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Short communication

Paleoparasitological results from XVIII century human remains from Rio de Janeiro, Brazil

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ABSTRACT

Paleoparasitological studies of the Brazilian colonial period are scarce. A paleoparasitological analysis was performed on human remains from the archeological site *Praça XV* Cemetery in Rio de Janeiro, dating from the early 18th to 19th Centuries. The samples were obtained from the Institute of the Brazilian Archaeology collection, and showed evidence of washing and brushing. Sediments were extracted from sacral foramina by scraping. Sediments from skulls were used as negative paleoparasitological controls. Spontaneous sedimentation method was performed prior to microscopic analysis. The results revealed that 8 of 10 individuals were infected with intestinal helminths and/or protozoa. Eggs of the nematodes *Trichuris* sp. and *Ascaris* sp. as well as a single taeniid egg were found. Protozoa cysts suggestive of *Entamoeba* sp. were also observed. *Trichuris* sp. was the most frequent and abundant parasite, found in 70% of individuals (26 eggs). The study showed the importance of analysis of sediment from human remains preserved in museum or scientific collections, even those subjected to a curating procedure. The levels of infection revealed here should be considered underestimations. This is the first paleoparasitological study from Rio de Janeiro city for the Brazilian colonial period and the first report of human *Taenia* sp. in the New World.

1. Introduction

During the Brazilian colonial period, Rio de Janeiro was the capital of Brazil and the main commercial center of the country and, as a consequence, underwent massive urbanization (Fragoso and Florentino, 2001). Located on the coast, the city's port was a trading center for the entire southeast region, primarily handling sugar, coffee and gold (Fragoso and Florentino, 2001). The accelerated population growth, combined with disordered expansion of the city and poor sanitary conditions, allowed the introduction and spread of infectious and parasitic diseases.

Paleoparasitology is the study of parasites in archeological or paleontological materials. Such research provides information on past and present diseases and contributes to understanding the evolution of human social structure, biology, and behavior (Pike, 1968; Bouchet et al., 2003a; Gonçalves et al., 2003). Many studies have shown the presence of parasites in human remains in Pre-Columbian Brazil (Ferreira et al., 1980; Araújo et al., 1981; Gonçalves et al., 2003; Iñiguez et al., 2003); however studies that demonstrate the presence and frequency of parasites in colonial periods are scarce. The most common biological samples for paleoparasitological analyses are coprolites (desiccated or mineralized feces), and sediments from latrines and the pelvic and abdominal regions of mummified bodies (Bouchet et al., 2003a). Nevertheless, Fugassa et al. (2008), working with bones that had undergone museum curation, recovered *Ascaris* sp. and *Capillaria* spp. eggs from organic material in the human sacrum foramina found at a Patagonian archeological site (850–500 years BP).

In the present study, a paleoparasitological analysis of human remains from the archeological site *Praça XV* Cemetery in Rio de Janeiro, Brazil, was conducted in order to verify the presence of intestinal parasites in osteological remains of the Brazilian colonial period, submitted to a curatorial process, and to generate new data on the epidemiology of parasitic diseases in Brazilian colonial period.

2. Materials and methods

The present day *Praça XV de Novembro* (November XV Square) was a central area of Rio de Janeiro city in colonial times. In 1996, during the construction of a tunnel in the port, human skeletons dating from the 18th and 19th centuries were discovered. There

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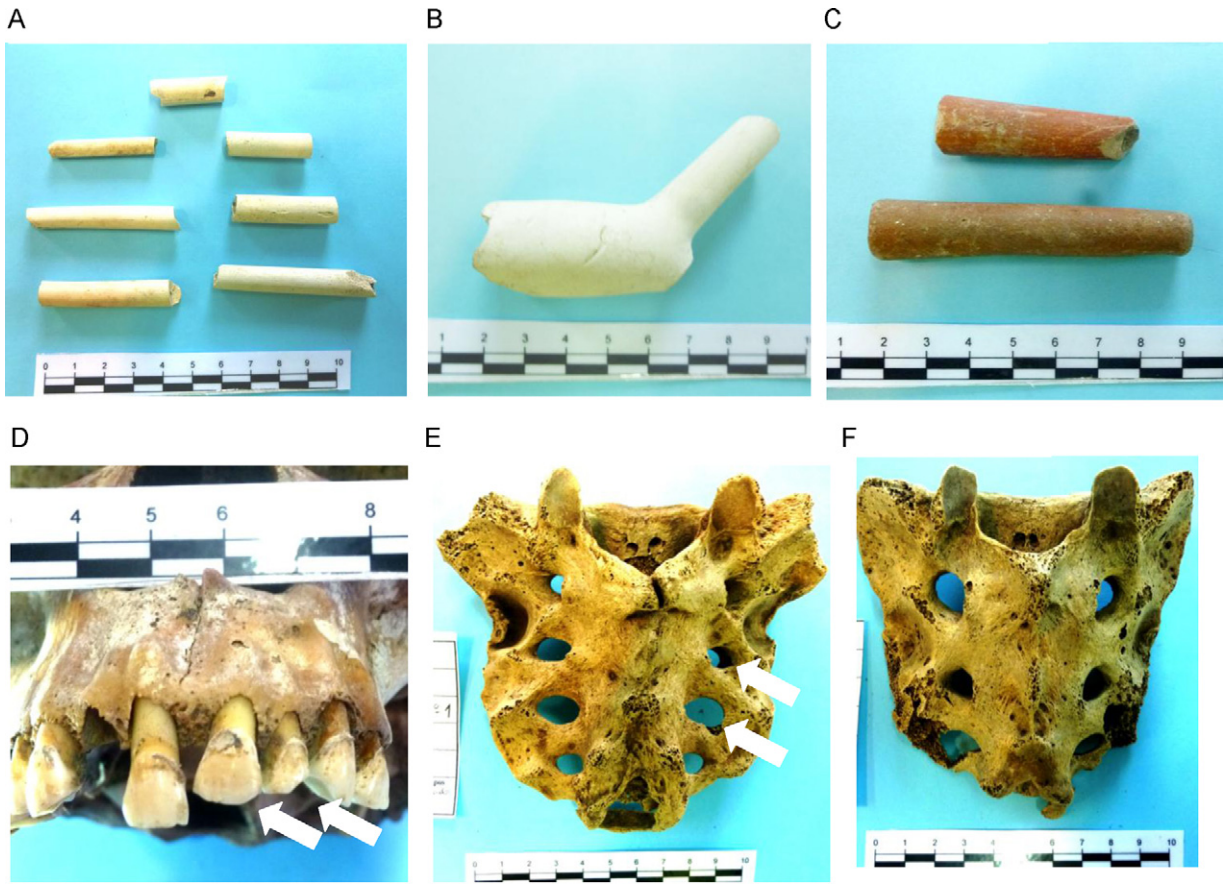

Fig. 1. Archeological remains from the archeological site *Praça XV* Cemetery, Rio de Janeiro, Brazil. IAB collection. (A–C) Cultural artifacts related to AfroBrazilian culture. (D) Intentional dental modifications. (E and F) Human sacrum and sacral foramina. Scale bars = 1.0 cm.
Photographed by A.M. Iñiguez.

was a burial ground for the general population in the region. African slaves who died in the slave markets located near the port were also buried in the cemetery. The archeological remains, including cultural artifacts (Fig. 1A–C), were collected by the Institute of Brazilian Archaeology (Instituto de Arqueologia Brasileira – IAB). Stratigraphic analysis performed during excavation confirmed that human skeletal remains were found in argillaceous and sandy soil, characteristic of a typical mangrove ecosystem. During excavation, due to a high degree of disaggregation of the burials and the anatomic separation of individuals, complete skeletal series were not identified. Instead, a series of types of bones, skulls and mandibles for example, were collected, stored at room temperature, and protected from light. All bones were well preserved and were submitted to the process of curation. In this process, the bones were cleaned of the original soil, but no chemicals were added.

The bio-anthropological analysis of 37 skulls showed that 59% were young adults, 27% mature adults, 11% adolescents, and 3% children. Forty-six percent were male, 38% female, and 16% undetermined. Craniofacial analysis of 14 skulls revealed that 11 showed evidence of ethnic African origin. The preliminary analysis of 12 skulls demonstrated that 9 individuals presented dental modifications (Fig. 1D).

The sediment samples were recovered from the interior of the sacral foramina of ten individuals (Fig. 1E and F). Three to 7 g of sediment, comprising chiefly sand, were used in the paleoparasitological analysis. Samples were transported to laboratory at 4 °C and kept at −20 °C until paleoparasitological analysis. The sediment samples were rehydrated (1:2 w/v) with 0.5% aqueous trisodium phosphate solution for 72 h at 4 °C. Paleoparasitological analysis was conducted by spontaneous sedimentation prior to microscopic examination. Ten to 20 slides of each sample were examined under 10× and 40× magnification (Axiostar Plus – Zeiss). Parasites eggs were measured and photographed with a Canon A650 IS and edited using the ImageJ 1.44p program (National Institutes of Health – USA). Descriptive statistical analyses were performed using the program R (version 2.13.2).

Sediment scrapings from the interior of skulls ($n = 5$) were used as negative controls for paleoparasitological analysis. The control sediments were processed following the methodology described above.

3. Results

Paleoparasitological examination revealed that 8/10 individuals were infected by intestinal parasites. *Trichuris* sp. eggs (Nematoda) were the most frequently found, in 7/10 individuals. Twenty-six eggs were recovered (Fig. 2A–E). Most of these *Trichuris* sp. eggs possessed no content or polar plugs. Eggs measurements were 40.84–56.1 μm in length and 22.0–33.0 μm in width (Table 1). Descriptive statistical analyses demonstrated that *Trichuris* sp. eggs of mean length, 47.83 ± 4.46 ($n = 24$) and mean width, 27.41 ± 3.23 ($n = 24$), corresponded to that established for *Trichuris trichiura* species by Confalonieri et al. (1988) (Table 2; Fig. 3). A single *Ascaris* sp. egg, measuring 79.2 μm × 56.9 μm, was found in sample XV31 (Fig. 2F).

A helminth egg, measuring 31.5 μm × 31.5 μm, belonging to the family Taeniidae was found in sample XV33 (Fig. 2G). The taeniid

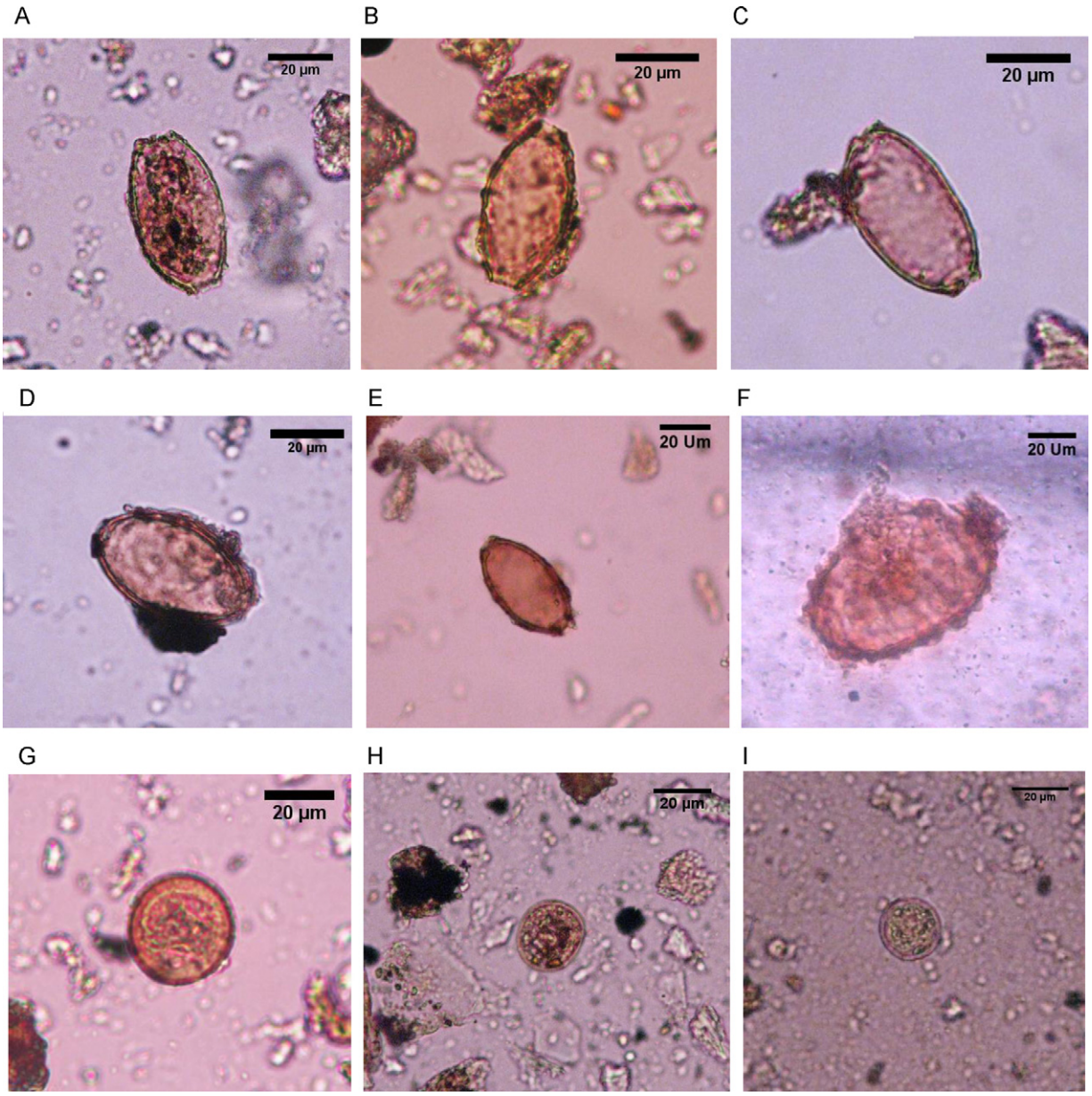

Fig. 2. Results Paleoparasitological analysis. A-E *Trichuris* sp. eggs; F *Ascaris* sp. egg. G *Taenia* egg; H-I Protozoan cysts. Scale bars are on the figures.

egg is characteristic of human *Taenia* sp., displaying a thick shell with a prism aspect and dimensions of 31–43 μm (WHO, 2007).

Two spherical structures similar to intestinal protozoa were observed in the samples XV29 and XV33, measuring 25.2 μm × 25.2 μm and 22.5 μm × 22.5 μm in diameter, respectively (Fig. 2H and I). These putative intestinal protozoa are suggestive of *Entamoeba coli*, due to the absence of flagella and fibrils, and the size of both mature and immature cysts of 10–35 μm, usually 15–25 μm (WHO, 2007). Control samples of skull sediment were negative.

4. Discussion

This is the first report of intestinal parasites in human remains from archeological sites in Rio de Janeiro from the Brazilian colonial period. *T. trichiura* and Trichostrongylide infection have been reported in archeological sites from the historic period of Brazil in Minas Gerais and Piauí states (Confalonieri et al., 1981; Araújo et al., 1984). The human remains retrieved from this site constituted an opportunity to study infectious and parasitic diseases during the Brazilian colonial period due to the absence of data related to this period, and the preservation of material. In general, the preservation of organic material is limited to places of low humidity or frozen conditions (Bouchet et al., 2003b). Tropical climates are usually unsuitable for the preservation of organic matter. The Rio de Janeiro city has a tropical climate with high temperatures and humidity. The combination of heavy rainfall, abundant insect life, and acid soil usually accelerate the process of decomposition of organic material (Bouchet et al., 2003b). Nevertheless, it was possible to find helminth eggs in organic material recovered from human remains from this site.

Ascaris lumbricoides and *T. trichiura* infections reflect poor sanitation and hygiene (Bouchet et al., 2003a). They are the most common human intestinal parasites in European archeological sites dated to medieval and historic times (Bouchet et al., 2003a). However, *Ascaris* eggs are rarely found in American prehistoric populations, while *T. trichiura* had a wide distribution in the New World in pre-Colombian times (Gonçalves et al., 2003). This

Table 1
Morphometric data for *Trichuris* sp. eggs found in *Praça XV* Cemetery, Rio de Janeiro, Brazil.

Sample (n = 24 eggs)	Length (μm)	Width (μm)
XV24	42.07	22.20
XV24	47.25	24.75
XV24	47.25	28.46
XV25	56.10	33.00
XV25	45.80	27.20
XV25	44.55	27.22
XV26	47.02	22.20
XV26	40.84	24.75
XV26	44.55	24.75
XV26	45.78	24.75
XV28	42.75	24.75
XV28	37.12	34.65
XV28	49.50	27.22
XV28	51.97	29.70
XV28	49.50	29.70
XV29	44.55	29.70
XV31	55.68	29.70
XV31	47.02	27.22
XV31	49.05	30.93
XV31	47.02	24.75
XV31	49.50	27.20
XV32	44.55	24.75
XV32	49.50	28.46
XV32	54.45	32.17
XV32	47.02	29.70
Mean	47.83 ± 4.46	27.41 ± 3.23

Table 2
Morphometric data for *Trichuris* sp. eggs (n = 24) found in *Praça XV* Cemetery, Rio de Janeiro, Brazil.

Measurements	Length (μm)	Width (μm)
Min.	40.84	22.20
1st Qu.	45.16	24.75
Median	47.02	27.22
Mean	47.83	27.41
3rd Qu.	49.50	29.70
Max.	56.10	33.00
SD	4.46	3.23

difference may be due to environmental and taphonomic factors that affect preservation of eggs (Leles et al., 2010) or by some inherent characteristic of the egg.

Taenia spp. eggs have been recovered from human coprolites and latrine sediments from Old World archeological sites (Bouchet et al., 2003b; Gonçalves et al., 2003). They have not been found in humans of the New World in pre-Colombian times prior to the introduction of pigs and cattle (Gonçalves et al., 2003). Paleoparasitological studies have demonstrated the presence of taeniid eggs in pre-Columbian Patagonia in zooarchaeological material, where human tapeworms have not been found (Beltrame et al., 2010, 2011). In the present study, because the parasite eggs were found in sediments from the sacrum, (Fig. 1E and F) with external deposits and/or contamination removed during the curatorial processes, the finding suggests infection by human taeniids, *Taenia solium* or *T. saginata*.

There is some evidence pointing to the human origin of the *Taenia* sp. egg: (1) Bio-anthropological analysis indicates that the *Praça XV* Cemetery archeological site was a human cemetery; (2) Control samples of skull sediment were negative for paleoparasitological analysis; and (3) the finding is statistically supported, of the human-specific parasite *T. trichiura*, corroborated the human origin of the samples. Therefore, this is possibly the first record of human *Taenia* sp. in the New World, corroborating with new data of the paleodistribution of tapeworms.

However, we cannot rule out the possibility that the *Praça XV* Cemetery was used for disposal of organic refuse, including animal excrement. Rocha et al. (2006) showed animal-specific parasites, such as *Capillaria* sp. and *Oxyuris equi* eggs, in the Place d Armes archeological site (Namur, Belgium) of the Gallo-Roman

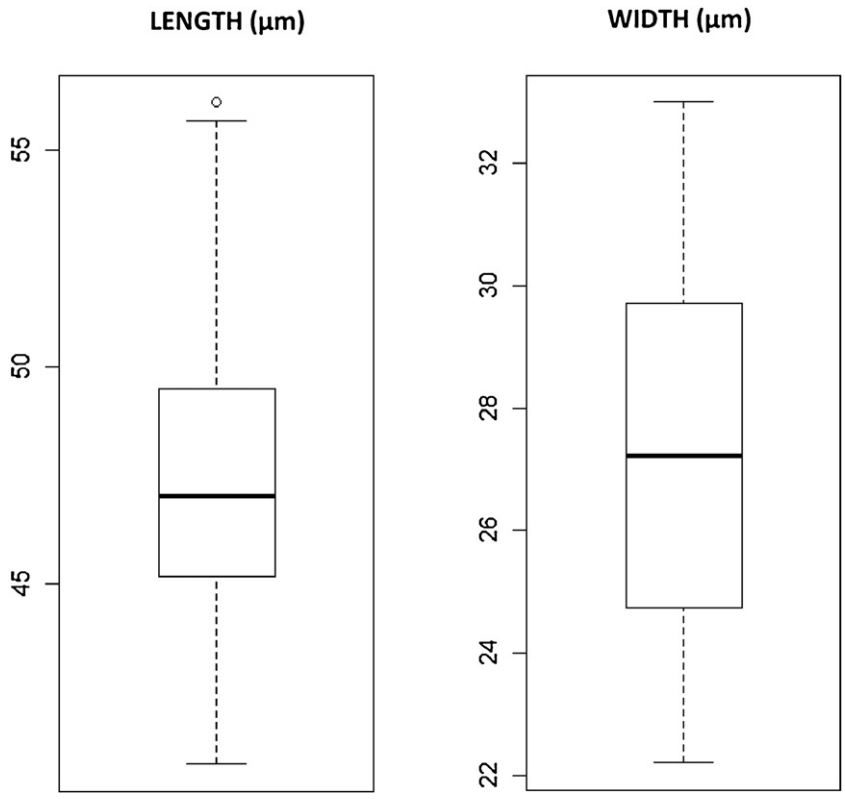

Fig. 3. Descriptive statistical analyses of morphometric data from *Trichuris* sp. eggs from this study.

Period through the 19th century, confirming the zoological origin of organic sediments. The presence of human and animal specific-eggs confirms the used of this site for disposal of various types of organic matter, including human and animal excrement. In the present study, although no human material contaminated with animal feces was found, we cannot rule out the possibility of a post-mortem artifact, or contamination, as only a single egg was found.

This study contributes to the understanding of parasitic diseases with zoonotic potential, especially in cities such as Rio de Janeiro that had increased population growth, crowding, and poor sanitation conditions in colonial times.

We found spherical structures that may be intestinal protozoan cysts. Cysts are fragile and less resistant to decay compared to helminth eggs (Gonçalves et al., 2003). Of 3 archeological samples positive for *Giardia duodenalis* antigen by immunoassay, Gonçalves et al. (2002) found only one positive by microscopic examination. We observed only two cysts that we attributed to protozoa. The vulnerability of cysts to decay may result in artificially low estimates of protozoa in paleoparasitology (Gonçalves et al., 2002). The location of *Praça XV* Cemetery, near the coastline in the port of Rio de Janeiro at Guanabara Bay, may have contributed to preservation of intestinal protozoa. Historical records show that the location corresponded to a vast mangrove swamp, and the human skeletal remains were found in argillaceous and sandy soil characteristic of a typical mangrove ecosystem. It is possible that the microenvironment of a mangrove swamp permitted differential preservation of cysts and eggs. In addition, the foramina sacrum trapped parasitic eggs and cysts and may also have provided protection against taphonomic effects, allowing better preservation of parasites.

The eggs of *Trichuris* sp. found were within the size range reported for both desiccated and non-desiccated eggs of *T. trichiura* species (Confalonieri et al., 1988). The average length expected for the human *T. trichiura*, without polar plugs, is 48.08 and 47.65 μm for non-desiccated and desiccated eggs, respectively (Confalonieri et al., 1988). The results confirmed the experimental observations of Confalonieri et al. (1988), who found that the size of *T. trichiura* eggs does not change with desiccation.

Paleoparasitological records suggest that, although the majority of studied intestinal parasites infected pre-Columbian Amerindians (Gonçalves et al., 2003), parasitosis seemed to have become a major public health problem after the arrival of Europeans settlers (Le Bailly et al., 2006). A considerable increase may have occurred with European colonization and the creation of new urban settlements (Fernandes et al., 2005). It would be likely to find these parasites in Brazilian historical archeological sites; however, there is no current data from this period. The present study demonstrated the frequent occurrence and diversity of intestinal parasites in historic times, and suggests a high intensity of parasites in this population, since the source of material examined was cleaned human remains.

The study showed the importance of analysis of sediment from human remains preserved in museum or scientific collections, even following the museum curating process. Our work was successful in recovering helminth eggs in 80% of individuals, even when the bone materials were cleaned. In addition, we found a large number of eggs, mainly of *T. trichiura*, suggesting that the results represent an underestimation of the prevalence of intestinal infection in historic times. If it were possible to access all organic material associated with the abdominal region, such as complete sacral sediment or coprolites, the prevalence, intensity, and/or diversity of recovered parasites would likely be much greater.

The study of sediment samples from the human sacral region submitted to the curatorial process opens opportunities for the study of past disease patterns and increased understanding of the introduction and spread of disease. The curatorial process should be regulated, and all organic material preserved, to enable future studies in paleoparasitology, as well as in paleogenetic, paleopalynology, and paleodiet.

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