Studies on protozoa in ancient remains - A Review

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Paleoparasitological research has made important contributions to the understanding of parasite evolution and ecology. Although parasitic protozoa exhibit a worldwide distribution, recovering these organisms from an archaeological context is still exceptional and relies on the availability and distribution of evidence, the ecology of infectious diseases and adequate detection techniques. Here, we present a review of the findings related to protozoa in ancient remains, with an emphasis on their geographical distribution in the past and the methodologies used for their retrieval. The development of more sensitive detection methods has increased the number of identified parasitic species, promising interesting insights from research in the future.

Key words: paleoparasitology - mummies - coprolites - infectious diseases - protozoa - paleoepidemiology

Since the beginning of the last century, paleoparasitology has been focused on understanding the origin and evolution of infectious diseases, relying on archaeological and paleontological material to do so. A wide diversity of intestinal parasites has been retrieved from ancient remains, primarily from helminths (Gonçalves et al. 2003). However, although protozoa exhibit a global distribution, they are not recovered easily from archaeological contexts. This scarcity might be related to difficulties in detecting these organisms using traditional optical microscopy and to the sensitivity of parasitic structures, which are less resistant to taphonomic processes, leading to a low estimation of protozoa in the archaeological record.

This literature review aims to identify and summarise the geographic distribution of protozoa in the archaeological record, with an emphasis on protozoa associated with humans, including both intestinal and tissue parasites and the methodologies used to study them in ancient remains. An electronic database search was performed targeting studies on protozoa in the fields of paleoparasitology, archaeology and paleopathology and authors showing previous research efforts on this subject. The search comprised all publications found on this topic in PubMed and ScienceDirect and their bibliographies were screened as well. The data extracted from the literature included parasite species, archaeological sites and dates, the methods applied and the results of the studies. There were no exclusions related to publication dates or languages.

Methodological approaches to the identification of protozoa

Although macroscopic examinations of lesions are generally limited to making observations of body preservation and the presence of specific landmarks, this technique is the most direct way of approaching disease in archaeological remains. For example, Chagas disease was diagnosed based on an altered large intestinal tract in a pre-Columbian mummy (Reinhard et al. 2003) and later confirmed via molecular biological methods (Dittmar et al. 2003). However, this finding was exceptional, as the majority of infectious diseases will not be detected using such methodology. Consulting historical documents provides an indirect method for approximating protozoan infections. By reviewing medical documents, autopsy reports and original death certificates recorded by court physicians, Gino Fornaciari et al. (2010a, b) reconstructed the medical history of one of the most influential families of the Italian Renaissance, the Medici (Nerlich et al. 2012).

In a similar manner, the origin of leishmaniasis in the Americas was discussed based on ethno-historical documents and anthropomorphic representations on Mochica ceramics (huacos) showing lesions similar to those found in mucous leishmaniasis (Altamirano-Enciso et al. 2003). Microscopy has been the traditional method for parasite identification in paleoparasitological analyses and the first protozoa found in fossilised faeces (coprolites) were described using this technique (Pizzi & Schenone 1954, Witenberg 1961, Fouant et al. 1982). Unfortunately, most of these early findings were not accompanied by photographs or images, preventing comparisons with later studies.

Immunofluorescence and enzyme-linked immunosorbent assays (ELISA) have been the most commonly employed techniques for antigen recognition in ancient remains. Biochemical techniques were initially used in this field in 1989, when Faulkner et al. (1989) applied indirect immunofluorescence to identify Giardia cysts from human coprolites dated to 2,177 ± 145 years before present (BP). Since that time, various intestinal parasites have been successfully identified via these techniques in coprolites around the world (for a review, see Gonçalves et al. 2003).

With the development of methods for ancient DNA recovery, tracing parasitic diseases became possible. Analyses of ancient DNA in the field of paleoparasitology have shown that the diversity of infectious diseases in the past was greater than previously described.
tology were first performed in experimental animal mummies and demonstrated that molecular techniques could recover parasitic DNA from archaeological material (Bastos et al. 1996). In paleoparasitology, molecular biological methods have been used primarily for species confirmation, resulting in the identification of falciparum malaria, visceral leishmaniasis (VL) and Chagas disease. However, there are limitations to these techniques. The need to retrieve small DNA fragments from parasitic structures that are difficult to preserve and are usually associated with material of uncertain archaeological dates makes further analyses difficult.

Several parasites of animal species have been recovered from coprolites of human origin, suggesting false parasitism in some cases and zoonosis in others. Most of these studies have been performed on helminths. However, many of the infections considered to be zoonoses, such as cryptosporidiosis and giardiasis, can only be confirmed through molecular characterisation of genotypes and subgenotypes. No enteric protozoa have been identified by these methods to date. Nevertheless, beyond the application of these techniques for diagnostic purposes, they would expand the ability to study protozoan infections in the past.

**A brief history of studies on protozoa in ancient remains**

The analysis of protozoa in the archaeological record (Fig. 1) relies on the distribution and availability of ancient remains, the ecology of infectious diseases and the use of adequate detection techniques. Studies conducted in amber specimens have provided an idea of how old the association with protozoans is (Table I). The discovery of a trypanosomatid (of the genus *Paleoleishmania*) within a female sandfly in Cretaceous Burmese amber indicates that vector-borne parasites already existed by the Early Cretaceous (Poinar & Poinar 2004). The description of a trypanosomatid from faecal droplets adjacent to *Triatoma dominicana* provides the first fossil evidence of a triatomine-trypanosomatid vector association, dating to the mid-Tertiary era (Poinar 2005a). The presence of *Plasmodium dominicana* in a Tertiary Dominican Republic amber specimen establishes a minimum age for the genus *Plasmodium* and places avian malaria in the Americas by the mid-Tertiary, supporting earlier theories that some species responsible for primate malaria could have evolved in the Americas (Poinar 2005b). Indirect evidence based on the frequency of erosive lesions found in tyrannosaurids suggests infection by a *Trichomonas gallinae*-like protozoan and represents the first report of an avian-transmissible disease in non-avian theropod dinosaurs (Wolff et al. 2009). Cysts similar to those of the extant genus *Entamoeba* have been preserved in coprolites from the Early Cretaceous, enabling the description of two new genera and species, *Entamoebites antiquus* (Poinar & Boucot 2006) and *Endamoebites proterus* (Poinar 2009). Unsporulated coccidian oocysts (*Archeococcidia antiquus* sp. nov. and *Archeococcidia nothrotheriopsae* sp. nov.) have also been described in coprolites from a Shasta ground sloth (*Nothrotheriops shastensis*) (Schmidt et al. 1992).

In addition, *Eimeria* oocysts from various animal species have been retrieved from archaeological contexts. The first such report refers to oocysts in deer coprolites dated to 9000 BP from northeastern Brazil, for which a new species (*Eimeria lobatoi*) was suggested (Ferreira et al. 1992) and oocysts of *Eimeria macusaniensis* and *Eimeria vitasaensis* have been detected in mumified camelids from Peru (Legúía et al. 1995, Legúía 1999). More recently, *E. macusaniensis* was recovered from various archaeological sites in Santa Cruz, Argentina (Fugassa & Barberena 2006, Fugassa & Guichón 2006, Fugassa 2007, Fugassa et al. 2007, Beltrame et al. 2010), where the host specificity of this species enabled more reliable identification of camelids in archaeological deposits. Furthermore, by comparing the dimensions of oocysts from these archaeological sites, a temporal trend was established indicating a size reduction over time (Fugassa et al. 2008). This discovery offers insight into host-parasite coevolution and paleoenvironmental changes.

A large number of publications have addressed the study of mumified human remains, which have shown preservation varying from excellent to very poor (Lyn-
TABLE I

Summary of studies on protozoa in extinct animals

| Protozoan                        | Origin                                      | Period          | Host                        | References                      |
|----------------------------------|---------------------------------------------|-----------------|-----------------------------|---------------------------------|
| Archeococcidia antiquus sp. nov. | Rampart Cave, Grand Canyon (Arizona, USA)  | 10500 ± 180 BP  | Shasta ground sloth         | Schmidt et al. (1992)           |
| Archeococcidia nothrotheriopsae sp. nov. | Burmese amber  | Early Cretaceous  | Sandfly                      | Poinar & Poinar (2004)         |
| Paleoleishmania proterus gen. nov., sp. nov. | La Toca amber mine (Dominican Republic) | Mid-Tertiary    | Triatoma dominicana sp. nov. | Poinar (2005a)                  |
| Trypanosoma antiquus sp. nov.    | Burmese amber                               | Mid-Tertiary    | Triatoma dominicana sp. nov. | Poinar (2005a)                  |
| Plasmodium dominicana sp. nov.   | Dominican Republic amber                    | Mid-Tertiary    | Culex mosquito              | Poinar (2005b)                  |
| Entamoebites antiquus            | Belgium                                     | Early Cretaceous| Iguanodon                   | Poinar & Boucot (2006)         |
| Free living trypanosomatids     | Amber beds in Kachin (Burma)                | Early Cretaceous| Sandfly larvae              | Poinar (2007)                   |
| Endamoebites proterus gen. nov. | Burmese amber                               | Early Cretaceous| Termite                     | Poinar (2009)                   |
| Trichomonas gallinæ-like protozoan | North America                               | Latest Maastrichtian | Tyrannosaurids               | Wolff et al. (2009)            |
| Eimeria lobatoi                  | Perna I, São Raimundo Nonato (Paul, Brazil) | 9000 BP         | Deer                        | Ferreira et al. (1992)         |
| Eimeria macusaniensis, Eimeria ivitaensis | Peru                                      | 1000 BP         | Camelids                    | Leguia et al. (1995)           |
| E. macusaniensis                | Orejas de Burro 1                           | 3978-3720 cal. year BP | Camelids                    | Fugassa & Barberena (2006)     |
|                                 | Nombre de Jesús                             | XVI century AD  | Camelids                    | Fugassa & Guichón (2006)       |
|                                 | Cerro Casa de Piedra 7                     | 8000 BP         | Camelids                    | Fugassa (2007)                  |
|                                 | Cerro Casa de Piedra                       | Middle Holocene | Felines                      | Fugassa et al. (2009)          |
|                                 |                                             | Late Holocene   | Uncertain human origin      | Beltrame et al. (2010)         |

a: all studies used microscopy as identification method, except for Wolff et al. (2009) who analysed erosive lesions; AD: Anno Domini; BP: before present.
nerup 2007). Soft tissue preservation depends on rapid dehydration overtaking postmortem decay and can be brought about either by natural conditions (a hot or very cold dry climate) or via artificial means (mortuary practices preventing degradation). Hence, the dry and salty climate of the Saharan and Atacama Deserts, the cold winds and permanent ice of the Andean Cordillera and the aridity of the Argentinean Pampas and Brazilian Savannah (Cerrado and Caatinga) present ideal conditions for tissue preservation. Similarly, bodies within sealed tombs are generally well preserved, facilitating the identification of diseases that do not necessarily leave traces in bone (Cockburn et al. 1998, Auferheide 2003).

Enteric protozoa are expected to be found worldwide, as gastrointestinal infections represent one of the oldest and most common associations of infectious disease with humanity. In addition, these organisms do not require specific vectors, as they are generally transmitted by contaminated food and water. Blood protozoa, on the other hand, depend strongly on the distribution of their vectors and, consequently, on various environmental factors.

Forty-eight publications addressing protozoa found in human remains (Table II) were retrieved from the electronic databases, ranging from the year 1954-2012. The number of publications from the present century was equal to the number published from the 1950s-1990s. The first descriptions of enteric protozoa in archaeological remains were secondary to findings of larger parasites (Pizzi & Schenone 1954, Witenberg 1961, Faulkner et al. 1989). Subsequently and with the growing availability of commercial kits that enable parasite retrieval from coprolites, the number of studies on protozoa increased. For example, Giardia duodenalis, Cryptosporidium parvum and Entamoeba spp have been successfully identified in samples from both the New and the Old World, dating to between 5300 BP and the XIX century (Gonçalves et al. 2004, Le Bailly & Bouchet 2006).

Unlike enteric protozoa, blood protozoa have historically attracted the interest of more researchers, primarily because of their epidemiological importance in public health. The use of molecular techniques has enabled confirmation of Chagas disease in Andean mummies dating back to 9000 BP (Auferheide et al. 2004) and falciparum malaria in ancient Egyptian mummies dating to 5200 BP (Miller et al. 1994, Cerutti et al. 1999, Rabino Massa et al. 2000, Nerlich et al. 2008).

**TABLE II**

| Publications on protozoa in ancient human remains |
|--------------------------------------------------|
| Publications (n)                                 |
| New World | Old World | Total |
| Enteric protozoa | 10 | 5 | 15 |
| Blood protozoa | 16 | 17 | 33 |
| Total | 26 | 22 | 48 |

Fig. 2: paleodistribution of Trypanosoma cruzi studies in humans (white spots). Grey area approximately represents the current geographic extent of Chagas disease in Central and South America (adapted from Silveira 1999).
duced, would also have increased the risk of infection by triatomine species adapted to live in rocks (Araújo et al. 1998, Ferreira et al. 2000). The T. cruzi infections described from the archaeological record were reviewed by Ferreira et al. (2011) regarding the origin and spread of Chagas disease.

Malaria - Human malaria is one of the most common infectious diseases in the world. It is transmitted by infected female mosquitoes of the genus Anopheles, which inject malaria parasites while feeding. There are five species known to infect humans, among which Plasmodium falciparum accounts for the death of more than one million people every year (Snow et al. 2005). This infection exhibits a widespread distribution in tropical and subtropical areas, with the highest transmission currently found in the Amazonas, Sub-Saharan Africa, India and parts of Oceania (CDC 2012).

Studies in ancient remains have provided evidence of endemic malaria in Egypt and Italy (Fig. 3, Table IV), where proximity to river valleys would have resulted in a high risk of acquiring malaria, as river flooding produces perfect breeding sites for mosquitoes. Despite the lack of treatments for malaria and references to disease symptoms in ancient Egyptian texts, some texts do note the presence of mosquitoes and the use of nets to avoid them (Strouhal 1992, Nunn 2001, Herodotus 2008). Symptoms including an enlarged spleen accompanied by fever are mentioned in the Papyrus Ebers (Ebbell 1937), but no clear description of malaria is given. In the vicinity of the Tiber, the discovery of a large Roman children’s cemetery, dating to 430 BC (Soren et al. 1995), suggests that an epidemic outbreak of malaria occurred, as falciparum malaria is known to cause a high rate of premature deliveries in non-immune pregnant women.

It is worth noting that malaria antigen detection tests are not as sensitive as microscopy. Although some researchers have been able to recover P. falciparum histidine-rich protein 2 using the ParaSight™-F test (Miller et al. 1994, Cerutti et al. 1999), some of these results were not reproducible in further investigations (Taylor et al. 1997). Subsequently, studies on living patients showed cross-reaction of the monoclonal IgG antibody used in this test with the rheumatoid factor in blood, resulting in false positive tests for malaria (Iqbal et al. 2000, Moody 2002).

The occurrence of malaria in the Americas has been subject to great debate among historians (for a review, see Bruce-Chwatt 1965). Those defending its pre-Columbian presence argue that there is linguistic evidence indicating the symptoms of the disease (Guerra 1964, cited in Bruce-Chwatt 1965, p. 378) and botanical evidence of the therapeutic use of cinchona bark (Jaramillo-Arango 1950, cited in Bruce-Chwatt 1965, p. 379). Nevertheless, the historical evidence for and against a pre-Columbian existence of malaria is controversial; there are no known references to the disease nor to the cinchona plant in the available written records from the Incas, Mayas or Aztecs. Moreover, for one or two generations after the first arrival of the Spaniards, there were no reports of diseases that might be considered to be ma-

| TABLE III |
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| Summary of studies on Trypanosoma cruzi in ancient human remains |

| Origin (archaeological site) | Period | Methods | Results (positive/total analysed) | References |
|---|---|---|---|---|
| Tarapaca Gully (Chile) | 2400-1600 BP | Paleopathology | 11/22 | Rothhammer et al. (1984) |
| Inca mummy (Peru) | 470 BC-600 AD | Paleopathology | 12/22 | Rothhammer et al. (1985) |
| Atacama mummies (Chile) | XV-XVI century AD | Immunohistochemistry and electron microscopy | 1/1 | Fornaciari et al. (1992) |
| Andean mummies | ~2000 BP-4000 AD | DNA | 9/27 | Guhl et al. (1997, 1999) |
| Andean mummies | ~2000 BP-1400 AD | DNA | 4/6 | Guhl et al. (2000) |
| Chihuahuan Desert (Texas, USA) | ~4000 BP | DNA | 1/1 | Naarden et al. (2001) |
| Chihuahuan Desert (Texas, USA) | ~1150 BP | DNA | 1/1 | Harkins et al. (2003) |
| Andean mummies | ~560 ± 40 BP | DNA | 7/7 | Fernandes et al. (2008) |
| AD: Anno Domini; BC: before Christ; BP: before present. | | | | |
laria, not even in localities that were later known to be associated with a high malaria burden (Ashburn 1947). Regarding the use of bark, it is believed that the native Indians of Peru would have transmitted their knowledge of its use to Jesuit missionaries after the Conquest, in 1527 (Bruce-Chwatt 1965). Recent phylogenetic analyses and Approximate Bayesian Computation methods suggest independent introductions of two clusters of \textit{P. falciparum} from African origins in South America, favouring multiple introductions from Africa during the transatlantic slave trade (Yalcindag et al. 2012).

**Leishmaniasis** - Leishmaniasis is a parasitic disease caused by protozoa of the genus \textit{Leishmania}. It is endemic in southern Europe, North Africa, the Middle East, Central and South America and India (Piscopo & Azzopardi 2007). Infections involving this parasite are regarded as cutaneous (CL), mucocutaneous (ML) or VL, which present different geographic distributions and clinical manifestations. More than 90% of all VL cases occur in India, Bangladesh, Nepal, Sudan, Ethiopia and Brazil, 90% of all CL is reported in Afghanistan, Algeria, Iran, Saudi Arabia, Syria, Brazil, Colombia, Peru and Bolivia and more than 90% of all cases of ML occur in Bolivia, Brazil, Ethiopia and Peru (WHO 2012).

The antiquity of leishmaniasis in the New World has been inferred from the existence of \textit{huacos} with facial mutilations, references from chroniclers of the Conquest and Colonial Period and the persistence of some quechua words that make allusions to the disease (Altamirano-Enciso 2000). The evidence of \textit{Leishmania} in the archaeological record is scarce. The presence of these parasites in the high-altitude Atacama Desert, where the disease is not normally found, suggests a pattern of mobility from endemic areas (Costa et al. 2009, Marsteller et al. 2011) dating to as early as 1000 BP (Fig. 4, Table V). An analogous situation was proposed for leishmaniasis in Egypt, where expeditions to Nubia (modern Sudan), currently a highly endemic country for VL (Zink et al. 2006), would explain the high incidence of \textit{Leishmania} DNA in the Middle Kingdom, as opposed to its absence in earlier or later periods. \textit{Leishmania infantum} was recently identified in Eleanor of Toledo (1522-1562), a Spanish noble woman and wife of Cosimo I de’Medici and in mummies from the Brazilian Colonial Period, 1530-1815 (ongoing research), which is in accordance with studies confirming the recent importation of this parasite into the New World from southwest Europe (Kuhls et al. 2011).

**Enteric protozoa** - Paleoparasitological evidence of protozoans is scarce. Because their cysts and oocysts are fragile microstructures compared to helminth eggs, the identification of these organisms from archaeological remains via optical microscopy has been infrequent. The application of ELISA greatly improved the detection of protozoa infections in coprolites and latrine soils in the Americas and Europe (Table VI) and Gonçalves et al. (2002) concluded that the sensitivity of this technique was greater than that offered by microscopy for diagnosing \textit{G. duodenalis}. \textit{Cryptosporidium} spp and \textit{G. duodenalis} have been identified based on immunofluorescence analysis in archaeological remains in Peru, dating to as early as 4300 BP (Ortega & Bonavia 2003), while in Europe, Le Bailly et al. (2008) identified \textit{G. duodenalis} in samples from medieval times using immunofluorescence and ELISA. More recently, the detection of \textit{G. duodenalis} and \textit{Entamoeba histolytica} in archaeological samples from the Middle East has confirmed written evidence of the occurrence of infective diarrhoea in the Crusader period (Mitchell et al. 2008).

The case of \textit{Toxoplasma gondii} - \textit{T. gondii} is a widespread zoonotic protozoan that infects most species of mammals, birds, fish, amphibians and reptiles. To detect this parasite in ancient remains, one of the following scenarios must occur. In the first scenario, the infective stage of the parasite (oocysts) must be found in the co-

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![Fig. 3: paleodistribution of \textit{Plasmodium falciparum} studies in humans (white spots). Grey area approximately represents the current geographic distribution of the disease (CDC 2012).](image-url)
TABLE IV

Summary of studies on Plasmodium falciparum in ancient human remains

| Origin (archaeological site) | Period | Methods | Results (positive/total analysed) | References |
|-----------------------------|--------|---------|----------------------------------|------------|
| Arab-Persian Gulf           | Hellenistic | Electronic microscopy | NI | Maat & Baig (1990) |
|                             | 5200-1450 BC | Immunoenzymatic assay | 7/18 | Miller et al. (1994) |
| Egyptian and Nubian mummies | 700 BC | DNA | 0/1 | Taylor et al. (1997) |
| Granville mummy (Kurna)    | 3200 BC | DNA | 34/80 | Cerutti et al. (1999), Rabino Massa et al. (2000) |
| Egyptian mummies (Assiut-Gebelein) | 3200 BC | DNA | NI | Zink et al. (2001) |
| Egyptian mummies (Abydos/Thebes) | 3500-500 BC | DNA | 2/91 | Nerlich et al. (2008) |
| Lugnano, Teverina (Italy)  | V century AD | DNA | 1/1 | Abbott (2001), Sallares & Gomzi (2001) |
| Egyptian mummy (Gebelein)  | 5200-1450 AD | Immunoenzymatic assay | 1/1 | Bianucci et al. (2008) |
| Egyptian mummy (Abydos/Thbes) | 1515-1567 AD | Immunoenzymatic assay | 2/2 | Formaciari et al. (2010a, b) |
| Medici family (Italy)      | XVI century AD | Immunoenzymatic assay | 4/6 | Fornaciari et al. (2010b) |
| Egyptian mummies           | 1550-1324 BC | DNA | 4/16 | Hawass et al. (2010) |

AD: Anno Domini; BC: before Christ; BP: before present; NI: not informed.

Toxoplasma has not yet been detected in ancient remains, although successful recovery of its DNA has been accomplished from desiccated mouse tissue (Terra et al. 2004). Although methodological difficulties must be considered, the worldwide dispersion of the infection today suggests the possibility of finding the parasite through systematic examinations of mummies and archaeological remains.

The ecology of infectious diseases in humans entails more than the risk of acquiring an infection. It also involves the likelihood of exposure, the conditions of establishment and favourable circumstances that lead to successful transmission. While adapting to harsh environments, human populations have become part of various parasitic life cycles. For malaria, proximity to marshy areas favours the incidence of disease, as seen in the Nile Delta (Rabino Massa et al. 2000) and the fringes of the Tiber valley (Sallares & Gomzi 2001). Additionally, members of the Medici family are known to have hunted in areas of Tuscany endemic for malaria (Fornaciari et al. 2010a, b). Chagas disease is thought to have originated from a human intrusion into the T. cruzi syl-
### TABLE V
Summary of studies on *Leishmania* spp in ancient human remains

| Protozoan        | Origin (archaeological site) | Period       | Methods         | Results (positive/total analysed) | References                        |
|------------------|------------------------------|--------------|-----------------|----------------------------------|-----------------------------------|
| *Leishmania* spp | Makat-tampu (Peru)           | Inca         | Paleopathology  | 5/241                            | Altamirano-Enciso (2000)           |
|                  | Peru                         | 800 BC       | Immunohistology | NI                               | Guillen & Allison (2005)           |
|                  | Coyo Oriente (Atacama)       | 1000-500 BP  | Paleopathology  | 4/255                            | Costa et al. (2009)                |
| *Leishmania* donovani | Egyptian mummies (Abydos/Thebes) | 3500 BC-500 BC | DNA             | 4/91                             | Zink et al. (2006)                 |
|                  | Nubian mummies (Kulubnarti)  | 1500-550 AD  | NI              | 9/70                             |                                   |
| *Leishmania* infantum | Eleonora from Toledo (Italy) | 1522-1562 AD | DNA             | 1/1                              | Nerlich et al. (2012)              |

AD: Anno Domini; BC: before Christ; BP: before present; NI: not informed.

### TABLE VI
Summary of studies on enteric protozoa in ancient human remains

| Protozoan         | Origin (archaeological site) | Period       | Methods         | Results (positive/total analysed) | References                       |
|-------------------|------------------------------|--------------|-----------------|----------------------------------|----------------------------------|
| *Giardia* duodenalis | Nahal-Mishmar (Israel)       | 160 AD       | Microscopy      | NI/2                             | Witenberg (1961)                 |
|                   | Big Bone Cave, Tennessee (USA) | 2177 ± 145 BP | IFA             | NI/8                             | Faulkner et al. (1989)           |
|                   | Pre-Columbian mummies (Andes) | 3000-500 BP  | ELISA           | 7/20                             | Allison et al. (1999)            |
|                   | Antelope House, Arizona (USA) | 1200-1300 AD | ELISA           | 3/83                             | Gonçalves et al. (2002)          |
|                   | Lübeck (Germany)             | 1500-1600 AD |                 |                                  |                                   |
|                   | Namur (Belgium)              | XVIII century AD |               |                                  |                                   |
|                   | Los Gavilanes (Peru)         | 2375-1525 BC | IFA             | 1/18                             | Ortega & Bonavia (2003)           |
|                   | Manache (Peru)               | 500-900 AD   |                 | 1/2                              |                                   |
|                   | Chevennez (Switzerland)      | VII-IX century AD | ELISA | 5/5                             | Le Bailly (2005)                 |
|                   | La Mothe (France)            | X-XI century AD | ELISA, IFA      | 1/9                              | Le Bailly et al. (2008)          |
|                   | Acre (Israel)                | XIII century AD | ELISA         | 1/8                              | Mitchell et al. (2008)           |
| Protozoan          | Origin (archaeological site) | Period          | Methods              | Results (positive/total analysed) | References                      |
|-------------------|-------------------------------|-----------------|----------------------|-----------------------------------|---------------------------------|
| **Entamoeba spp** | El Plomo (Chile)              | Pre-Columbian   | Microscopy           | 1/1                               | Pizzi & Schenone (1954)         |
|                   | Nahal-Mishmar (Israel)        | 160 AD          | Microscopy           | NI/2                              | Witenberg (1961)                |
|                   | Huari (Peru)                  | Pre-Columbian   | Microscopy, ELISA    | 2/7, 0/3                          | Fouant et al. (1982)            |
|                   | Alto Ramirez (Chile)          |                 |                      | 2/11, 0/9                         |                                 |
|                   | Atacama (Chile)               |                 |                      | 3/26, 0/21                        |                                 |
|                   | Cabuza (Chile)                |                 |                      | 3/29, 0/20                        |                                 |
|                   | Tihuanaco (Chile)             |                 |                      | 1.5, 0.5                          |                                 |
|                   | Fortin Minana (Argentina)     | XIX century AD  | ELISA                | 9/11                              | Gonçalves et al. (2004)         |
|                   | Namur (Belgium)               | XIV-XVIII century AD | ELISA             | 2/12                              |                                 |
|                   | Castillon-du-Gard (France)    | III century AD  | ELISA                | 2/14                              |                                 |
|                   | Gresine (France)              | 2500 BP         | ELISA                | 1/5                               |                                 |
|                   | Arbon (Switzerland)           | 5300 BP         | ELISA                | 3/5                               |                                 |
|                   | Canyon De Chelly (USA)        | 800-700 BP      | ELISA                | 3/17                              |                                 |
|                   | Hornstaad-Hörnle I, Stockwiesen, Torwiesen II, Taschenwiese, Gründwiesen (Germany) | 3900-2500 BC | ELISA                | 0/30                              | Le Bailly & Bouchet (2006)      |
|                   | Arbon-Bleiche 3, Chevenez (Switzerland) | 3400 BC-IX century AD | ELISA             | 5/11                              |                                 |
|                   | Chalain, Lattes, Pineuilh, Épinal (France) | 3200 BC-XVII century AD | ELISA             | 4/23                              |                                 |
|                   | Vilnius (Lithuania)           | XIX century AD  | ELISA                | 0/6                               |                                 |
|                   | Kouphovouno (Greece)          | 5000-2000 BC    | ELISA                | 5/5                               |                                 |
|                   | Alexandria, Saqqarah (Egypt)  | 715 BC-VII century AD | ELISA             | 0/11                              |                                 |
|                   | Sai (Nubia)                   | 275 BC-350 AD   | ELISA                | 0/3                               |                                 |
|                   | Shilourokambos (Cyprus)       | 7500-7000 BC    | ELISA                | 0/3                               |                                 |
|                   | Qumram (Israel)               | 100 BC          | ELISA                | 0/2                               |                                 |
|                   | Meadowlark (USA)              | XIX century AD  | ELISA                | 3/5                               | Le Bailly & Bouchet (2006),     |
|                   | Acre (Israel)                 | XIII century AD | ELISA                | 6/8                               | Mitchell et al. (2008)          |
|                   | Chilomastix mesnili           |                 |                      |                                   |                                 |
|                   | Nahal-Mishmar (Israel)        | 160 AD          | Microscopy           | NI/2                              | Witenberg (1961)                |
|                   | Isospora beli                 |                 |                      |                                   |                                 |
|                   | Cyclospora cayetanensis       |                 |                      |                                   |                                 |
|                   | Sarcocystis hominis           |                 |                      |                                   |                                 |

AD: Anno Domini; BC: before Christ; BP: before present; ELISA: enzyme-linked immunosorbent assay; IFA: indirect fluorescent antibody test; NI: not informed.
vatic cycle, gradually transitioning into a domestic cycle (Aufferheide et al. 2004, Araújo et al. 2009, Ferreira et al. 2011) and leishmaniasis would have increased in the New World due to travel to endemic zones or migration from such areas (Costa et al. 2009).

The probability of detecting parasites is sometimes enhanced by the methodology applied. Studies on enteric protozoa have increased with the availability of commercial kits that facilitate the processing of a large number of samples simultaneously. Molecular biological techniques offer a more sensitive method to retrieve information from archaeological contexts and even though limitations associated with ancient DNA must be considered (such as sample preservation, age and contamination), examination of enteric protozoa using these means would offer an interesting perspective on the zoonotic potential of Giardia spp and Cryptosporidium spp in the archaeological record, an emphasis that has not yet been explored in the literature.

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