2010); 18) Tomar (Relvado, 2015); **MODERN:** 19) Faro (Paredes et al., 2014); 20) Porto (Domingues, 2013); 21) Reguengos de Monsaraz (Gonçalves, 2004); 22) Santarém (Tereso, 2009); 23) Seixal (Lopes, 2002).

scanning electronic microscopy and spectrometric analysis, were related to an infectious process, possibly TB. These results combined with the evidence of periosteal new bone on the ribs and destruction of several vertebral bodies were interpreted by Fernandes and colleagues (2014) as being highly compatible with a tuberculous process.

Final comments

The (pre)history of tuberculosis is still incomplete and its writing depends on advances on paleopathological techniques and methods, in particular of genetic analysis because it can help to trace the origin and evolution of this disease, and to identify individuals who suffered from tuberculosis, even in the absence of suspicious bone lesions.

Written and clinical sources reveal that tuberculosis has caused a great number of victims over the last 2000 years. However, there is no parallel in the paleopathological record, including in the Portuguese territory. It is necessary to re-study skeletons exhumed in the 19th–20th centuries and to carry out new excavations in burial places all over the world.

Further research on human and animal osteological remains has been intensified in recent years and certainly will bring new information about paleotuberculosis.

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