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Mummy Studies and Parasite Infections in Brazil

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Mummified bodies are unexpected finds in archaeological sites of tropical countries. Rain forests and humid climate provide poor environmental conditions to preserve organic remains. However, South American tropical environments also include dry savanna, such as the *caatinga* and the *cerrado* Brazilian biomes, as well as cold Highlands, where naturally mummified human bodies have been found in archaeological sites. Unique environmental conditions created in some historical sites also helped to preserve organic remains, some of which have been studied in our laboratories. This kind of environmental condition is found mainly in the Brazilian northeast, but also in Central Brazil, especially in Minas Gerais State, where the oldest mummified corpse in Brazil is dated to near 3,000 years ago.

Some mummifies from Minas Gerais have been studied by the paleoparasitologist team in Rio de Janeiro. One of them was a partially mummified female body found in the site Gruta do Gentio II, Unaí, Minas Gerais State. The body was completely wrapped in plant tissue, covered with leaves and other funeral rituals. Using a forceps we get extracted samples with minimal damage to the corpse, as earlier reported by Ferreira et al. (1983a).

Parasitological studies are easily carried out with coprolites extracted from mummies. This is because coprolites from mummies have a known zoological origin. Coprolites found free in archaeological sediments are not so easily identified as human. Morphology, food remains, specific parasites and other aspects such as archaeological context must be used to determine the zoological origin of coprolites from domestic or trash contexts. The morphology of fecal samples of modern animals can be used to identify animal coprolites and separate them from human's, as done in the Brazilian northeast (Chame, 2003).

Paleoparasitology in Brazil is mainly concerned with internal parasites, but ectoparasites have also been studied. Head and crab lice have been found in mummies and scalps dated up to 11,000. Shrunken heads, trophy heads, naturally mummified remains and even hairs found free in the archaeological soils have been searched for parasites with positive results in the recent years (Araujo et al., 2000; Rick et al., 2002; Santoro et al., 2003).

Ancient DNA has been recovered from mummified soft tissues opening new avenues of research. Even in the absence of morphological evidence of parasites, ancient infections may be studied in different samples such as faeces, bone, soft tissues and so on. Thanks to the PCR techniques, not only mummified remains, but also skeletonized remains are now available for parasite analysis. We anticipate that it will be possible to recover ancient genetic material to compare with modern specimens and trace evolutionary events through time (Araujo et al., 1998). Actually, Donoghue et al. (2004) have clearly demonstrated results of *Mycobacterium tuberculosis* evolution through paleoparasitological analysis. A fast progress in this field is bringing clear benefits to paleoparasitology field of research.

The aim of paleoparasitological research in Brazil is to understand which parasite infections have been spread from Africa to Europe and Asia with human ancestors, and from the Old to the New World, during the peopling of the Americas up to the time of the Great Navigations. Other goals of our study include understanding the impact, the burden of the disease, and the evolution of host-parasite relationships in pre-historic populations.

In Brazil, as elsewhere, skeletons are more often found than preserved corpses. Because of that, molecular paleoparasitologists have turned their attention to aDNA extraction from bones and teeth. Teeth preserve DNA more often because of their resistance to most of the taphonomic factors in the external environment that causes DNA decay. Besides identifying traces of the parasites even in the absence of morphological preservation, the DNA identification support research lines studying the origin and evolution of parasitic diseases.

Two interesting examples of Brazilian mummified bodies studied for paleoparasitological purposes are the historical corpses from Itacambira and the Botocudo mummy from Sant´Ana, both from Minas Gerais State. The historical mummies from Itacambira came from a Colonial cemetery close to a church of the eighteenth century, and provided *in situ* coprolites (Ferreira et al., 1980) positive for *Trichuris* (Fig. 1). The Botocudo female found in a rockshelter in Sant´Ana, Rio Novo, today in the National Museum collection, was accompanied by two small mummy bundles of young children. The female was autopsied following standard procedures proposed for the study of Egyptian mummies

Fig. 1 - mummified bodies found in a colonial church in Itacambira, Minas Gerais, Brazil.

(Cockburn et al., 1998), by a team of archaeologists, anthropologists, pathologists, paleoparasitologists and dermatologists. The corpse (Fig. 2) was in bad state of preservation and no parasites were recovered either from the woman or from the two children in the bundles. Although negative for morphological parasitic search, the three corpses may be positive for parasitic aDNA analysis

Fig. 2 - Pre-Columbian female mummified body found in Minas Gerais, Brazil, with two bundles containing children.

and new samples are being collected for this purpose. The mummies from Minas Gerais were the focus of the most interesting debate in paleoparasitology and established the importance of mummies in the field. The mummy of Unai provided an interesting case. At the very beginning of paleoparasitological research, some coprolites were found in archaeological levels in a cave in Minas Gerais. They were sent to our laboratory for paleoparasitological examination and we found *Trichuris* and hookworm eggs, different kinds of food remains, and charcoal fragments from cooking. Based on the evidence, the coprolites were determined to be human. The shape and size of *Trichuris* eggs were consistent with *T. trichiura*, a very specific human parasite. Hookworm eggs were also defined by morphology. Results were published (Ferreira et al., 1980), presenting the first paleoparasitological findings in Brazilian archaeological sites, with interpretation. Two years later a letter was published criticizing our paper by Michael Kliks (1982) and proposing that the coprolites could be of a bear or a dog, and that the parasites were not human parasites. Concluding his comments, Kliks said "all of us who work with ancient dung must remember all that glisters is not gold". An definitive rebuttal to his criticisms was possible when a mummified child in the same site was studied. The same parasite species were found in the mummy. The same journal published our paper with the new results, and a letter answering the critique. Of course the following final comment was registered: "the archaeological site is in Minas Gerais, the Brazilian State where most of our gold mines were found, and there, very often, what glisters is really gold!" (Ferreira et al., 1983b).
From outside of Brazil, other materials have been studied in our laboratory. We examined 37 Chilean mummies found in the Atacama Desert following standard procedures of collecting a minimal tissue and coprolite amount. These mummies from Atacamenho people were found in San Pedro de Atacama dating up to 2,000 years ago. People from the Pacific coast, from the Andean Cordillera, from urban centers close to the Titicaca Lake, as well as from the tropical forest on the other side of the Andean Cordillera crossed the desert bringing goods to exchange. San Pedro village attracted people from different ecosystems playing the role of a dispersal center for different infectious diseases, some of which still prevailing over the years. Coprolites collected in all these mummies were processed following recommended techniques (Araujo et al., 1998; Reinhard and Bryant Jr., 1992; Bouchet et al.,2003).
Atacama Desert mummies were examined for ectoparasites, as well as for intestinal parasites and Chagas disease. Head lice were found on the hair of female and male mummified bodies, and crab louse eggs were found on pubic hairs of a female body (Rick et al., 2002). No intestinal parasites were found in the coprolites. On the other side, visceral tissues samples from the chest and the abdomen of these mummies were tested for *Trypanosoma cruzi* infection by PCR technique. Four of twelve were positive, suggesting a high prevalence of Chagas disease at the region (Ferreira et al., 2000).
In fact, the first steps to investigate Chagas disease in other parts of the Americas actually began in 1984, when our team observed triatomine trying to attack the archaeologists who were copying rock paintings in the archaeological site of Pedra Furada, at the National Park of Serra da Capivara, Brazil. We were concerned with triatomine attacks on the archaeologists, and fortunately, none of them was infected by these vectors of *T. cruzi*, but the idea that the ancient artists had also been attacked by triatomines was established in our minds. At that time nothing could be done to investigate the disease in ancient material, as Chagas disease leaves no pathology in bones, but the PCR techniques improved the investigations based in DNA remains and we can finally see beyond the bones. The National Park of Serra da Capivara has more than 700 known archaeological sites. Most are rock-shelters covered with paintings. Skeletons have been found in urns, but also buried in the archaeological layers (Fig. 3) dating up to 11,000 years (Araujo et al., 2000; Lessa & Guidon, 2002).

Fig. 3 - Human skeleton dated of 11,000 years ago, excavated from the National Park of Serra da Capivara, Piaui State, Brazil. Hairs were found infested with head lice eggs.

Among them are the oldest testimonies of human presence in South America and our plans for the future include the exciting investigation of genetic sequences of *T. cruzi* over time in the human remains.

In 1997, we analyzed coprolites and mummies from Chile and Peru as part of an CNPq-sponsored research collaboration between ENSP/FIOCRUZ and the University of Nebraska. These data were written-up in 2001 as part of a Fulbright Commission/CAPES sponsored continuation of this collaboration. Some of the data have now been published. The description of fish tapeworm infection with consideration of the peculiarities of egg preservation in mummies was done for Chinchorro sites in Chile (Reinhard and Urban 2003). The parasitology of the Chiribaya culture was defined during those years (Martinson et al, 2003).

The first results for Chagas outside the Andean region was unexpectly obtained from a partially mummified corpse found many years ago in the border of Mexico/Texas. This occurred during a CAPES-sponsored continuation of the collaboration between ENSP/FIOCRUZ and the University of Nebraska. We had the opportunity to examine the mummy and we saw clear evidence of an enlarged colon, clearly suggesting Chagas disease (Reinhard et al., 2003). That is presently being investigated and some interesting results were obtained regarding DNA sequences of *T. cruzi* (Dittmar et al. 2003). More recently, we evidenced *T. cruzi* aDNA (Araujo et al., 2005) in another mummified body excavated in Minas Gerais, Brazil, showing that Chagas disease was present among lowland pre-Columbian South American Indians, as well as in the Andes.

Paleoparasitology in Brazil was strongly supported by mummy investigations, as far as most of the important results we have in conventional parasitological or DNA techniques were obtained in mummified remains. Many papers have been published since we started to investigate mummies and their parasitological histories, and a recent review has been published by two of the authors in *Memorias do Instituto Oswaldo Cruz* (2003).

Modern trends like molecular paleoparasitology are opening the opportunity to investigate materials that were never studied, as well as to review mummies that have already been studied. Molecular analysis based on PCR and other techniques make mummies the best source to investigate the origin and evolution of host-parasite relationships. Thanks to recent advances, paleoepidemiology is now a possible plan for the future of paleoparasitology, opening opportunities to study the origin and evolution of infectious diseases.

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