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Review

Archaeological occurrences and historical review of the human amoeba, *Entamoeba histolytica*, over the past 6000 years

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ABSTRACT

Understanding parasite history and the evolution of host/parasite relationships is one of the most important aspects of paleoparasitology. Within the framework of this research topic, this paper focuses on the human pathogenic amoeba, *Entamoeba histolytica*. The compilation of all the available archaeological data concerning this parasite leads to a first glimpse of the history of this parasite of current medical importance. Paleoparasitological investigation into this parasite uses immunological techniques and shows that the modern strain of *E. histolytica* has been present in Western Europe since at least the Neolithic period (3700 years BCE), and could have originated in the Old World. The appearance of the modern amoeba strain in the pre-Columbian Americas and the Middle East around the 12th century CE gives rise to hypotheses as to how human migrations (Atlantic or Pacific routes) contributed to the diffusion of this pathogen, resulting in its current distribution. This compilation proves that parasites are valuable proxies for studying past human and animal migrations, and should be given more consideration in the future.

Contents

1. Introduction

For more than a century, data have been collecting related to ancient parasites, including arthropods, helminths and protozoans. This research field, named paleoparasitology, is a branch of parasitology combining archaeology, anthropology, biology, and health sciences, and is based on the study of samples taken from archaeological and paleontological excavations (Bouchet et al., 2003a; Dittmar et al., 2012). Since the early stages of this research, advances in sample diversity, extraction methods, identification tools, and the treatment of data have led to broadening the sphere of pathogen detection from humans and animals (Bouchet et al., 2003b; Goncalves et al., 2003; Sianto et al., 2009; Frias et al., 2013). Many ancient parasite occurrences have been recorded from periods spanning the past ten millennia and it is possible to propose a reconstruction of parasite history. This is already the case for some human gastrointestinal helminths, such as the pinworm (Araujo and Ferreira, 1995; Araujo et al., 2008, 2015), the hookworm (Araujo et al., 1988, 2008, 2015; Ferreira and Araújo, 1996), and animal-linked helminths, such as the lancet liver fluke (Le Bailly and Bouchet, 2010), or the horse pinworm (Dufour et al., 2015). Understanding the history of parasites and the evolution of the host/parasite relationship presents major challenges for paleoparasitologists (Araujo et al., 2013; Mitchell, 2013), and concurs with the challenges of other related specialities as paleomicrobiology or paleopathology (Drancourt and Raoult, 2005, 2008; Dutour, 2013).

Immunology was introduced into paleoparasitological analyses in the 1980s to test the presence of protozoans of medical importance in

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ancient samples (Fouant et al., 1982; Faulkner et al., 1989). As oocysts are very fragile and do not resist well to natural degradation processes (taphonomy), protozoans cannot be retrieved by conventional microscopic analyses, except in extremely good preservation conditions (Goncalves et al., 2002). Among these protozoans, the human pathogenic amoeba, *Entamoeba histolytica*, has been detected several times during the past twenty years. This article presents a review of the past occurrences of this parasite, and discusses hypotheses explaining its past and present distribution.

2. The human pathogenic amoeba

E. histolytica is an invasive intestinal pathogenic protozoa (Eukaryota, Amoebozoa, Archamoeba) parasitizing humans, and in lesser proportions, animals such as primates, cats, dogs and some rodents (Acha and Szyfres, 2005; Singh et al., 2009). This parasite infects five hundred million people and kills about one hundred thousand humans per year, making it one of the most important causes of parasite morbidity and mortality in the world (Baxt and Singh, 2008). The diagnosis of amoebiasis in present-day medicine can be made on patient stool or blood samples, with light microscopy, but more commonly immunological (Haque et al., 1995, 1998) or molecular biology techniques (Beck et al., 2002; Roy et al., 2005) are used due to their ability to differentiate pathogenic forms of *Entamoeba* from the harmless species that appear similar under microscopy.

In ancient samples, the detection of *E. histolytica* is also based on the recovery of parasite markers from coprolites (preserved ancient faeces) or sediment samples taken from the pelvic region of skeletons, cesspits, latrines and all contexts potentially containing human faecal matter. Microscopy were successfully used in very rare cases with exceptionally good preservation conditions whereas immunology, particularly Enzyme Linked ImmunoSorbent Assays (ELISA), has yielded positive results for the detection of preserved specific antigens of the parasite. Most of the analyses conducted by paleoparasitology units in the world to detect ancient *E. histolytica* use the "*Entamoeba histolytica* II" test, commercialized by TechLab (Blacksburg, Virginia, US). This test can detect a specific epitope of an adhesin (N-acetyl-D-galactosamine-binding lectine) produced by pathogenic *E. histolytica* (Haque et al., 2000; Mirelman et al., 1997). It has good sensitivity and specificity, and presents no cross-reactivity with the non-pathogenic species *Entamoeba dispar* or *Entamoeba moshkovskii*, or with other related microorganisms, such as *Giardia intestinalis* or *Entamoeba coli* (Furrows et al., 2004).

3. Review of past records of *E. histolytica*

Published mentions of amoeba recoveries are relatively scant. Only nine publications, one book chapter and one doctoral thesis are known to date to record the discovery of *E. histolytica* or *Entamoeba* sp. traces, using immunology and, to a lesser extent, microscopy. In this paragraph, we present an overview of these occurrences, classified by order of publication. Details of the mentions of the human pathogenic amoeba classified by date or chronological period are given in Table 1. For all the dates, the term CE (Common Era) or BCE (Before Common Era) is used.

Pizzi and Schenone (1954) were the first to observe *Entamoeba* sp. cysts in archaeological samples (possibly *E. coli* according to the authors' hypotheses), whereas Witenberg (1961) was the first to attest the presence of *E. histolytica* in ancient samples. He found amoeba cysts using light microscopy in two human coprolites from a Judean Desert cave in Nahal Mishmar, dated from the Bar-Kokhba period (132–135 CE). However, no image was presented in the paper to illustrate this diagnosis. It is important to recall here that the pathogenic *E. histolytica* and the commensal, harmless *E. dispar* and *E. moshkovskii* appear identical under the microscope.

Fouant et al. (1982) analysed eighty coprolite samples from Chilean and Peruvian pre-Columbian mummies. The authors identified *Entamoeba* sp. cysts in ten samples using microscopy. In a second stage, the authors then used *E. histolytica*-specific ELISA tests to confirm the amoeba species. Immunological tests were all negative for *E. histolytica*-specific antigens. Consequently, the authors raised the possibility that the cysts belonged to a different species, or that cyst antigenicity was lost over time. This paper relates the first use of immunology in paleoparasitological research and was followed by a twenty-two year time lapse before a second article attempted to detect this pathogen in archaeological samples.

Goncalves et al. (2004) published a collective research work on *E. histolytica* carried out by three paleoparasitology laboratories in Brazil, the USA and France. ELISA was used to test ninety samples covering a wide range of dates (radiocarbon dates or cultural contexts extending from 7000 BCE to the 19th century CE) and geographic locations in the Americas (52% of the samples), Europe (42% of the samples), and Africa (6% of the samples). Among these samples, twenty were positive for *E. histolytica* antigens. The oldest positive results were found in a Neolithic site from Switzerland dating to around 3400 years BCE.

During a PhD thesis conducted between 2002 and 2005, a hundred and two samples were tested, collected from nineteen archaeological sites of different dates and geographic locations. Among these samples, ELISA-specific tests revealed the presence of amoeba antigens in seventeen samples. The earliest positive records were found in samples collected from a Greek site dating from between 5000 and 2000 BCE (Le Bailly, 2005, 2011; Le Bailly and Bouchet, 2006).

Mitchell et al. (2008) identified *E. histolytica* using ELISA in a set of soil sediment samples from a latrine in the Crusader Acre (Israel). The latrine, dated to the 13th century CE, was located in a hospital used by European crusaders belonging to the military order of St. John. Another set of samples from a large communal latrine outside the hospital tested negative for the dysentery agent.

Yeh et al. (2014) also identified *E. histolytica* using immunology (ELISA) in a sediment sample from a medieval latrine recovered from the historical center of Riga (Latvia). In the same sample, dated to 1356 CE by dendrochronology, the authors also used light microscopy and observed cysts attributed to the human pathogenic amoeba.

Le Bailly et al. (2014) revealed evidence of *E. histolytica* by using immunology in two cemeteries from the colonial period in the Caribbean (Guadeloupe). Around 17% (16.6 %) of the samples ($n = 48$) dated to the colonial period tested positive for the *E. histolytica* antigen. During this analysis, one sample dated to the pre-colonial period also tested positive for the amoeba, thereby confirming the presence of the parasite before the arrival of Europeans during the 15th century CE.

Le Bailly and Bouchet (2015) reviewed early occurrences and used ELISA to test materials from archaeological sites in Africa (Egypt, Sudan), Asia (China, Israel, Kazakhstan, and Lebanon), the Pacific (Samoa) and South America (Chile). Fifteen samples tested positive from seven archaeological sites in France, Belgium, Italy, and Switzerland. One Swiss sample from the archaeological site of Concise (Lake Neufchatel), dated to the Neolithic period (3700 years BCE), was positive for *E. histolytica*-specific antigens, thus confirming the presence of the parasite in prehistoric Switzerland.

Finally, Yeh et al. (2015) used ELISA to test the presence of *E. histolytica* in samples from a cesspool excavated in medieval Jerusalem. One coprolite sample tested positive for the parasite, dated to 1304–1413 CE (Mamluk period).

4. Immunology versus microscopy in light of ancient protozoan detection

The use of immunology for the detection of ancient protozoans offers many advantages for interpreting results. In the present cases, the tests used exclusively detect pathogenic *E. histolytica*,