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 paleoparasi-

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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 ivitaensis* have been detected in mummified camelids from Peru (Leguía et al. 1995, Leguía 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 mummified human remains, which have shown preservation varying from excellent to very poor (Lyn-

Fig. 1: paleodistribution of enteric protozoa (white spots) and blood protozoa (black spots) in humans.

TABLE I

Summary of studies on protozoa in extinct animals[a]

Protozoan	Origin (paleontological/archaeological site)	Period	Host	References
Archeococcidia antiquus sp. nov. *Archeococcidia nothrotheriopsae* sp. nov.	Rampart Cave, Grand Canyon (Arizona, USA)	10500 ± 180 BP	Shasta ground sloth (*Nothrotheriops shastensis*)	Schmidt et al. (1992)
Paleoleishmania proterus gen. nov., sp. nov.	Burmese amber	Early Cretaceous	Sandfly	Poinar & Poinar (2004)
Trypanosoma antiquus sp. nov.	La Toca amber mine (Dominican Republic)	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 (*Kalotermes burmensis* sp. nov.)	Poinar (2009)
Trichomonas gallinae-like protozoan	North America	Latest Maastrichtian	Tyrannosaurids	Wolff et al. (2009)
Eimeria lobatoi	Perna I, São Raimundo Nonato (Piauí, Brazil)	9000 BP	Deer	Ferreira et al. (1992)
Eimeria macusaniensis, *Eimeria ivitaensis*	Peru	1000 BP	Camelids (*Lama glama*, *Lama alpaca)*	Leguía 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, Aufderheide 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 (Aufderheide 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

Blood protozoa - Chagas disease in the pre-Columbian Americas - Trypanosoma cruzi, the causative agent of American trypanosomiasis, or Chagas disease, is transmitted through the faecal droppings of infected vectors from the subfamily Triatominae. *T. cruzi* is geographically restricted to the Americas and occurs primarily in Latin America, where it is endemic (Moncayo & Silveira 2009). Its paleodistribution was also constrained to the New World, comprising the Andean area, a small region in the Brazilian savannah and part of the Chihuahuan Desert in North America (Fig. 2, Table III).

Descriptions of cases of *T. cruzi* infections in the past are relatively abundant in the literature. The identification of amastigote nests in cardiac fibres from a Peruvian mummy (Fornaciari et al. 1992) and visceral lesions in Chilean mummies (Rothhammer et al. 1985) confirms the occurrence of both the infection and disease in pre-Columbian times. Humans were infected early in their history and were likely infected in various ways, depending on how they interacted with their environment. The existence of Chagas disease in pre-Columbian populations predates sedentism and domestication by several thousand years, suggesting other means of initial contagion. Some authors propose that accidental infection of humans occurred due to contact with natural *T. cruzi* foci (Guhl et al. 2000) and that human dwellings and domestication would have subsequently facilitated its establishment in domestic settings (Aufderheide et al. 2004). Various alternatives have been put forth regarding how this would have happened. The ingestion of raw infected meat was suggested as a potential route of infection by Neghme (1982), but archaeological evidence was not provided until almost 20 years later, when Reinhard et al. (2003) reported finding unburned bones and hair from woodrats in coprolites from an area where a case of Chagas disease was described and later molecularly confirmed (Dittmar et al. 2003). The occupation of caves and rock shelters, before dwellings were intro-

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).

TABLE III

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)
	470 BC-600 AD	Paleopathology	12/22	Rothhammer et al. (1985)
Inca mummy (Peru)	XV-XVI century AD	Immunohistochemistry and electron microscopy	1/1	Fornaciari et al. (1992)
Atacama mummies (Chile)	2000 BC-200 BP	DNA	9/27	Guhl et al. (1997, 1999)
	4000 BP	DNA	11/31	Guhl et al. (2000)
	2000 BP-1400 AD	DNA	4/6	Ferreira et al. (2000)
Andean mummies	~4000 BP	DNA	25/27 fragments	Madden et al. (2001)
Chihuahuan Desert (Texas, USA)	1150 BP	Paleopathology	1/1	Reinhard et al. (2003)
		DNA	1/1	Dittmar et al. (2003)
Northern Chile, southern Peru	~9000 BP-450 BP	DNA	115/283	Aufderheide et al. (2004)
Pre-Columbian mummies (Bolivia)	3600-900 BP	DNA	11/29	Orellana (2008)
Peruaçu Valley (Minas Gerais, Brazil)	7000-4500 BP	DNA	1/1	Lima et al. (2008)
	560 ± 40 BP	DNA	7/7	Fernandes et al. (2008)

AD: Anno Domini; BC: before Christ; BP: before present.

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-

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 *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 *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 *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 *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 *Leishmania* DNA in the Middle Kingdom, as opposed to its absence in earlier or later periods. *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 *G. duodenalis*. *Cryptosporidium* spp and *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 *G. duodenalis* in samples from medieval times using immunofluorescence and ELISA. More recently, the detection of *G. duodenalis* and *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 Toxoplasma gondii - *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-

Fig. 3: paleodistribution of *Plasmodium falciparum* studies in humans (white spots). Grey area approximately represents the current geographic distribution of the disease (CDC 2012).

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)
Egyptian and Nubian mummies	5200-1450 BP	Immunoenzymatic assay	7/18	Miller et al. (1994)
Granville mummy (Kurna)	700 BC	DNA	0/1	Taylor et al. (1997)
Egyptian mummies (Assiut-Gebelein)	3200 BC	Immunoenzymatic assay	34/80	Cerutti et al. (1999), Rabino Massa et al. (2000)
Egyptian mummies	1800-1400 AD, 1500-500 BC	DNA	NI	Zink et al. (2001)
Lugnano, Teverina (Italy)	V century AD	DNA	1/5	Abbott (2001), Sallares & Gomzi (2001), Sallares et al. (2004)
Egyptian mummy (Gebelein)	2820-2630 BC	Immunoenzymatic assay	1/1	Bianucci et al. (2008)
Egyptian mummies (Abydos/Thebes)	3500-500 BC	DNA	2/91	Nerlich et al. (2008)
Francesco I of Medici (Italy)	1531-1587 AD	Immunoenzymatic assay	2/2	Fornaciari et al. (2010a)
Medici family (Italy)	XVI century AD	Immunoenzymatic assay	4/6	Fornaciari et al. (2010b)
Ancient Egyptian mummies	1550-1324 BC	DNA	4/16	Hawass et al. (2010)

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

prolites of felids, as they are the only definitive host for *T. gondii*. In the second scenario, encysted forms of the parasite (bradyzoites) have to be retrieved from various tissues of the body, either from intermediary hosts (animals, including humans) or infected cats. The complex life cycle of *T. gondii* limits the potential for its identification in coprolites because, although its oocysts are shed in the faeces of adult cats in some cases, oocyst excretion usually occurs only in young felids, which are less immunocompetent (Dubey et al. 1977, Dubey 1995). *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-

Fig. 4: paleodistribution of *Leishmania* spp studies in humans (white spots). Grey areas approximately represent the current distribution of visceral leishmaniasis (dark grey) and cutaneous-mucocutaneous (light grey) in the New World.

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)
			DNA	3/4	
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)
			protein assay	1/1	

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	IFA	7/20	Allison et al. (1999)
			ELISA	2/7	
	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)

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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 Ramírez (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	
	Shillourokambos (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), Le Bailly et al. (2006)
	Acre (Israel)	XIII century AD	ELISA	6/8	Mitchell et al. (2008)
Cryptosporidium parvum	Andes (Chile-Peru)	3000-500 BP	ELISA	8/15	Allison et al. (1999)
	PV35-4 (Peru)	770-830 AD	IFA	1/2	Ortega & Bonavia (2003)
	Acre (Israel)	XIII century AD	ELISA	0/8	Mitchell et al. (2008)
	La Mothe (France)	X-XI century AD	IFA	0/9	Le Bailly et al. (2008)
Chilomastix mesnili	Nahal-Mishmar (Israel)	160 AD	Microscopy	NI/2	Witenberg (1961)
Isospora beli	Andes (Chile-Peru)	3000-500 BP	IFA	16/20	Allison et al. (1999)
Cyclospora cayetanensis			IFA	2/20	
Sarcocystis hominis			IFA	1/20	

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 (Aufderheide 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 methods 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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