J. Biol. Res. 2005 n. I - Vol. LXXX - Rubbettino - Soveria Mannelli

Protozoan Infection in Archaeological Material

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KEY WORDS: paleoparasitology, coprolites, mummies, paleopathology, diseases in the past

Abstract

The identification of parasites in ancient human feces is compromised by differential preservation of identifiable parasite structures. However, protein molecules can survive the damage of the environment. It was possible to detected antigen of Entamoeba histolytica and Giardia duodenalis in historic and prehistoric human fecal remains using two enzyme immunoassay (ELISA) kits with monoclonal antibodies specific for E. histolytica and G. duodenalis, respectively. Specimens of desiccated feces and ancient latrine sediment from the New and the Old World were examined. The ELISA detected E. histolytica antigen in samples from Argentina, USA, France, Belgium, and Switzerland, dated to about 5300 years BP to the 19th Century AD. G. duodenalis antigen was detected in samples from USA, Belgium, and Germany, dated to about 1200 AD, 1600 AD, and 1700 AD. The detection of protozoan antigen using immunoassays is a reliable tool for the study of intestinal parasites in the past.

Introduction

Protozoan diagnosis is not easily achieved in paleoparasitology. Intestinal protozoan cysts are not well preserved in archaeological material. Therefore, they are rarely found (Witenberg, 1961; Reinhard et al., 1986; Faulkner et Al., 1989; Ferreira et al., 1992; Gonçalves et al., 2003). There have been many attempts to detect protozoan cysts in coprolites and archaeological sediments, but only recently new techniques proved to be effective (Gonçalves et al., 2002; Gonçalves et al., 2004). Microscopic examination is not effective, and up to now immunological tests have been showing cross-reactions with dubious results (Fouant et al., 1982; Allison et al., 1999;). Molecular biology techniques applied to paleoparasitological research opened new possibilities in the field. Monoclonal antibodies immunoassays have been tried in the same way.

Monoclonal antibodies are prepared in the laboratory, and they are very specific. Monoclonal antibodies can detect specific antigens. Enzyme-linked immunosorbent assay (ELISA), using monoclonal antibodies, is a quite simple reaction and can be done in the laboratory with a minimum of specialized equipment.

The ELISA principle is the link of the antigen with the specific monoclonal antibody, and then with the enzyme-linked polyclonal antibody, which is marked and results in a yellow-color reaction that can be seen by visual inspection (but preferable under a spectrophotometer). Negative reaction results in no color.

First we applied a commercially available ELISA kit to diagnose *Giardia duodenalis* infection in mummies, coprolites and latrine sediment from archaeological sites. Positive results were obtained for three archaeological sites, including one where *Giardia* cysts were also found in microscopic analysis (Gonçalves et al., 2002). Following this experiment, we applied a commercially ELISA kit to *Entamoeba histolytica* diagnosis in the same samples, as well as to other samples sent to our Laboratory of Paleoparasitology at Oswaldo Cruz Foundation, Brazil. The ELISA detected 20 positive samples (Gonçalves et al., 2004).

These results point to interesting possibilities for monoclonal tests. The detection of protozoan antigen using immunoassays is a reliable tool for the studies of intestinal parasites in the past.

We are currently applying the monoclonal tests to detect *E. histolytica* infection among South American Indian tribes. We aim to compare ancient with modern sequences prevalent among ancient American inhabitants.

Material and methods

We tested coprolites and sediments from different archaeological sites of the Old and New World, dated from 400,000 years in Europe up to American Colonial times

(Tabs. 1-2). Coprolites and sediments were rehydrated in 0.5% aqueous solution of trisodium phosphate for 72 hours, as recommended by Callen and Cameron (1960).

G. duodenalis assay:

GiardiaThe ProSpecT *Giardia* Microplate assay (Alexon, Inc., Sunnyvalle, CA, USA), a monoclonal antibody assay, was performed on each sample according to the manufacturer's directions. It detects the GSA65 *G. duodenalis*-specific antigen.

Country	Archaeological samples
Brazil	42 coprolites, dated from 5,230 BC to 1,730 AD
Chile	28 coprolites, dated from 4,100 BC to 800 AD
USA	5 coprolites, dated from 1,200 AD to 1,300 AD
Germany	3 cesspit soil samples, dated from 1,500 AD to 1,600 AD
Argentina	2 coprolites, pre-Colombian time
France	1 coprolite, dated to about 400,000 BC
	1 cesspit soil sample, dated to about 1,400 AD
Belgium	1 cesspit soil sample, dated to the 18th Century

Table 1 - Country of the archaeological site, type and age of archaeological sample examined for Giardia duodenalis BC: Before Christ / AD: Anno Domini.

Country	**Archaeological samples**
Brazil	9 coprolites, dated from 8,760 BP to 2,000 BP
Chile	8 coprolites, dated from 6,110 BP to 2,950 BP
USA	17 coprolites, dated from 700 BP to 1,100 BP
Germany	1 cesspit soil sample, dated to the 15th Century AD
Argentina	11 cesspit soil samples, dated to the 19th Century AD
	2 coprolites, from pre-Colombian time
France	15 cesspit soil samples, dated from the 3rd Century AD to the 16th Century AD
	5 sediment samples, dated to 2,500 BP
Sudan	5 coprolites, from 2,700 BP to 500 BP
Switzerland	5 sediment samples, dated to 5,300 BP
Belgium	12 cesspit soil samples, dated from the 9th to the 18th Century AD

Table 2 - Country of the archaeological site, type and age of archaeological sample examined for Entamoeba histolytica antigen BP: Before Present / AD: Anno Domini.

E.histolytica assay:

The *E. histolytica* II assay (TechLab, Blackburg, VA, USA) was used according the manufacturer´s directions. It detects an amoebic adherence lectin.

Results

The *G. duodenalis* kit detected positive results in 3 archaeological samples. A positive sample from a cesspit of Anasazi culture, Canyon De Chelly (USA), dated to about 1200 *Anno Domini* (AD), and samples from a medieval latrine in Lubeck (Germany) and Namur (Belgium), dated to the 17th and 18th Century respectively.
The *E. histolytica* kit detected a total of 20 positive samples. In Europe, the kit detected positive samples from cesspits in a roman villa, at Castillon-du-Gard (France), occupied from the Roman Period until the High Medieval Period, and samples from medieval latrines in Namur (Belgium). *E. histolytica* antigen was also found in organic sediment of a Neolithic settlement close to Lake Constance (Switzerland), and from a Bronze Age settlement in Gresine (France). In the New World, we detected positive samples in cesspits from Fortin Minana (Argentina), a military settlement from the 19th century and from cesspits of Anasazi culture, Canyon De Chelly (USA), dated from 1200 to 1300 AD.

Discussion

The kit for *G. duodenalis* assay utilized monoclonal antibody against GSA65 antigen, a glycoprotein. It is stable and is secreted in large amounts by encysting trophozoites (Rosoff & Stibbs, 1986). According to Aldeen et al. (1998), the sensitivity and specificity of the assay is 100% in fresh feces. According to our results, this antigen survives the destruction of cysts and trophozoites. It was shown to be present for at least 800 years in human remains.
The kit for *E. histolytica* utilized monoclonal antibody against *E. histolytica* lectin. The sensibility and the specificity of the method are very high (Haque et al., 2000; Sharp et al., 2001), from 96.9% to 100% and 94.7% to 100%, respectively, when compared with culture of stool samples and isoenzyme analysis, according to the manufacturer. The protein epitopes from the *E. histolytica* lectin can remain stable for at least 5,300 years, according to our findings from Switzerland.
Although very common parasites worldwide, the finding of *E. histolytica* and *G. duodenalis* cysts in ancient human feces are very rare, probably due to a faster decay than helminth eggs and larvae. Caution should be exerted in the interpretation of negative results in ancient samples as antigen structure may be compromised due to uncontrolled environment conditions.
Results obtained proved that monoclonal tests could be applied to detect protozoan infections in ancient populations, and that protein sequences are preserved

through times. The utility of this technique, combined with a new generation of molecular methods, will reveal the temporal and spatial distribution of protozoan parasites in ancient times.

Supported by CNPq; CAPES/COFECUB; Fulbright Commission; CNRS

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