Karl Reinhard Papers/Publications   Natural Resources, School of

2002

Detection of *Giardia duodenalis* antigen in coprolites using a commercially available enzyme-linked immunosorbent assay

Marcelo Luiz Carvalho Gonçalves
Escola Nacional de Saúde Pública, Fundação Oswaldo Cruz, Rio de Janeiro, Brazil

Adauto Araújo
Fundação Oswaldo Cruz, adauto@ensp.fiocruz.br

Rosemere Duarte
Escola Nacional de Saúde Pública, Fundação Oswaldo Cruz, Rio de Janeiro, Brazil

Joaquim Pereira da Silva
Escola Nacional de Saúde Pública, Fundação Oswaldo Cruz, Rio de Janeiro, Brazil

Karl Reinhard
University of Nebraska-Lincoln, kreinhard1@mac.com

See next page for additional authors
Follow this and additional works at: http://digitalcommons.unl.edu/natresreinhard

 Part of the Archaeological Anthropology Commons, Ecology and Evolutionary Biology Commons, Environmental Public Health Commons, Other Public Health Commons, and the Parasitology Commons

Authors

Marcelo Luiz Carvalho Gonçalves, Adauto Araújo, Rosemere Duarte, Joaquim Pereira da Silva, Karl Reinhard, Françoise Bouchet, and Luis Fernando Ferreira

Detection of *Giardia duodenalis* antigen in coprolites using a commercially available enzyme-linked immunosorbent assay

Marcelo Luiz Carvalho Gonçalves,[1] Adauto Araújo,[1] Rosemere Duarte,[1] Joaquim Pereira da Silva,[1] Karl Reinhard,[2] Françoise Bouchet,[3] and Luiz Fernando Ferreira[1]

1 Escola Nacional de Saúde Pública, Fundação Oswaldo Cruz, Rio de Janeiro, Brazil
2 School of Natural Resources Sciences, University of Nebraska–Lincoln, Lincoln, NE USA
3 Laboratoire de Páleoparasitologie EA3308, Associé CNRS ESA 8045, U. F. R. de Pharmacie, Université de Reims, Reims, France

Correspondence — Adauto Araújo, Escola Nacional de Saúde Pública, Fundação Oswaldo Cruz, , Rua Leopoldo Bulhões 1480, CEP 21041-210, Rio de Janeiro, Brazil; fax +55 21 25982610, email adauto@ensp.fiocruz.br

Abstract
The objective of this experiment was to assess the utility of a commercially available enzyme-linked immunosorbent assay (ELISA) kit for diagnosis of giardiasis in archaeological human remains. The kit, a monoclonal antibody assay, is used to detect the presence of *Giardia*-specific antigen 65 (GSA65) in human feces. We utilized the assay in ancient fecal material. The material included desiccated feces found in mummies or in archaeological sites, and sediments from latrines. A total of 83 specimens, previously examined microscopically for parasites, were examined. The ELISA detected 3 positive samples, dated to about 1200 AD, 1600 AD, and 1700 AD. The ELISA was superior to direct observation. It was possible to identify *G. duodenalis* cysts by direct microscopy in only one of these samples. The results did not show cross-reactivity between this protozoan and helminths. The use of ELISA to detect *G. duodenalis* coproantigen could help the diagnosis of giardiasis in ancient human remains.

Keywords: giardiasis, diagnosis, ELISA, paleoparasitology, coprolites, ancient feces

Introduction

Paleoparasitology is the study of parasites in archaeological material. Normally, paleoparasitologists in the Americas analyze coprolites which are desiccated fecal material found in mummies or in archaeological sites. Detection of protozoa in coprolites poses a challenge to paleoparasitology. Unlike helminth eggs and larvae, protozoan cysts are not so resistant to the environmental conditions of archaeological sites. The vulnerability of cysts to decay may result in artificially low estimations of protozoa in paleoparasitology as indicated by the infrequent finding of protozoan cysts in coprolites compared to helminth eggs (Reinhard *et al.*, 1986). Also there is considerable day to day variation in cyst excretion that decreases the sensitivity of conventional coprolite examination. A single microscopical examination of fresh faeces from patients infected with *Giardia duodenalis* (syn. *G. lamblia, G. intestinalis)* yields false negative results in up to 50% of patients (Burke, 1977; Wolfe, 1978). Therefore, it is particularly difficult to identify *G. duodenalis* in archaeological sites.

Some *G. duodenalis* antigens appear to remain detectable for long periods of time. Faulkner *et al.* (1989) succeeded in obtaining positive indirect immunofluorescent antibody test to *G. duodenalis* in a coprolite 2177 years old. Allison *et al.* (1999), using fluorescent monoclonal antibody and enzyme-linked immunosorbent assay (ELISA), obtained positive results in coprolites 500 to 3000 years old.

ELISA for the detection of *G. duodenalis* antigens in fresh or preserved feces has been useful in the diagnosis of giardiasis. Several studies have found ELISA to be a tool with very high sensitivity and specificity for *G. duodenalis* coproantigen detection Ganoff *et al.,* 1989; ROSOFF *et al.,* 1989; ADDISS *et al.,* 1991; Scheffler & Van Etta, 1994; Aldeen *et al.,* 1998; Garcia *et al.*, 2000).

We conducted a study to assess the utility of a commercially available ELISA kit for the detection of a *G. duodenalis*-specific stool antigen, the *Giardia*-specific antigen 65 (GSA65), in archaeological samples. ELISA results were compared with those from conventional coprolite examination. The detection of *G. duodenalis* coproantigen in coprolites could be a method for giardiasis diagnosis in ancient human remains.

Materials and Methods

Specimens

A total of 83 coprolites and sediment samples, previously examined microscopically for helminth eggs and larvae at the paleoparasitology laboratory, National School of Public Health, FIOCRUZ, Brazil, were tested for *G. duodenalis* antigen. Their archaeological origin and dates are listed in Table 1. The samples were dated either by ^{14}C method or by cultural context. A sample of fresh feces, positive for *G. duodenalis* cysts by direct wet mount method, was used as a positive control. It was desiccated at 37° C for 2 weeks (an experimental coprolite) and was tested following the same procedures used for coprolite samples. This method of experimental production of coprolites has proven useful in identifying diagnostic criteria for other parasites (Confalonieri *et al.*, 1985). All specimens were also re-examined for helminth eggs and larvae, as well as for protozoan cysts.

Conventional examination of coprolites

The specimens were rehydrated by immersion in a 0.5% aqueous solution of trisodium phosphate for 72 h, following the technique of Callen & Cameron (1960). The rehydrated sample solutions were mixed approximately 10:1 in acetic formalin solution (Raillet Henry solution) to retard fungal and bacterial growth (Reinhard *et al.*, 1986). The material was allowed to sediment following the technique proposed by Lutz (1919). A portion of each sediment was used for microscopical examination. The material was placed on a slide and covered with a coverslip (22 × 22 mm) and examined for the presence of parasites. Twenty slides for each sample were examined at magnification of ×100 and ×400.

A portion of each rehydrated sample was also examined by the zinc-sulphate flotation method (Faust *et al.*, 1938). Five slides for each sample were examined at magnification of ×100 and ×400. All wet preparations were examined by at least 2 of the authors.

Table 1. Country of the archaeological site, type, and age of archaeological samples examined for *Giardia duodenalis*

Country	Archaeological samples
Brazil	39 coprolites from archaeologic layers and 3 coprolites from colon contents of mummies, dated from 5230 BC to 1730 AD
Chile	21 coprolites from archaeologic layers and 7 coprolites from colon contents of mummies, dated from 4100 BC to 800 AD
USA	5 coprolites from archaeologic layers, dated from 1200 AD to 1300 AD
Germany	3 medieval latrine soil samples, dated from 1500 AD to 1600 AD
Argentina	2 coprolites from colon contents of mummies, pre-Colombian time
France	1 coprolite from archaeologic layer, dated to about 400 000 BC and 1 medieval latrine soil sample, dated to about 1400 AD
Belgium	1 medieval latrine soil sample, dated to the 18th century

Enzyme-linked immunosorbent assay

The ProSpecT *Giardia* microplate assay (Alexon, Inc, Sunnyvale, CA, USA), a monoclonal antibody assay, was performed on each sample (100 µL of rehydrated sample solution, as described above), according to the manufacturer's directions. It detects the GSA65 *G. duodenalis*-specific antigen in stools. Results were interpreted by visual inspection, following the color panels included in the kit. Any detectable color generation was regarded as a positive result. Following visual inspection, the absorbance of each specimen was measured at 450 nm wavelength with a spectrophotometer. The absorbance values were adjusted by subtracting the optical density (OD) of the negative control from the OD of the samples. Specimens which produced an adjusted OD ≥ 0.050 were considered positive, according to the manufacturer's instructions.

Results

The immunoassay and the conventional examination results are given in Table 2. The microscopical examination was positive for *G. duodenalis* in only 1 of the 3 archaeological samples positive for *G. duodenalis* antigen detection by immunoassay. Two of the 3 positive samples (latrine soils) were retested using the same steps and procedures as above and the ELISA was concordant in both samples. Visual and spectrophotometer readings were concordant in all samples.

The feces used for the experimental coprolite had abundant cysts of *G. duodenalis* before desiccation. After desiccation and rehydration, identifiable cysts were very scarce.

Discussion

The observed decline of identifiable cysts in the experimental coprolite is very important. This indicates that the dehydration process, which is the reason coprolites preserve, and the rehydration process used for the analysis of most coprolites, destroys the majority of cysts. Therefore, the near absence of protozoa in coprolite studies is not surprising.

The results show no cross-reactivity between *G. duodenalis* and helminths, as can be seen in Table 2.

Table 2. Results of microscopic examination and enzyme-linked immunosorbent assay for GSA65 *Giardia duodenalis* antigen in human coprolites from archaeological sites

Country	Microscopic examination	ELISA result (OD)[a]
Brazil	13 samples with *Trichuris trichiura* eggs + Ancylostomidae larvae and/or eggs	neg. (<0.050)
	12 samples with *T. trichiura* eggs	neg. (<0.050)
	8 samples with Ancylostomidae larvae and/or eggs	neg. (<0.050)
	3 samples with free-living larvae	neg. (<0.050)
	6 negative samples (Ferreira *et al.,* 1980, 1983, 1987)	neg. (<0.050)
Chile	6 samples with *Diphyllobothrium pacificum* eggs	neg. (<0.050)
	1 sample with *Enterobius vermicularis* eggs	neg. (<0.050)
	1 sample with *T. trichiura* eggs	neg. (<0.050)
	20 negative samples (Ferreira *et al.,* 1984; Araújo *et al.,* 1985)	neg. (<0.050)
USA	2 samples with *E. vermicularis* eggs	pos. (1 sample) (0.092)
		neg. (1 sample) (<0.050)
	3 negative samples (Reinhard, 1985)	neg. (<0.050)
Germany	3 samples with *T. trichiura* + *Ascaris lumbricoides* + *D. latum* + *Fasciola hepatica* eggs (Herrmann, 1985)	pos. (1 sample) (0.237)
		neg. (2 samples) (<0 .050)
Argentina	1 sample with Ancylostomidae eggs	neg. (<0.050)
	1 sample with free-living larvae (unpublished data)	neg. (<0.050)
France	1 sample with *T. trichiura* + *A. lumbricoides* + *D. latum* + *Schistosoma haematobium* eggs	neg. (<0.050)
	1 negative sample (Bouchet & Paicheler, 1995)	neg. (<0.050)
Belgium	1 sample with *T. trichiura* + *A. lumbricoides* + *D. latum* + Ancylostomidae larvae and eggs + *G. duodenalis* cysts (unpublished data)	pos. (0.496)
Positive control	1 sample with *G. duodenalis* cysts (experimental coprolite)	pos. (0.741)

a. Enzyme-linked immunosorbent assay; optical density. Reader adjusted by subtracting the OD of the negative control (OD = 0.019) from the OD of the samples.

The use of 0.5% aqueous solution of trisodium phosphate and acetic formalin solution in coprolites did not prevent positive results in the immunological test.

The ELISA was superior to direct observation. The imunoassay detected *G. duodenalis* in 2 samples in which the direct examination was negative. In one sample the results were concordant. The finding of human-specific parasites in these samples confirms their human origin.

The sensitivity of direct microscopy to detect protozoa in coprolites is poor. There are not many well-documented papers referring to protozoa in coprolites (Witenberg, 1961; Fouant *et al.,* 1982; Faulkner *et al.,* 1989; Ferreira *et al.,* 1992; Allison *et al.,* 1999). Even in fresh feces, in order to obtain a reliable result of giardiasis by direct examination, one should examine at least a series of 3 or more stool samples, as cysts of *G. duodenalis* are shed from the intestinal tract on a periodic basis. Antigens, however, are shed in a more continous way (Rosoff & Stibbs, 1986a).

The previous use of ELISA for protozoan detection in coprolites has not been encouraging. Fouant *et al.* (1982) observed possible *Entamoeba* sp. cysts in 4 coprolites from pre-Colombian Indians from Chile. None were reactive to an ELISA utilized to detect *E. histolytica* antigens. They argued that the cysts found could be from *E. coli* species rather than *E. histolytica* species. Alternatively, they argued that the negative ELISA findings could result from decay of cyst antigenicity with time. Allison *et al.* (1999) compared a fluorescent antibody kit (Meridian Diagnostics, Cincinnati, OH, USA) to an ELISA kit (Meridian Diagnostics) for *G. duodenalis* detection in coprolites from preColombian South American mummies 500 to 3000 years old. The fluorescent antibody kit yielded more positive results than the immunosorbent assay. It was not stated if the ELISA kit used monoclonal or polyclonal antibodies. The findings from Fouant *et al.* (1982) and from Allison *et al.* (1999) could be explained by the relative low sensitivity of old generation enzyme immunoassays.

The assay evaluated here utilizes monoclonal antibody against GSA65 antigen. The GSA65 *G. duodena*lis-specific stool antigen is a glycoprotein. It is stable and is secreted in large amounts by encysting trophozoites (Rosoff & Stibbs, 1986b). According to Aldeen *et al.* (1998), the sensitivity and specificity of the assay is 100% in fresh feces. The immunological test performed was rapid and simple, although expensive. According to our results, GSA65 survives the destruction of cysts and trophozoites. It was shown to be present for at least 800 years in human remains.

Although many species of *Giardia* parasitize virtually all classes of vertebrates, only *G. duodenalis,* a species complex, is identified from humans and most other mammals (Thompson *et al.,* 1993; Thompson, 2000). Molecular characterizations indicate that isolates from humans belong to assemblage A (genetically equivalent to 'Polish' group or Groups 1/2*)* or assemblage B (genetically equivalent to 'Belgian' group or Group 3). Major assemblages have distinct clusters. These clusters appear to have typical patterns of dispersion and potential for zoonotic transmission (Thompson *et at.,* 2000). The use of such molecular markers in coprolites with *G. duodenalis* could raise interesting issues concerning the origin and dispersion of this parasite. Mayrhofer *et at.* (1995) pointed out the relevance of studying the genetic divergence in *Giardia* assemblages concerning the phylogenetic or ecologic route of the giardiasis in humans.

If further studies confirm our results, the use of immunological methods for antigen detection can become the "gold standard" for diagnosis of protozoan infection in coprolites. As more sensitive *G. duodenalis* detection tools arrive, a more comprehensive picture of this infection in ancient populations could be done.

References

Addiss, D. G., Mathews, H. M., Steward, J. M., Wahlquist, S. P., Williams, R. M., Finton, R. J., Spencer, H. C. & Juranek, D. D. (1991). Evaluation of a commercially available enzyme-linked immunosorbent assay for *Giardia lamblia* antigen in stool. *Journal of Clinical Microbiology,* 29, 1137–1142.

Aldeen, W. E., Carrol, K., Robinson, A., Morrison, M. & Hale, D. (1998). Comparison of nine commercially available enzyme-linked immunosorbent assays for detection of *Giardia lamblia* in faecal specimens. *Journal of Clinical Microbiology,* 36, 1338–1340.

Allison, M. J., Bergman, T. & Gerszten, E. (1999). Further studies on fecal parasites in Antiquity. *American Journal of Clinical Pathology,* 112, 605–609.

Araújo, A., Ferreira, L. F., Confalonieri, D., Nunez, L. & Ribeiro-Filho, B. M. (1985). *Enterobius vermicularis* eggs in precolumbian human coprolites from Chile. *Memórias do Instituto Oswaldo Cruz,* 80, 141–143.

Bouchet, F. & Paicheler, J. C. (1995). Paléoparasitologie: présomption d'un cas de bilharziose au XVᵉ siècle à Montbéliard (Doubs, France). *Comptes Rendus Hebdomadaires des Sceances de l'Academie des Sciences, Paris, Series III,* 318, 811–814.

Burke, J. A. (1977). The clinical and laboratory diagnosis of giardiasis. *Critical Reviews in Clinical Laboratory Sciences,* 7, 373–391.

Callen, E. O. & Cameron, T. W. M. (1960). A prehistoric diet as revealed in coprolites. *New Scientist,* 8, 35–40.

Confalonieri, D., Ribeiro-Filho B. M., Ferreira, L. F. & Araújo, A. (1985). The experimental approach to paleoparasitology: Desiccation of *Trichuris trichiura* eggs. *Paleopathology Newsletter,* 51, 76–77.

Faulkner, C. T., Sharon, P. & Johnson, S. S. (1989). Prehistoric parasitism in Tennessee: Evidence from the analysis of desiccated faecal material collected from Big Bone cave, Van Buren, Tennessee. *Journal of Parasitology,* 75, 461–463.

Faust, E. C., D'antoni, J. S., Odom, V., Miller, M. J., Peres, C., Sawitz, W. & Thomen, L. F. (1938). A critical study of clinical laboratory technics for the diagnosis of protozoan cysts and helminth eggs in faeces. *American Journal of Tropical Medicine,* 18, 169–183.

Ferreira, L. F., de Araújo, A. J. *G.* & Confalonieri, U. E. C. (1980). The finding of eggs and larvae of parasitic helminths in archaeological material from Unaí, Minas Gerais, Brazil. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* 74, 798–800.

Ferreira, L. F., de Araújo, A. J. *G.* & Confalonieri, U. E. C. (1983). The finding of helminth eggs in a Brazilian mummy. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* 77, 65–67.

Ferreira, L. F., Araújo, A., Confalonieri, U. & Nunez, L. (1984). The finding of eggs of *Diphyllobothrium* in human coprolites (4,100–1,950 BC) from Northern Chile. *Memórias do Instituto Oswaldo Cruz,* 79, 175–180.

Ferreira, L. F., Araújo, A., Confalonieri, U., Chame, M. & Ribeiro, B. (1987). Encontro de ovos de ancilostomideos em copró1itos humanos datados de 7.230 ± 80 anos, Piauí, Brasil. *Anais da Academia Brasileira de Ciéncias,* 59, 280–281.

Ferreira, L. F., Araujo, A., Confalonieri, U., Chame, M. & Ribeiro, B. (1992). *Eimeria* oocysts in deer coprolites dated from 9,000 years BP. *Memórias do Instituto Oswaldo Cruz,* 87, 105–106.

Fouant, M. M., Allison, M., Gerszten, E. & Focacci, G. (1982). Parasitos intestinales entre los indigenas precolombinos. *Revista Chungará,* 9, 285–299.

Garcia, L. S., Shimizu, R. Y. & Bernard, C. N. (2000). Detection of *Giardia lamblia, Entamoeba histolytica/Entamoeba dispar,* and *Cryptosporidium parvum* antigens in human faecal specimens using the triage parasite panel enzyme immunoassay. *Journal of Clinical Microbiology,* 38, 3337–3340.

Herrmann, B. (I 985). Parasitologisch epidemiologische auswertungen mittelalterlicher kloaken. *Zeitschrift für Archäologie des Mittelalters,* 13, 131–161.

Janoff, E. N., Craft J. C., Pickering, L. K., Novotny, T., Blaser, M. J., Knisley, C. V. & Reller, L. B. (1989). Diagnosis of infections by detection of parasite-specific antigens. *Journal of Clinical Microbiology,* 27, 431–435.

Lutz, A. (1919). O *Schistosomum mansoni* e a schistosomatose segundo observações feitas no Brasil. *Memórias do Instituto Oswaldo Cruz,* 11, 121–150.

Mayrhofer, G., Andrews, R H., Ey, P. L. & Chilton, N. B. (1995). Division of *Giardia* isolates from humans into two genetically distinct assemblages by eletrophoretic analysis of enzymes encoded at *loci* 27 and comparison with *Giardia muris. Parasitology,* 111, 11–17.

Reinhard, K. J. (1985). *Recovery of helminths from prehistoric feces: The cultural ecology of ancient parasitism.* MS thesis, University of Northem Arizona, Flagstaff, Arizona, USA.

Reinhard, K. J., Confalonieri, U., Herrmann, B., Ferreira, L. F. & Araujo, A. (1986). Recovery of parasite remains from coprolites and latrines: Aspects of paleoparasitological technique. *Homo,* 37, 217–239.

Rosoff, J. D., Sanders, C. A., Sonnad, S. S., De Lay, P. R, Hadley, W. K, Vincenzi F. F., Yajko, D. M. & O'Hanley, P. D. (1989). Stool diagnosis of giardiasis using a commercially available enzyme immunoassay to detect *Giardia*-specific antigen 65 (GSA 65). *Journal of Clinical Microbiology,* 27, 1997–2002.

Rosoff, J. D. & Stibbs, H. H. (1986a). Physical and chemical characterization of a *Giardia lamblia*-specific antigen useful in the coprodiagnosis of giardiasis. *Journal of Clinical Microbiology,* 24, 1079–1083.

Rosoff, J. D. & Stibbs, H. H. (1986b). Isolation and identification of a *Giardia lamblia*-specific stool antigen (GSA 65) useful in coprodiagnosis of giardiasis. *Journal of Clinical Microbiology,* 23, 905–910.

Scheffler, E. H. & Van Etta, L. L. (1994). Evaluation of rapid commercial enzyme immunoassay for detection of *Giardia lamblia* in formalin-preserved stool specimens. *Journal of Clinical Microbiology,* 32, 1807–1808.

Thompson, R. C. A. (2000). Giardiasis as a re-emerging infectious disease and its zoonotic potencial. *International Journal for Parasitology,* 30, 1259–1267.

Thompson, R C A., Reynoldson, J. A. & Mendis, A. H. S. (1993). *Giardia* and giardiasis. *Advances in Parasitology,* 32, 71–160.

Thompson, R C A., Hopkins, R. M. & Homan, W. L. (2000). Nomenclature and genetic groupings of *Giardia* infecting mammals. *Parasitology Today,* 16, 210–213.

Witenberg, *G.* (1961). Human parasites in archaeological findings. *Bulletin of the Israel Exploration Society,* 25, 86.

Wolfe, M. S. (1978). Giardiasis. *New England Journal of Medicine,* 298, 319–321.