Archaeol Anthropol Sci (2018) 10:75–81
DOI 10.1007/s12520-016-0337-9

ORIGINAL PAPER

Evidence of contact between New and Old World: paleoparasitological and food remains study in the Tagus river population of Sarilhos Grandes (Montijo, Portugal)

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Received: 25 July 2015 / Accepted: 25 April 2016 / Published online: 11 May 2016

Abstract The period of European maritime expansion that started in the fifteenth century had a great impact on trading, on human migrations and consequently in the dispersion of infectious diseases. Portugal was at the core of this expansion; however, studies about parasitic infections, especially helminths, are lacking. This study aims to help reduce this gap presenting the results of microscopic analysis of soil sediments collected from the São Jorge churchyard of Sarilhos Grandes (Montijo). Consecrated in the fourteenth century AD, it remained as a burial ground until the nineteenth century. Soil samples collected from the pelvic girdle of five adult individuals and samples taken as control were analysed under the microscope after current conventional methodological procedures were undertaken. Eggs from *Ascaris lumbricoides* were identified. Also eggs of trichostrongyle type species were identified in two individuals and may represent the first report in archaeological European samples. Food remains include potato and rice starches, muscle fibres, bivalves, pollen grains and fungi spores. The stratigraphy interpretation together with potato findings put the oldest skeletons to a chronology around the sixteenth century AD. These results are consistent with historical sources that documented the prominence of Tagus river nearby villages in maritime expansion.

Keywords Paleodiet · Helminths · Paleoparasitology · Paleopathology · Potato · Infectious diseases

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Introduction

Portugal began the maritime expansion due to multiple factors, including its geographical position in Western Europe, which had its starting point with the conquest of Ceuta in 1415 (Ramos et al. 2009). New maritime routes considerably increased the previously existing trade of people and goods via land or river. Portugal, especially Lisbon, became a multicultural metropolis. For instance, in 1551, Christovam de Oliveira calculated that 10% of the 100,000 inhabitants of Lisbon were slaves (Veiga 1887).

As people migrate, they carry with them food and disease so the European modern expansion had a great impact on parasite transmission (Reinhard et al. 2013). Even though this renders paleoparasitological studies in Portugal fundamental to understand parasite transmission between Europe and other continents during maritime expansion, so far no studies have been conducted.

This paper presents the parasitological and dietary results of the analysis of soil samples recovered from five individuals exhumed from the funerary area associated with the Church of São Jorge, Sarilhos Grandes (Montijo). The objective of this analysis is to start inferring the impact of these voyages on the dispersion of parasites and food available to a Tagus river population.

The historical background

Sarilhos Grandes lies on the left bank of the Tagus River, distant 16 km in straight line from Lisbon. Its earliest historical references date from 1304 A.D. and mention the presence of water mills and salt evaporation ponds (Pimentel 1908; Graça 1989). The Setúbal Peninsula, where it is located, was occupied by peoples of different geographic origins and faiths, such as Muslims, Jews and Christians (Costa 2015).

In 2008, during an archaeological survey previous to the building of wastewater infrastructures, a burial site associated to the Church of São Jorge was identified and later excavated (Pereira et al. 2008). The present building is the result of a reconstruction in 1740 A.D. which was later renovated (Pimentel 1908). The original temple dates probably from the Middle Age and later integrated the 1500's chapel of Nossa Senhora da Piedade (Almeida 2004). In historic sources, the associated cemetery was first mentioned in 1390 A.D., when the Bishop of Lisbon granted permission for its construction ensuring that the population could be buried in holy ground (Dias 2000). This burial area was used until the foundation of the municipal cemetery in 1864 (Pimentel 1908). A precise chronological of the burials was not obtained; however, the associated artefacts, which include a King John III ceitil coin (1502–1557 AD), suggest a period spanning from the Middle Age to the nineteenth century (Pereira et al. 2008).

The archaeological survey covered 23 m^2; the area affected by the construction works for wastewater piping. This area evidenced an intense funerary use that resulted in the loss of the precise location of some graves and in the disturbance of most skeletons leading to the commingling of the human remains. This precluded soil sampling for analysis in 17 of the 22 individuals in primary deposition. In addition to these very fragmented individuals, four ossuaries were also excavated. Individuals from both sexes and all age categories were identified, including newborns that were spatially concentrated (Pereira et al. 2008).

The funerary characteristics of the burial site are consistent with other Christian necropolises (Pearson 2003), with most individuals inhumed in a supine position with their heads pointing to the West and feet pointing to the East. Invariably, their legs were extended but some variation in the positioning of the arms was found (Pereira et al. 2008).

Materials and methods

Samples

Soil samples were collected from five individuals (Fig. 1) with preserved pelvic girdles (skeletons 8, 9, 13, 17 and 22). Because of the reutilization of the burial ground, it was only possible to collect control samples from three individuals, taken from the anatomical regions further away from the pelvic girdle (e.g. cranium and humerus). One soil sample was taken also from an area unrelated to the burials.

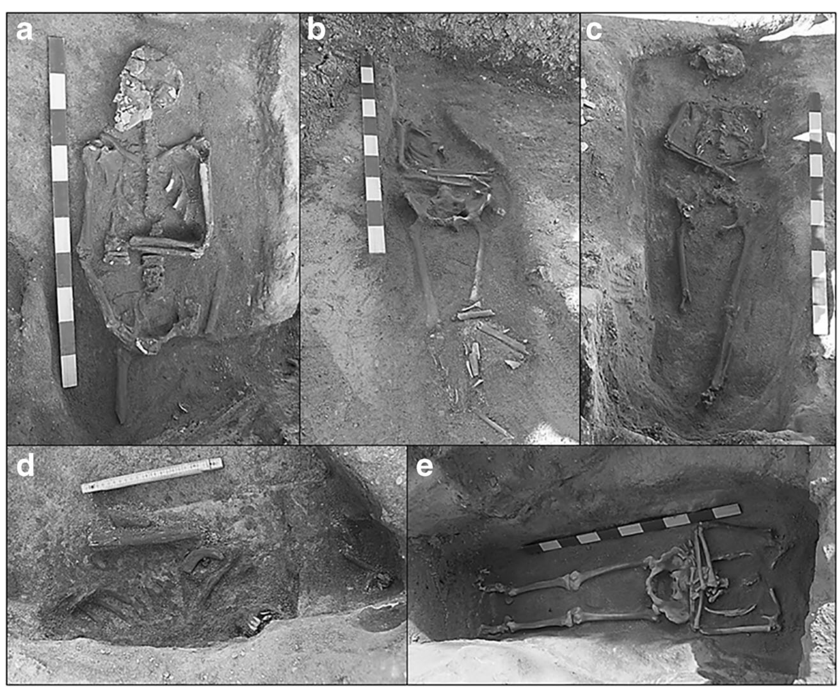

Fig. 1 Skeletons (*Sk.*) sampled for this study. **a** Correspond to Sk. 8, **b** to Sk. 9, **c** to Sk. 13, **d** to Sk. 17 and **e** to Sk. 22

Each soil sample was collected using latex gloves and plastic spoons that were discarded after each individual sampling. The samples were later sent to the Laboratories of Paleoparasitology and Palynology of the Oswaldo Cruz Foundation, Brazil, for paleoparasitological and paleodietary analysis.

Osteoarchaeological study

The paleobiological and paleopathological profiles of the individuals were determined by macroscopic observation of bones and teeth and used to cross with the results from the paleoparasitological and paleodietary analysis. Age-at-death estimation was obtained by using standard macroscopic methods (Albert and Maples 1995; Fazekas and Kósa 1978; MacLaughlin 1990; Scheuer et al. 2000; Webb and Suchey 1985; Ubelaker 1989). Whenever possible, sex diagnosis of adult individuals was based on morphological traits of the innominate bone and the skull (Bruzek 2002; Buikstra and Ubelaker 1994). When these bones were absent, sex was diagnosed using osteometric cut-off points calculated on Coimbra identified skeletal collection (Wasterlain 2000). Identification of caries was based on Lukacs (1989). Degenerative joint diseases—such as osteoarthritis and Schmorl's nodes—and differential diagnosis of the pathological lesions were based on Aufderheide and Rodríguez-Martín (1998), Ortner (2003) and Waldron (2009).

Paleoparasitological and paleodietary analysis

Approximately 10 to 30 ml of each soil sample was rehydrated using a 0.5% aqueous solution of trisodium phosphate (Na3Po4) with *Lycopodium* commercial spores (batch 124, 961). After 72 h, the samples were homogenized and sieved through metal sieves of 250 μm. The sieved liquid was then manually shaken and rested for 30 s, after which the liquid in suspension was carefully poured into another recipient so that the biological remains could be separated from the sand in the sample. This process was performed three times, as proposed by Reinhard et al. (2008). The liquid containing the biological remains was centrifuged for 1 min at 2000 rpm. Twenty microscope slides containing one drop of the concentrated sediment were prepared and observed for identification of parasites at 100 and 400 magnifications using a Nikon E200 microscope with polarized light. A Lumenera® camera and the software Image Pro Express® were used for image collection and measurement of microfossils.

The parasite eggs were quantified per millimetre (epml) of dry sediment by counting all identified eggs and the introduced *Lycopodium* spores based on Maher's (1981) formula for pollen grains quantification (parasite eggs/ml dry sediment = ((eggs counted/*Lycopodium* counted) × spores added)/sediment volume). The paleodietary analysis was qualitative and based on identification of dietary remains present in the microscope slides prepared for paleoparasitological study.

Results

Skeleton 17 had no associated parasites. Helminths were found in the samples collected from the pelves of the remaining four individuals (Fig. 2). All control samples tested were negative for intestinal parasites. Individuals 9 and 13 had translucid thin shelled Nematoda eggs, morphologically and morphometrically (length: 103–114.3; width: 46.2–54.9 μm (n = 8)) consistent with trichostrongyle probably to genus *Trichostrongylus* or strongyle, with completely formed larvae. Yellowish structures with ornamented shell and a possible operculum at one end measuring 157.7–165.6 × 95.7–95.9 μm (n = 3) were classified as unidentified helminth eggs associated to individual 22. *Ascaris lumbricoides* eggs, measuring 66–77 × 37–54.5 μm (n = 5), were identified in individuals 22 and 8. The latter had a Nematoda larva that could not be identified at a species level. It could be either from an *A. lumbricoides* or from the other Nematoda found (Fig. 3).

Dietary remains were found in all five pelvic samples, which included potato starches (*Solanum tuberosum*), rice (*Oryza* sp.), bivalves, *Perenospora* cf. *pisi* oospore, muscle fibres, insects, Asteraceae pollen grains, Gramineae phytoliths and *Morchella* sp. ascospore (see Table 1 for details).

Paleopathological analysis of the skeletal remains was hampered by the poor preservation of the individuals (Fig. 1). Despite this, pathological lesions were observed in skeletons 8, 9 and 22. Individual 8 was an adult male, with dental caries, significant wear of the anterior dentition and degenerative joint disease in the axial skeleton. Individual 9 was an adult female with Schmorl's nodes in the lower thoracic vertebrae, along with laminar spur. Lastly, individual 22 was an adult male with osteoarthritis in most of the present joints, with eburnation of the first metatarsal-phalangeal joint of the right foot. Unfortunately, no bone lesions specifically associated with parasites and/or diet were identified, besides dental caries.

Discussion

The Montijo region was characterized by low population density and high circulation of people and goods (Pimentel 1908; Costa 2015). In the study of Costa (2015), about the neighbour village of Aldeia Galega, documents referred to a well with drinking water, with an attached tank for animals and the existence of a dump out of the village. Agricultural production focused on cereal, vegetables and fruits (e.g. melon, chestnut, plum, almond, pine nut, hazelnut, acorn, lentil, flax, onion,

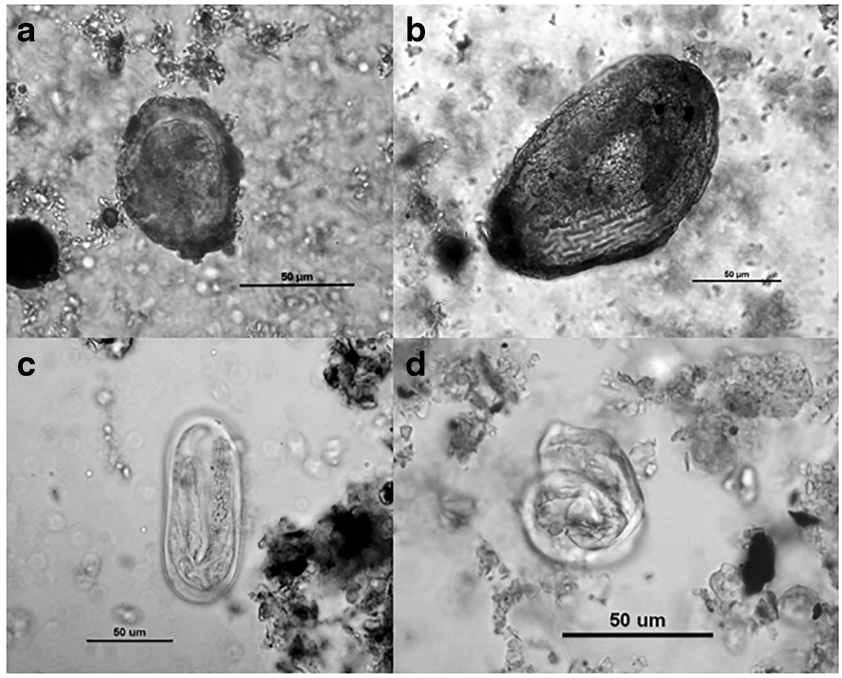

Fig. 2 Helminths found in pelvic girdle soil samples from skeletons exhumed from Church of São Jorge burial ground. **a** *A. lumbricoides*. **b** Unidentified egg. **c** Probably trychostrongyle or strongyle egg. **d** Nematoda larva. Scales equal 50 μm

garlic, mustard and grape). In addition, there was also production of cheese and honey (Costa 2015). These products were consumed by local populations, which had a diet that relied on wine, olive oil, bread and especially cereal. The latter were also produced in the area and were consumed by sailors during maritime expansion (Costa 2015). The pine woods from the region were essentials on shipbuilding (*Leitura Nova* 1502).

Considering the strategic location of Sarilhos Grandes, the presence of trichostrongyle eggs is particularly relevant because this parasite is unreported in paleoparasitological studies of European populations. Species of the family Trichostrongylidae are often present in the intestine of all classes of vertebrates, especially ruminants, causing significant losses in the husbandry of species affected by that parasite. Humans may also become infected by several species of *Trichostrongylus* (eggs 81–104 × 40–48 μm) and other genera by consuming water and foods that are contaminated by larvae which, once ingested, complete their development in the small intestine of the host (Roberts and Janovy, 2008). The presence of adult parasites is usually asymptomatic to the human host, but it may cause digestive disorders and anaemia. Individuals in direct contact with farm animals in poor sanitary conditions are usually the most susceptible (Acha and Szyfres 2003). Eggs of *Trichostrongylus* spp. are seldom found in archaeological remains and they have only been reported in American countries, such as Brazil (Araújo et al. 1984), Chile, Argentina (Gonçalves et al. 2003), México (Reinhard et al. 1989) and the USA (Reinhard et al. 1985; Reinhard et al. 1987). In the USA, the first finding was interpreted as false parasitism associated with the consumption of rabbit offal because only one egg was

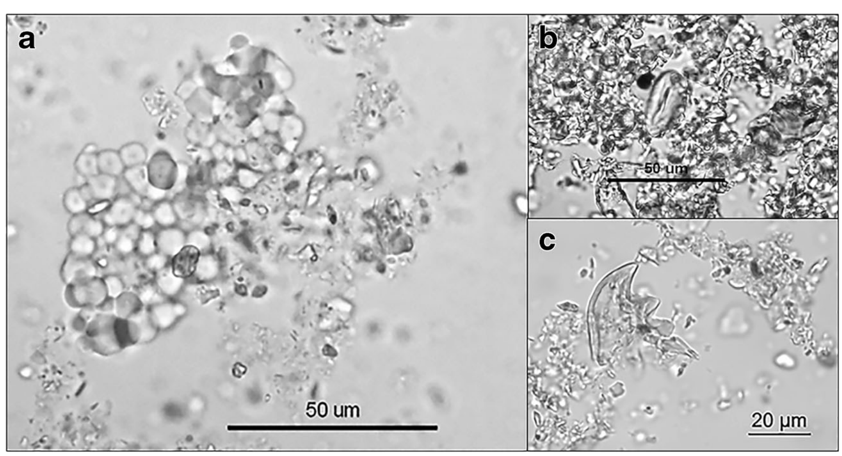

Fig. 3 Dietary remains identified in pelvic girdle soil samples from skeletons exhumed from São Jorge churchyard. **a** Rice starches. **b** Potato starch. **c** Remains of the mouthparts of bivalve radula or insect mandibula

Table 1 Paleoparasitological and paleodietary results obtained from soil samples collected from the pelvic girdle of 5 individuals and 2 control samples, Church of São Jorge

Sample	Sex	Sampling point	Dietary remains	Parasites found	Parasites per millimetre (ppml)
Skeleton 8	Male >25 years	Sacrum	Rice starch, muscle fibres, bivalve, pollen grains of Asteraceae	*A. lumbricoides* Nematode larva	17.7 3.8
		Skull	x x	x x	x x
		Right humerus	x x	x x	x x
Skeleton 9	Female >29 years	Sacrum	Potato starch (*S. tuberosum*), *Perenospora* cf. *pisi* oospore	Tricostrongyle/Strongyle	123.5
		Right humerus	x x	x x	x x
Skeleton 13	Undetermined >29 years	Sacrum	Potato starch (*S. tuberosum*), insects	Tricostrongyle/Strongyle	6.9
		Right humerus	Insects	x x	x x
Skeleton 17	Female >25 years	Sacrum	Potato starch (*S. tuberosum*), muscle fibres, bivalve	x x	x x
Skeleton 22	Male >25 years	Sacrum	Potato starch (*S. tuberosum*), *Morchella* spp. ascospores, Gramineae phytoliths, insects	*A. lumbricoides* Unidentified helminth	1.6 2.4
UE 144		Soil	x x	x x	x x

found in 100 studied coprolites; the second one was considered to be a true infection related to the riverside diet of the Antelope House populations settled in the Chelly Canyon, which presents a moist soil that favours parasite multiplication. In Montijo, both possibilities were plausible because on the one had the area was—and still is—a waterside region, which promotes spreading of the parasite; on the other hand, hunting of rabbits, wolves and deer (ruminant) is historically documented, as is animal husbandry focused on cattle, sheep, goats and pigs (Costa 2015). Although most strongyle eggs has smaller sizes than those found in this study, morphological similarities between these two groups support this alternative diagnosis which is an interesting possibility since there are only two records in human material from the Old World, one in Egypt dated 1000 cal BC and in Netherlands between 1370 and 1425 AD (Gonçalves et al. 2003). Symptoms for the human host can be very similar to those of Trichostrongylidae while natural hosts include ruminants and other husbandry animals (Roberts and Janovy, 2008).

A. lumbricoides is a human parasite, commonly known as roundworm, that is transmitted by contact with faeces of a contaminated person or via consumption of water or food that are contaminated by eggs of the parasite. The eggs, which are extremely resistant and may remain infective for up to 10 years (Roberts and Janovy, 2008), have been found in European archaeological remains dating from the late Neolithic and especially the Middle Ages (Anastasiou 2015; Bouchet et al. 2003). Eggs of *A. lumbricoides* were identified in the samples collected from the skeletons of individuals 8 and 22 with very different concentrations: 17.7 and 1.6 eggs per ml. Skeleton 22 was well preserved despite being one of the ancient individuals, inhumed wearing shoes with metal buckles. Until recent times, shoe materials were quite hierarchical thus a metal buckles can presumably be related to some economic status of this male individual. Perhaps, a high economic status has facilitated his access to deworming medication, reflected in a lower number of eggs found, as suggested by Fisher et al. (2007). Another factor that may had contributed to the small concentration of *A. lumbricoides* is the low population density in Sarilhos Grandes, which in 1527 had approximately 184 inhabitants (Dias 2000), and about 819 in 1879 (Pimentel 1908). Likewise, individual 8 could be using some natural treatment to eliminate any possible discomfort caused by the presence of roundworms, since his diet revealed the presence of grains of pollen of the family Asteraceae with several species with medicinal properties (Rai et al. 2012) and that may have been the reason for its consumption. It is interesting that individual 22 was the only one that presented unidentified eggs. Some helminths have ornamentations on the eggshell such as Ascaridida and Acanthocephala. Although we could not correlate the structure found with any specific taxon, it is most probably that this finding reflects the consumption of an animal infected by this parasite as reported by Sianto et al. (2012). As such, the presence of this parasite in individual 22 may be related to the consumption of some exotic meat only accessible to wealthy people.

Other paleodietary results are also consistent with what is available from the literature about diet in this region. It is known that, due to the proximity of the Tagus river estuary, the local population has historically relied on fishing (fish and shellfish, including oysters), exploration of salt ponds and the production of wine (Câmara Municipal de Montijo 2015). As such, the evidence of consumption of bivalves in the Sarilhos Grandes individuals is not surprising. Bivalves are also an important source of calcium, iodine, zinc and potassium, among other nutrients (Pathou-Mathis 1997). Surprisingly, no fish parasites were found in the sample although it is likely that this population was exposed to this type of infection. Fish is an important source of protein in waterside settled populations and multiple paleoparasitological studies have reported infection of human population with fish parasites in Europe (Le Bailly et al. 2005, 2007), America (Araújo et al. 2011) and Asia (Han et al. 2003; Matsui and Kanehara 2003; Seo et al. 2008). The absence of fish parasites may be related to food habits of this population since cooking of fish and seafood kills parasites that may be present.

The presence of the *Morchella* sp. ascospores is probably the result of consumption of the *Morchella* spp. mushroom, which has been reported as eatable by Petithory and Ardoin-Guidon (1995) and may have been an important source of protein. On the other hand, *Perenospora* cf. *pisi* is a species of the group known as downy mildew that grows on several crucifers, which are widely consumed by human populations (Gupta 2004), and the presence of its oospores is probably related to the consumption of a vegetable in which it grew. The insect remains could not be identified at lower taxon either because they were very fragmented. Their presence should, however, not be related to diet but probably to post-burial intrusion which is consistent with their presence in one negative control sample.

Lastly, several species of the Gramineae family are widely consumed by human populations. One example is rice, of which starches were identified in the dietary analysis of the individuals sampled. Rice (*Oryza sativa*) was first introduced in the Iberian Peninsula in the eighth century A.D. by Muslim populations and it has been farmed at least since the thirteenth century. Since then it has, along with the potato (*Solanum tuberosum*), traditionally been used in the Portuguese gastronomy (Salaman et al. 1985; Sharma 2010). However, the latter was only introduced in Europe during the second half of the sixteenth century (Bracht et al. 2011). According to the stratigraphic interpretation, Sk. numbers 13, 17 and 22 were dated at or below the sixteenth century. The presence of potato in four skeletons (9, 13, 17 and 22) not only helps to narrow the chronology of the studied individuals but also indicates that Sarilhos Grandes population soon had access to this product from the New World.

Conclusions

This is one of the first paleoparasitological studies performed in Portuguese samples. Unfortunately, the number of sampled individuals is small, which might explain the low diversity of identified parasites. Nonetheless, the results suggest that the geographic location of Sarilhos Grandes impacted decisively on the nutritional intake of its past populations. Carbohydrates were probably acquired via rice and potato starches. Animal protein was possibly a substantial part of the diet, as suggested by the presence of bivalves and eggs of parasites whose natural hosts are animals. The absence of parasites from fish in the samples analysed it is probably related to fish cooking prior to consumption. Similar systematic studies targeting a wider chronological, geographical and cultural span are necessary to provide a more complete scenario of the Portuguese parasitological past and the impact of the maritime expansion.

Acknowledgments The authors thank João Costa from the Instituto de Estudos Medievais. The research is funded by CNPq post-doctoral fellowship process 201416/2014-0, and the Research Centre for Anthropology and Health—UID/ANT/00283/2013—FCT.

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