Paleoparasitology and the Antiquity of Human Host-parasite Relationships

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Paleoparasitology may be developed as a new tool to parasite evolution studies. DNA sequences dated thousand years ago, recovered from archaeological material, means the possibility to study parasite-host relationship coevolution through time. Together with tracing parasite-host dispersion throughout the continents, paleoparasitology points to the interesting field of evolution at the molecular level. In this paper a brief history of paleoparasitology is traced, pointing to the new perspectives opened by the recent techniques introduced.

Key words: paleoparasitology - coprolites - mummies - parasitism - infectious diseases

Paleoparasitology appeared as a new branch of parasitology when the first parasite eggs were recovered from archaeological material. In the beginning of the century, the development of a technique to rehydrate desiccated tissues allowed the finding of *Schistosoma haematobium* eggs in infected kidneys of Egyptian mummies dated of 3,200 years old (Ruffer 1910).

What is known now as the pioneer period of paleoparasitology can be characterized mainly by the findings of parasites in fecal material recovered from archaeological sites. Although organic remains may be found in a variety of environments, preservation seems to be best in moist anaerobic environments or desiccating environments. Latrines readly provide for the former conditions while caves and rock-shelters in arid regions provide for the later (Reinhard et al. 1988).

Coprolites are desiccated or mineralized feces and especial techniques were developed to search parasites in their contents. To rehydrate desiccated coprolites, a trissodium phosphate solution is used (Callen & Cameron 1960), and for fossilized coprolites, modified polen analysis techniques are used (Reinhard et al. 1985, Ferreira et al. 1993, Duarte et al. 1999).

Coprolites can be recovered from archaeological layers or cesspits, and directly from mummified bodies. They are the main source for parasite remains. Desiccation, and sometimes mineralization, results in excelent preservation of parasite larvae and eggs, whereas protozoan cysts are rarely found (Ferreira et al. 1992). Helminth species that normally hatch out of their eggs and leave feces are trapped by drying, providing records of hookworm (Araújo et al. 1981, Ferreira et al. 1987) and *Strongyloides* infection in ancient human (Reinhard et al. 1987) and animal (Araújo et al. 1989) populations.

With the cooperation among archaeologists and paleoparasitologists, pictures of parasite infections have been traced from ancient times to present days (Araújo et al. 1981, Horne 1985, Nozais 1985).

From the pioneer period, when the reporting of a parasite in archaeological materials deserved attention for the finding itself, sometimes as a curiosity, emerged interpretations and hypothesis for the presence of a given parasite in a determined place and time. Cockburn (1967) was the first to call attention for the potentiality of parasite studies in coprolites, and Ferreira et al. (1979) named paleoparasitology this new field of science.

Paleoparasitology advances by interpreting findings and making inferences about the impact of parasitic diseases among prehistoric populations. Hunter-gatherers were found to be less infected by helminths, whereas agricultural groups showed comparatively higher prevalences of intestinal parasites in archaeological sites in the United States (Reinhard 1992). Food habits of prehistoric inhabitants in the Chilean desert were discussed by Ferrreira et al. (1984) by the finding of *Diphyllobothrium pacificum* eggs, a sea lion parasite, in human coprolites dated of 6,000 years before present (B.P.).

Heirloom parasites are well exemplified by *Enterobius vermicularis* studies. This helminth infection was recorded in Norht America dated up

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to 10,000 years B.P. (Moore et al. 1969) through colonial times (Reinhard et al. 1997). *E. vermicularis* infection was found in cold climates, in Greenland, dated 1400 A.D. (Anno Domini) (Hausen 1986), and in the South American Pacific coast from 4,000 to 2,000 years B.P. (Pizzi & Schenone 1954, Araújo et al. 1985). Its absence in Brazilian archaeological material remains to be explained. Human-parasite coevolutionary studies based on paleoparasitological finds were performed showing the antiquity of parasite-host relationships, and tracing the origin and dispersion of the infection in prehistorical times (Confalonieri et al. 1991, Araújo & Ferreira 1995, Hugot et al. 1999).

The finding of human hookworm and whipworm eggs in pre-Columbian America (Allison et al. 1974, Ferreira et al. 1980,1983, Araújo et al. 1981) raised an interesting debate (Hawdon & Johnston 1996, Ferreira & Araújo 1996). Both are geohelminths, with an obligatory passage in the soil. They can only maintain their biological cycle to infect a new host if environmental conditions are suitable.

Hookworm eggs were found in human coprolites in South American archaeological sites radiocarbon dated as old as 7,200 years (Ferreira et al. 1987). *Trichuris trichiura* eggs were found in the Andean region and in the other side of the continent, in Brazilian Northeast, dated 2,000 years B.P. (Ferreira et al. 1989). In both cases, eggs were recovered from intestinal contents of South American mummies (Pizzi & Schenone 1954, Allison et al. 1974, Ferreira et al. 1983).

Prehistoric migrations crossing the Bering Land Bridge would not be responsible for the introduction of hookworm and whipworm infection during the first steps to the peopling of the Americas. Cold climate and nearly frozen conditions of the soil would have impaired larvae and eggs to evolve and be transmissible to a new host. Prehistoric transpacific contacts of Asian groups with South American Indians, proposed by archaeologists (Rivet 1926, Meggers & Evans 1966) based on cultural similarities may now be established also by a biological indicator. Dating the first Americans is under an intense debate and the recent archaeological findings in South America (Guidon & Delibrias 1986, Guidon & Arnaud 1991) turned the peopling of the New World a story to be rewritten (Gruhn 2000). Paleoparasitology, especially focused as an interdisciplinary science, certainly is contributing to this issue (Araújo & Ferreira 1996, 1997), together with paleogenetics (Ribeiro et al. 1996, Bonatto & Salzano 1997, Callegari-Jacques & Salzano 1999).

Paleoparasitological findings in the Old World revealed infections recorded in peat bog mummies (Fischer 1980, Jones 1986, Jones & Nicholson 1988, Hill 1990) and latrine soils (Szidat 1944, Taylor 1955, Pike 1967, 1968, 1975, Herrmann 1986, Reinhard et al. 1988). Interesting results were obtained by soil analysis and intestinal contents of mummified bodies by the French paleoparasitological team (Bouchet & Paicheler 1995, Bouchet et al. 1996, 1999).

Classical paleoparasitology based on coprolite rehydration and microscopic analysis after parasite concentration techniques is still contributing to the knowledge of parasitc disease past distribution throughout ancient times. But new techniques point to new perspectives, more sensible and capable to detect parasite traces in archaeological remains to the level of DNA fragments. Molecular paleoparasitology constitutes a powerful tool to the research of parasitic diseases in the past.

During the last ten years infectious diseases started to be diagnosed using technologies based on nucleic acid. Ancient DNA (aDNA) are nucleic acids recovered from archaeological material or museum specimens. In a broader sense it can be applied to any nucleic acid recovered after death when the autolysis process was started (Herrmann & Hummel 1994). aDNA was amplified from human bones and mummified tissues (Horay et al. 1989, Hagelberg et al. 1989, 1991, Hänni et al. 1990, Hagelberg & Clegg 1991, Pääbo 1993) and perspectives and limits were discussed with the new technology (Brown & Brown 1992). The polymerase chain reaction (PCR) was then incorporated in paleoparasitological analysis and adapted to detect parasite DNA fragments (Araújo et al. 1998).

The amplification of *Borrelia burgodorferi* DNA isolated from 13 of 1,036 mite museum samples in the United States (Persing et al. 1990), and the positive PCR for *Leishmania amazonensis* in taxidermized rodents sampled from museum specimens (Cantarino et al. 1998) showed the potentiality of this technique for the diagnosis of parasitic infections in preserved museum collections. PCR was used to study *Mycobacterium tuberculosis* infection in mediaeval skeletons in Europe (Spigelman & Lemma 1993), in a Peruvian precolumbian mummy (Salo et al. 1994), and in Chilean prehistoric skeletons (Arriaza et al. 1995).

Chagas disease is an excelent example for the development of this technique applied to the diagnosis of a parasitic infection in prehistorical times.

The first histological microscopic examination of suspected amastigote nests in mummified tissues were discussed (Rothhammer 1985, Rothhammer et al. 1985), but later confirmed in a Peruvian mummy (Fornaciari et al. 1992).

Mummies are a scarce remain of ancient cultures, but bones can be found more abundantly.

The search for *Trypanosoma cruzi* DNA fragments started with experimental approachs to test techniques in laboratory desiccated infected mice (Bastos et al. 1995) and then applied to the diagnosis of Chagas disease in mummified tissues of South American mummies (Guhl et al. 1997, 1999, Ferreira et al. 2000).

The focus is now directed to attempts to isolate *T. cruzi* DNA from bones and skeletons as they may be representative samples of prehistoric populations in an epidemiological sense (Guhl et al. 2000). Moreover, they are in most of the cases the only available material for analysis. One or two mummies or a handfull of bones found to be positive for some infection may indicate a infectious disease in a population, but rather a paleoepidemiological approach. What is possible now with the molecular paleoparasitology is the possibility of investigating significative samples of a skeleton population where traces of a parasite were not visible to a microscopic level.

This is really a new branch of science: the perspective of recovering a genome of a parasite that infected a human being living more than 10,000 years ago, and the comparisons that may be made with present lineages are opening possibilities to study the genome evolution through time. Origin, dispersion, virulence, and pathogenicity will certainly be more clearly understood under molecular paleoparasitological approaches.

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