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A novel approach: combining dental enamel hypoplasia and paleoparasitological analysis in medieval Islamic individuals buried in Santarém (Portugal)

Uma nova abordagem: conjugar a análise de hipoplasias lineares dentárias e o estudo paleoparasitológico de indivíduos exumados de uma necrópole medieval Islâmica de Santarém (Portugal)

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Abstract Paleopathological and paleoparasitological studies seek evidence to understand health and disease in past populations. These two approaches are often used independently despite their complementarity. In this paper, we aim to explore the possible relationship between a common indicator of childhood stress and intestinal parasite infection. Thirty

Resumo Os estudos paleopatológicos e paleoparasitológicos procuram evidências para compreender a saúde e a doença em populações do passado. Estas duas abordagens são frequentemente usadas independentemente, apesar da óbvia importância da sua complementaridade. Neste trabalho, pretendemos explorar a possível relação entre as hipopla-

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adult individuals from the Islamic necropolis of Santarém (9th–12th century AD) were macroscopically examined for linear enamel hypoplasia. Sediments from the pelvis and skull of each skeleton were observed under the optical microscope in search of helminth eggs. Hypoplasic defects were identified in 46.67% of the individuals, mostly on canines and incisors. Eggs from Ascaris lumbricoides and Trichuris trichiura were identified respectively in four and two individuals. The Fisher's exact test was performed to analyze whether the individuals with evidence of stress in early childhood were more prone to helminth infections or death at younger ages. Although these variables were shown to be independent, this exploratory study highlights the contribution of combining paleopathological and paleoparasitological methods to address the long-term impact of physiological stress exposure in early life on the immune system. Furthermore, a variety of factors that could have influenced these results are discussed and interpreted in a biocultural perspective.

sias do esmalte dentário formadas na infância e a infeção por parasitas intestinais na idade adulta. Em 30 indivíduos adultos, exumados da necrópole islâmica de Santarém (séculos IX-XII), foram pesquisadas macroscopicamente hipoplasias do esmalte dentário. Os sedimentos da pélvis e do crânio de cada esqueleto foram observados ao microscópio ótico na tentativa de identificar ovos de helmintos.
Os defeitos hipoplásicos existem em 46,67% dos indivíduos, principalmente em caninos e incisivos. Os ovos de Ascaris lumbricoides e Trichuris trichiura foram identificados, respetivamente, em 4 e 2 indivíduos. O teste exato de Fisher foi realizado para analisar se os indivíduos com hipoplasias foram mais propensos a infeções por helmintos ou se faleceram mais jovens. Embora estas variáveis tenham mostrado ser independentes, este estudo exploratório destaca a contribuição da combinação de métodos paleopatológicos e paleoparasitológicos para abordar o impacto de adversidades ocorridas na infância no sistema imunitário do adulto. Foram discutidos também os fatores que podem ter influenciado estes resultados e interpretados numa perspetiva biocultural.

Keywords: Parasite; cemetery; helminths; Middle Ages; "cumulative advantage/adversity"; developmental origins of health and disease.

Palavras-chave: Hipoplasias do esmalte dentário; helmintos; Idade Média; "vantagem cumulativa/adversidade"; origens do desenvolvimento da saúde e da doença.

Introduction

Paleopathology and paleoparasitology emerged as "sister disciplines", both initiated by the pioneering studies of Ruffer in ancient Egyptian mummies (Dutour, 2013: 147). Despite their complementarity, these scientific fields have mostly grown apart (Dutour, 2013). This trend has been reversed in recent years (e.g. Martinson et al., 2003; King and Henderson, 2014; Coolidge, 2015; Reinhard and Araujo, 2015; Sianto et al., 2015a; Sianto et al, 2016; Sianto et al., 2017). In this study, we propose a novel approach to understand the possible consequence of non-specific indicators of

disease, by analyzing the osteological and parasitological remains, as well as other sources of documentary and archeological evidence. This methodology integrates the study of the human host with possible infectious agents and environment conditions where both coexisted.

The non-specific indicators of disease have been extensively studied as a way to understand implicit relationships between past populations and their surroundings (Reitsema and McIlvaine, 2014). There are many skeletal indicators that can be used to infer 'physiological stress' in early life but, in the current study, the linear enamel hypoplasias (LEH) were selected as a non-specific indicator of childhood developmental problems due to the overall good preservation of the teeth in the sample. LEH are deficiencies in enamel thickness produced by physiological perturbations during the secretory phase of amelogenesis (Goodman and Rose, 1990). They appear as linear grooves around the tooth crown, seen macroscopically at the buccal surface of the enamel (Hillson and Bond, 1997).

The disruption of the ameloblasts can be originated due to multiple etiologies, such as deficient nutrition, infectious diseases, hereditary abnormalities or traumatic events (Goodman and Rose, 1990; Wong, 2014). The location of the defect can further inform about its origin (Goodman and Rose, 1990). While congenital abnormalities are visible in all teeth (Witkop, 1957), defects caused by trauma are

only present in adjacent teeth (Wong, 2014). In addition, those lesions due to systemic 'stress' are seen in teeth that are formed simultaneously (Wong, 2014). LEH are initiated by episodic events, generally the result of deficient nutrition and/or periods of disease that delay the tissues' development (Goodman and Rose, 1990; Hillson and Bond, 1997; May et al., 1993).

Health outcomes can be influenced by early development events (Armelagos et al., 2009). Barker was the first to introduce this hypothesis by demonstrating that fetal and infants' development was related to cardiovascular and respiratory diseases in later life (Barker et al., 1989, 1991; Barker, 1995). This hypothesis is now generally accepted and expanded to other anatomical structures, under the "developmental origins of health and disease" (DOHaD) paradigm (Cameron and Demerath, 2002; Mcmillen, 2005). This hypothesis suggests that exposure of an organism to physiological problems during a phase of developmental plasticity interacts with its genotype, modulating it and, probably, restraining its capacity to cope with the environment through lifespan (Gluckman and Hanson, 2006).

Duray (1996) was the first to provide archeological evidence within this paradigm, demonstrating the correlation between 'stress' periods in childhood (through the presence of LEH) and premature mortality. Various studies on LEH have identified this relationship with reduced longevity, yet most of them did not

consider the DOHaD approach (Good-man, 1989; Goodman and Armelagos, 1989; Steckel, 2005; Boldsen, 2007; Armel-agos et al., 2009). When controlling for the cause of death, economic status and the year of birth, Amoroso et al. (2014) found no relationship between LEH and prema-ture death. They found, however, a posi-tive association with infectious diseases and concluded that differential exposure to stressors in infancy created a pattern of "cumulative adversity" throughout the lifespan (Amoroso et al., 2014). In the last years, researchers have been testing the possible relationship between early life experience and adult health. The results have been contradictory (e.g. DeWitte and Wood, 2008; Blevins et al., 2017).

Some parasite species can promote the appearance of these so-called 'stress indicators' by disturbing the host's de-velopment in different ways. Parasites can consume nutrients essential to the normal development of the host, and/ or provoke nutritional malabsorption due to gastrointestinal diseases (Blom et al., 2005; Sullivan, 2005). Parasitic load is also a good general indicator of infectious diseases because they are exacerbated by the same factors: malnutrition, high population density, poor hygiene and sanitation (Reinhard, 1988). Thus, the pa-leoparasitological analysis of sediments associated with human remains can be used as a proxy to analyze infectious diseases in adulthood and contribute to clarifying the outcomes of physiological

'stress' in early development on later life.

The aim of this exploratory study is to search for hypoplasic defects in the teeth of adult individuals excavated from an Islamic necropolis of Santarém and to evaluate whether these individuals were more prone to have intestinal parasites or to die at a younger age. The mechanisms underlying these relations will be dis-cussed in light of the archeological and documentary evidence available for this population.

Materials and Methods
Archeological site

The skeletal individuals derive from a necropolis excavated, between 2004 and 2005, in Santarém (Portugal) (Matias, 2007). At this site, 639 graves were identi-fied, where 422 individuals were buried following the Islamic burial rituals (Matias, 2009a), meaning the bodies were at right lateral decubitus, oriented south-east to north-west, in narrow single graves di-rectly excavated in clay and limestone geological substrate (Matias, 2009b).

This burial area was one of the 5 Maqâbir of *Shantarîn*, the Islamic denomi-nation of the city from the 9th–12th centu-ries AD (Matias, 2009a, 2009b). *Shantarîn* was an important military, administrative and political center, located on a plateau at the northern margin of the Tagus river (Santos, 2011). The river provided a cru-cial trade route and fertile soil, due to

seasonal flooding (Conde, 1997; Santos, 2011). Consequently, the city was densely populated and subsisted on agriculture, pastoralism and commerce (Ramalho et al., 2001; Santos, 2011).

Skeletal analysis

The skeletal sample consists of 30 individuals, currently housed at the Department of Life Sciences, University of Coimbra. The selection of skeletons was based on the preservation of the skull and pelvic girdle because these are the anatomic regions for pathological analysis and sediment sampling (Cunha, 2015). The amount of sediment that could be retrieved was also a selection criterion since these skeletons were previously cleaned.

The sex of the individuals was estimated by the morphometric characteristics proposed by Bruzek (1991, 2002). The age at death was estimated by the metamorphosis of the pubic symphysis (Brooks and Suchey, 1990), auricular surface (Lovejoy et al., 1985), acetabulum (Calce, 2012) and by the root transparency of mono-radicular teeth (Lamendin et al., 1992). Individuals were grouped in three broad age categories: young adults (18–34 years old), middle age (35–49 years old), and older adults (+50 years old).

The permanent teeth were macroscopically searched for LEH under natural light. LEH was recorded when one or more lines of reduced enamel thickness were seen in the buccal surface of the teeth. The LEH were registered using the standard recommendations and scales of severity proposed by Steckel et al. (2006). The location of the hypoplasic lesions was recorded across each tooth type, following the FDI (Fédération Dentaire Internationale) notation. Teeth with damaged or obscured crowns were not included in the analysis.

Paleoparasitological analysis

Sediments from the sacral foramina and iliac fossa were recovered from the pelvis of each individual. These regions were chosen because these are the anatomic areas where the viscera, most probably, decomposed during the skeletonization process (Reinhard et al., 1992; Sianto and Santos, 2014). Control samples were taken from the soil on the interior of the skull (Reinhard et al., 1992; Sianto and Santos, 2014). Since this skeletal sample had been previously cleaned, all the available sediment was collected. The soil sample sizes ranged from 0.7 to 4.4 grams.

Each sediment sample was weighed and commercial tablets of *Lycopodium* sp. spores (batch 3862) were added (Maher, 1981; Reinhard et al., 1992; Warnock and Reinhard, 1992). These mixtures were rehydrated in a solution of sodium triphosphate, with 0.5% concentration, during 72 hours (Callen and Cameron, 1960). Subsequently, each sample was homogenized

with a glass rod, sieved through gauze and sedimented for at least 2 hours. To further concentrate the biological material, samples were centrifuged at 2000 rpm for 1 minute (Warnock and Reinhard, 1992).

At least 20 microscope slides were observed from the pelvic sediment of each individual. The slides, prepared with one drop of the samples and a drop of glycerol, were inspected using a microscope with 100x and 400x optic magnifications. The paleoparasitological findings were measured, photographed and egg morphology analyzed to identify helminth species. For each individual positive for intestinal parasites, 20 slides of control samples were also scrutinized. The concentration of eggs found in each individual's sediment was quantified in relation to the number of *Lycopodium* sp. spores counted using the formula suggested by Maher (1981).

Data analysis

All data were analyzed using R version 3.2. An exploratory analysis was performed to search for patterns within the data. Due to the small sample size, conditional exact tests based on the hypergeometric distribution were used (Fisher, 1935). Although this test is conservative on small samples, and it is still regarded as more reliable because it "preserves test size, so the true significance level does not exceed the nominal level" (Lydersen et al., 2007: 4328–4329). The Fisher–Freeman–

Halton's exact test (Freeman and Halton, 1951) was applied at 0.95 confidence level, using the two-sided hypothesis to evaluate whether higher the presence of LEH or higher scores of LEH were related to early age at death. The one sided Fisher's exact test was performed to assess whether individuals with evidence of dental enamel hypoplasia were more prone to parasitic infections (Fisher, 1935). The Fisher–Freeman–Halton's exact test (Freeman and Halton, 1951) at a 0.95 confidence level was also used to determine if there was a positive relationship between higher LEH scores and the presence of helminth eggs in the sacral region.

Results
Linear enamel hypoplasia

From the 30 adult individuals under analysis, 518 preserved teeth were examined. The frequency of LEH varied across teeth and is summarized in Table 1. Hypoplasic defects were identified in 14 (46.67%) individuals, eight (8/16=50%) males and six (6/14=42.86%) females. LEH were seen in 55.55% (5/9) of the young adults, 43.75% (7/16) of middle age and 40% (2/5) of the older adults. The Fisher–Freeman–Halton's test revealed that the presence of LEH (p value=0.8885) was not related to age at death. The frequency of LEH varied across teeth and is summarized in table 1.

Table 1. Proportions of the teeth recovered from a sample of 30 Islamic individuals from a medieval necropolis in Shantarîn showing linear enamel hypoplasias across each tooth type, in accordance with the FDI notation system.

Teeth	18	17	16	15	14	13	12	11	21	22	23	24	25	26	27	28
LEH (%)	0	6.67	8.33	17.65	0	26.32	16.67	23.81	27.78	23.53	25	17.65	13.33	0	0	0
	(0/17)	(1/15)	(1/12)	(3/17)	(0/16)	(5/19)	(3/18)	(5/21)	(5/18)	(4/17)	(4/16)	(3/17)	(2/15)	(0/9)	(0/11)	(0/13)
Teeth	48	47	46	45	44	43	42	41	31	32	33	34	35	36	37	38
LEH (%)	0	0	0	12.5	9.53	32	35	27.78	20	19.05	33.33	19.05	13.33	0	10	0
	(0/15)	(0/12)	(0/6)	(2/16)	(2/21)	(8/25)	(7/20)	(5/18)	(4/20)	(4/21)	(8/24)	(4/21)	(2/15)	(0/7)	(1/10)	(0/16)

The tooth types showing most hypoplastic defects on their crowns were the canines and the incisors, respectively. LEH were seen in 29.76% (24/84) of the canines and 24.18% (37/153) of the incisors. The molars showed the lowest frequency of enamel hypoplasia, visible only in 2.03% (3/148). Only two individuals (Sk. 355 and 146) manifested LEH on the molars and both showed two or more hypoplasic lines on the anterior dentition.

Most individuals (9/14=64.29%) presented one hypoplasic line (score 2) while five (5/14=35.71%) showed two or more lines per tooth and thus were scored 3 (Figure 1). The number of insults (scores) (p value=0.8586) was independent from the age at death.

Figure 1. Linear enamel hypoplasia on the dentition of a middle aged female (Sk. 483) from Shantarîn necropolis. At the right side of the jaw, the arrow shows a score 3 lesion, three visible linear groves. The left arrow indicates a score 2 lesion..

Helminths

Eggs from two species of helminths were identified in these individuals: *Ascaris lumbricoides* (Figure 2A) and *Trichuris trichiura* (Figure 2B). Another helminth egg was seen, although due to taphonomic damage the species could not be identified. The control samples were all negative for parasites. Results of the paleoparasitological analysis are summarized in table 2.
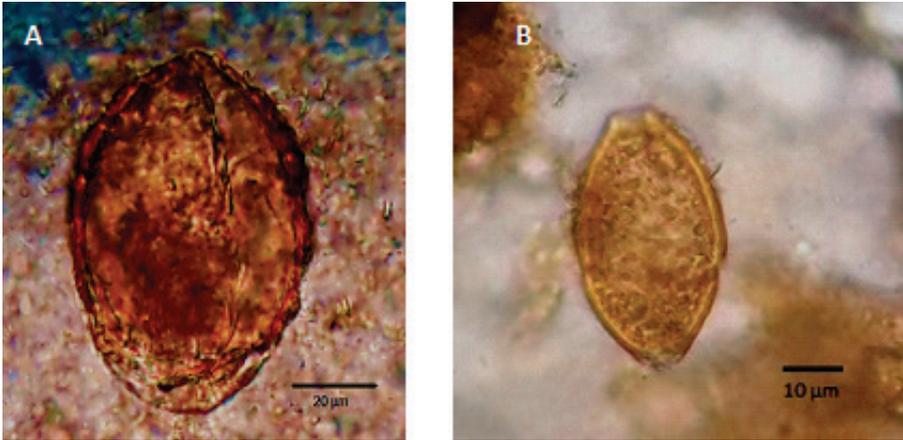

Figure 2. A) Eggs from A. A. lumbricoides, identified on an old male (Sk. 147); and B) T. trichiura, identified on an old female (Sk. 136) from the medieval Islamic necropolis of Shantarîn.

Table 2. Helminth species recovered from Shantarîn. Concentration of eggs per gram of sediment and the corresponding demographic data for each individual who tested positive for intestinal parasites.

Parasite species	Skeleton #	Sex	Age categories	Concentration (eggs/g)
Ascaris lumbricoides	147	Male	Older	14
	407	Male	Young	65
	529	Female	Middle age	41
	507	Female	Middle age	32
Trichuris trichiura	136	Female	Middle age	11
	402	Male	Young	7
Indeterminate	135	Male	Middle age	12

A. lumbricoides was seen in four (4/30=13.33%) individuals and *T. trichiura* in two (2/30=6.67%). No individual had both parasites. The concentrations of *Ascaris sp.* eggs were greater than from *Trichuris sp.*, reaching 65 eggs per gram of sediment. *T. trichiura* eggs were observed in concentrations of 7 and 11 eggs/gram, on individuals 402 and 136, respectively. No differences were seen in the occurrence of parasitic infections across sex or age groups.

Dental enamel hypoplasia and parasitic infection

Helminth infections were found in 4 of the 14 (28.57%) individuals exhibiting hypoplasias and in 3 of the 16 (18.75%) individuals without this marker. However, the Fisher exact test demonstrated that the difference was not significant at a 0.95 confidence level, with a p value of 0.419 and an odds ratio of 1.701. The number of insults, given by the scores of LEH, was also independent (Fisher–Freeman–Halton's test, p = 0.173) from the presence of helminth eggs in the sacral region.

Discussion

The variation and function of the immune system are largely a product of the environment in which the individual develops (McDade, 2003). There are "critical periods", representing transitional points in development and maturation, when specific environmental stimulus can produce "permanent change and predict long-term outcomes" (Cameron and Demerath, 2002: 160). Childhood is a "critical period" in development because, at this stage, a steady growth process occurs as well as an increasing exposure to a variety of novel pathogens (Cameron and Demerath, 2002; McDade, 2003). These processes result in great energetic demands for the children that often impose a trade-off between growth and maintenance effort (McDade, 2003).

The current macroscopic evaluation of 30 adult skeletons allowed the identification of hypoplasic defects in 46.67% (14/30) of the individuals analyzed. The analysis of LEH allows the examination of individuals who survived (Liebe-Harkort, 2012; Wood et al., 1992). Canines and incisors were, respectively, the most affected tooth types (Table 1). These teeth have similar formation periods (Reid and Dean, 2006), indicating that these lesions are probably due to systemic 'stress' during amelogenesis (Goodman and Rose, 1990). The time of the defect formation, based on its location, was not estimated. However, the complete formation of permanent canines and incisors occurs between 1 and 6 years old (Reid and Dean, 2006), suggesting that the LEH in this skeletal sample can be regarded as a non-specific indicator of disease in infancy and early childhood (Rose et al., 1978). Weaning age occurs within this time-

frame (1 to 6 years old) and represents a period of vulnerability for infants because they stop receiving antibodies from their mothers through breastfeeding and start new diets (Goodman and Armelagos, 1989; McDade, 2003; Lewis, 2007).

No significant differences on LEH were observed between sex or age groups. This result is in agreement with other studies showing that both sexes are equally exposed to risk factors (Stuart-Macadam, 1985; Liebe-Harkort, 2012). Various studies on the distribution of LEH showed an association with decreased lifespan (Goodman, 1989; Goodman and Armelagos, 1989; Duray, 1996; Steckel, 2005; Boldsen, 2007; Armelagos et al., 2009). However, the opposite relationship was also found (Amoroso et al., 2014; Watts, 2015) and thus the relationship between early insults and premature mortality is still to be fully understood. Despite the proportion of affected individuals being slightly higher at a younger age, this difference was not significant (Fisher's exact test, p value=0.8885). Moreover, the number of insults was also unrelated to the age of death (Fisher's exact test, p value=0.8586). In this study, a possible relationship between LEH and longevity could have been obscured by the large age range of each category. The time of the insult could also be important in this assessment.

Following the osteological paradox, individuals that overcome stress at younger ages could, has a result, became more resilient to further paleopathological prob-

lems throughout life (Wood et al., 1992). The DOHaD hypothesis anticipates that 'stress' exposure at specific age windows of developmental "sensitivity" can have detrimental long-term effects (Cameron and Demerath, 2002). When analyzing 'stress' episodes between 1 and 6 years of age, the results can be obscured because outside of the "critical periods" of development the relationship can be nonexistent or even be the opposite, which could explain the contradictory findings reported when analyzing LEH and age at death. This time window comprises two possible periods of vulnerability: infancy and early childhood (Cameron and Demerath, 2002). In infancy (0–2 years old), the thymus volume, lymphocytes proliferative potential and both T and B-cell counts are maximized (McDade, 2003). The thymus is the main organ of the lymphoid system, were T-cell precursors undergo differentiation and maturation, leading to the migration of selected thymocytes to the peripheral lymphoid system (Savino, 2002; 2006), thus representing a window of vulnerability for the infant. During early childhood, a steady decrease occurs in thymus volume, lymphocytes proliferative potential, T and B-cell counts. Simultaneously the concentrations of immunoglobulin and the proportion of memory lymphocytes increase (McDade, 2003). Epigenetic imprinting is an important factor for T-cell activation and memory response (Portela and Esteller, 2010; Netea et al., 2011). The high demands of the immune system at this age make it

a window of susceptibility to dysregulation that may be manifested in adulthood via epigenetic programming (Palmer, 2011; MacGillivray and Kollmann, 2014).

Nutrition is a determinant factor of immune function due to its high energetic costs (Beisel, 1996; McDade, 2003; Calder, 2013). An effective immune response to pathogens involves increased cell differentiation and replication, requiring a supply of nutrients such as protein, vitamin A, iron and zinc (Calder and Jackson, 2000). For instance, protein depletion in infancy is known to cause the atrophy of the thymus and, consequently, reduce immune cell counts and deterioration of the peripheral lymphoid system (Schaible and Kaufmann, 2007). The analysis of four Islamic silos recovered from Santarém, dated from 10th–11th centuries AD, showed faunal remains from various species of mammals (cattle, sheep, goat, rabbit), birds, fish (from river and sea) and molluscs (Moreno-García and Davis, 2001; Ramalho et al., 2001). In addition to this, there are remains of both juvenile goat and/or sheep with cut marks, suggesting the population had reasonable access to animal protein (Ramalho et al., 2001). *Shantarîn* was also known for its constantly fertile soil as a result of the seasonal flooding of this area (Conde, 1997; Santos, 2011). The 21 silos recovered in the urban area of *Shantarîn* with materials from the Islamic period further support the crops abundance and its contribution to the city's impregnability (Ramalho et al., 2001: 151).

Although the archeological evidence indicates an adequate access to food resources, the nutritional status of the population could still have been impaired due to exposure to infectious agents (Katona and Katona-Apte, 2008; Calder, 2013). When dealing with an infection, individuals suffer from greater energy expenditure and other detrimental changes in behavior, such as anorexia, which further reduces the caloric intake (Calder and Jackson, 2000). The pathogens and their related immune response can further prejudice nutritional status by diverting nutrients, promote nutrient loss and impair its absorption (Whitfield, 1993; Lunn, 2000). Children have immature immune systems, being more susceptible to multiple infections, which can become more severe and/or frequent (Beisel, 1996). During weaning, even well-nourished children often develop gastrointestinal infections and diarrhea from the incorporation of new foods (Mata et al., 1971; Walker et al., 2009). Infection in childhood promotes severe malnutrition, compromising immunity in the long-term (Schaible and Kaufmann, 2007) and thus favoring the emergence of other infectious diseases, contributing to further nutritional deficiencies (Pereira, 2003). Medieval Islamic medicine was on the raise, at which time various infectious diseases were first described (Syed, 2002), including some that are known to be caused by parasites (Cox, 2002). Texts from Salerno, a physician from 12th century AD, described worms as a result of humoral imbalance

caused by the excess of phlegm (Saffron, 1972). Treatments commonly prescribed were changes in diet, bloodletting and remedies to reduce phlegm in order to rebalance the humors (Harington et al., 1920). These treatments are now known to have no effect, although the concern and detailed descriptions of infectious diseases by these academics suggest they were a fairly recursive problem at that period (Sullivan, 2005).

Helminth eggs were found in 23.3% (7 out of 30) of the individuals analyzed and were not present in the control samples. These results indicated that the recovered eggs were the consequence of intestinal infection and not due to soil contamination (Reinhard et al., 1992). In this study, parasitic infections occurred equally in both sexes and all age groups (Table 2). *A. lumbricoides* (Figure 2A) and *T. trichiura* (Figure 2B) are human parasites that are transmitted from one host to the other through the oral-fecal route after a maturation period in the soil (Whitfield, 1993). Most infections are asymptomatic, but moderate to heavy infections may cause abdominal pain, anemia, malnutrition, and affect cognitive function in children (Roberts et al., 2009). According to these authors, *T. trichiura* infection can led to rectal prolapse, while massive infections with *A. lumbricoides* can cause fatal intestinal blockage. They are both identified as geohelminths and are protected by a chitin shell, adapted to their life stage in the soil (Wharton, 1980), allowing for better preservation in

archeological contexts (Harter et al., 2003). Both species are widely spread across European archeological sites (Bouchet et al., 2003a; Gonçalves et al., 2003), including in the Iberian Peninsula (Hidalgo-Argüello et al., 2003; Botella et al., 2010; Sianto et al., 2015a; 2015b; 2015c). The concentration of eggs per gram of sediment found in this sample is similar to those reported in other sites from Portugal and Spain (Sianto et al., 2015a; 2015b; 2015c).

Access to potable water, hygiene and sanitation are central to prevent the spread of the helminth species found in this study (Mara et al., 2010). Medieval Islamic writings refer to the importance of personal hygiene to maintain health (Leclerc, 1876). In Santarém, there is archeological evidence of an Islamic cistern connected to a well for water capture and storage (Batata et al., 2004). Islamic public bath houses were mentioned in historic documents, but few were identified (Serra, 2013). However, these buildings were found in other archaeological sites from the *Gharb al-Andalus*, in both the modern territories of Spain (Avila, 2008) and Portugal (Luzia, 2008). Islamic structures associated with sewage management were not yet recovered in Santarém. However, in Mérida (Spain), latrines which were directly open to the streets were identified, dating from the 8th-9th centuries AD (Alba et al., 2008), while, in Évora, latrines connecting to sewers were found, dating from the 10th–12th centuries AD (Filipe, 2012). Evidence of consecutive improve-

ments in sewages systems were seen in Silves (Portugal) after the 11th century AD, where latrines were connected to public cesspools (Serra, 2013). These structures from urban areas across the *Gharb al--Andalus* suggest a gradual improvement in the way these populations dealt with human waste. However, collecting waste from cesspits to use as fertilizers on the fields was a common practice in medieval times (Sterner, 2008). The consumption of raw vegetables contaminated by these fertilizers could easily spread parasites to new hosts (Whitfield, 1993). This practice allowed geohelminths to repeat their lifecycle and so could have represented a major source of transmission (Mitchell, 2015; Rácz et al., 2015).

The helminth infections were found to be independent from the development of LEH in early life (p value=0.4186), but the odds ratio (1.701449) seems to suggest a slight positive tendency for parasite infection in individuals exposed to 'stress' in early life. The number of incidences of LEH observed in each individual was not related to the probability of having parasitic infections (p value=0.1731). These results could however have been biased by the small sample size (N=30) and the small amounts of sediment that could be retrieved from these skeletons. In paleoparasitology, a positive result is always indicative of the presence of parasites, but a negative result does not always mean that the individual was not infected by parasites at the moment of death, espe-

cially when analyzing soil samples. In this type of sample, the eggs are dispersed in the sediment, so the probability of encountering them, in the small subsample analyzed from each individual, is reduced. In this study, the problem was also exacerbated because the skeletons were previously cleaned, which further reduced the concentration of eggs per gram and could result in false negatives. Pelvic sediments are also prone to taphonomic factors that reduce the chances of finding parasite eggs (Bouchet et al., 2003b). Taphonomic processes could have damaged some less resistant eggs or washed them away (Bouchet et al., 2003b), contributing to its absence and/or low concentrations. These problems can be reduced in future studies by analyzing more individuals and larger sediment samples, preferably retrieved prior to cleaning the skeletons, following the recommended recovery procedures (Sianto and Santos, 2014).

In a Portuguese sample from Lisbon, dated from the 19th and 20th centuries, Amoroso et al. (2014) found LEH to be related with death by infectious diseases, but not with decreased longevity. There are two distinct hypotheses that can explain this relationship: the "cumulative advantage/adversity" hypothesis and the DOHaD hypothesis. The first recognizes these associations as a reflection of continuous exposure to risk factors throughout life (Goodman and Armelagos, 1989). The bidirectional relations between infection, nutrition and immunity (Calder, 2013)

can create a continuous detrimental cycle (Beisel, 1996), which could have been facilitated by confounding variables, like access to food, living conditions or social status. In the Lisbon identified collection, Amoroso et al. (2014) concluded that the association between LEH and death by infectious diseases was better explained by the "cumulative adversity" model because early mortality was related with socioeconomic status, but not with non-specific indicators of disease in infancy. In the archeological sample from Santarém, there are no spoil in the graves because the Islamic burial ritual promotes equality in death, which invalidates any associations with social classes (Matias, 2009b, 2009c). Yet, other studies on the association of LEH and longevity have reported no relationship with socioeconomic status (Duray, 1996; Watts, 2015).

The second hypothesis states that exposure to 'stress' factors during "critical periods" of the development can have long-term consequences on health and longevity (Barker et al., 1989, 1991; Cameron and Demerath, 2002). Given the immune system's sensitivity to function change, it is indeed vulnerable to programming (Savino, 2002; 2006; Palmer, 2011; MacGillivray and Kollmann, 2014). Few authors have concluded that the relationship between hypoplasic defects and increased risk of death was shaped by early immune modulation (Duray, 1996; Armelagos et al., 2009). However, some epidemiological studies had also addressed the asso-

ciation between non-specific indicators of disease in early development and decreased immune function through adulthood. A study on seasonality of birth in rural Gambia has showed that individuals born during the wet/hungry season were linked to premature deaths by infectious diseases (Moore et al., 1999; 2006). A small thymus (associated with the wet season) was shown to predict early deaths by infectious related causes in infants from Guiné-Bissau and Bangladesh (Aaby et al., 2007; Garly et al., 2008; Moore et al., 2014). Given the absence of an effect at birth, Moore et al. (2014) concluded that this relationship could be influenced by developmental problems in early infancy. However, seasonal influences on long-term immunity were not observed in an epidemiological study in rural Bangladesh (Moore et al., 2004).

Given the plasticity of the immune system, its development is highly context-sensitive (McDade, 2003), which could have driven the discrepancy among different populations, both in the present and the past. Thus, further studies are necessary to determine if there is in fact a relationship between LEH developed at younger age and long-term risk of infection, and which mechanisms are responsible for it. The research should focus on diverse contexts across space, time, societies and cultures. Epidemiological studies often include monitoring individuals throughout their development, which is very time consuming. Here, we propose

that bioarchaeologists can also contribute to this debate because the study of the human skeleton offers the unique possibility of simultaneously monitoring markers of developmental problems and health outcomes.

Conclusion

A holistic approach to disease ecology can benefit from interdependencies between the host, parasite, and environment. The results, obtained in medieval individuals from the Islamic urban area of Santarém, did not reveal significant relations between LEH and premature mortality or the risk of infectious diseases. Despite these results, we can conclude that exposure to infectious agents must have had a detrimental effect on nutritional status, despite the reasonable access to food sources, and shaped the overall health of this Islamic medieval sample that lived in *Shantarîn*. However, more research is needed in order to understand the impact of early exposure to diseases on the immune system and its underlying mechanism, in both modern and archeological samples. Together with demographic and documentary evidence, the study of non-specific markers of diseases and the identification of helminth infections can be useful proxies to address this question in archeological settings. To further increase the accuracy of the results, the use of larger skeletal samples, the as-sessment of the age of LEH formation and the use of larger quantities of sediment in the paleoparasitological analysis are recommended.

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