THE ROLE OF MUMMY STUDIES IN PALEOPARASITOLOGY

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Paleoparasitology has advance during the past decade to the status of a statistically based science focused on problems of disease ecology and geographic distribution of parasitism. For most of its development, paleoparasitology has focused on the analysis of coprolites and latrine sediments. During the past few years, mummies have been increasingly included in paleoparasitology studies. We evaluate in this paper the interpretive value of mummies relative to other sources of paleoparasitological data.

Key words: Parasites, bioarchaeology, paleopathology.

El estudio de la paleoparasitología ha logrado transformarse, en esta última década, en una ciencia basada en la estadística, enfocada a temas tales como la ecología de enfermedades y la distribución geográfica parasítica. Los primeros estudios se basaron en el análisis de coprolitos y sedimentos letrinos. Ultimamente, se ha incorporado restos humanos momificados en los estudios paleoparasitologicos. En este trabajo, se analiza el valor interpretativo de este tipo de muestra en relación a otras fuentes de información paleoparasitológica.

Palabras claves: *Parásitos, bioarqueología, paleopatología.*

Paleoparasitology was borne from heresy (Ferreira 1988: 7-8). The heresy stemmed from the believe of a few parasitologists that the prevailing notion concerning the prehistoric distribution of parasites was wrong. That notion, stated simply, was that the prehistoric New World was essentially free of parasitic disease. There was a strong belief that the migration through Beringia eliminated human parasites in the New World (Stewart 1960). Paleoparasitologists in the late 1970s and 1980s proposed that parasitic disease was present prehistorically, and if we looked hard enough then the evidence of such disease would come to light.

Heretical ideas are by their nature opposed to accepted belief systems. For many years, and up until recently, there was resistance to Paleoparasitological findings. Mummy studies had an important role in overcoming this resistance. Now, after twenty years of continued study, the goals of paleoparasitology have grown from an exploratory stage to a statistically based science. Also, our past heresy is now accepted as scientific fact. In the future, we believe that mummies will become an ever increasingly important source of data concerning ancient parasites. We are summarizing the history of the field to explain the importance of mummies in the past, present, and future.

We recognized early on that because we were heretics, we had to be able to defend our evidence with absolute scientific empiricism. Thus, much of our early work was designed towards developing diagnostic criteria for identifying prehistoric parasite infections. These efforts were very important at the time because most of our data came from coprolites or latrines, and therefore the human origin of our findings was always suspect (Confalonieri et al. 1991). Therefore, great effort was expended in the description of larval forms of parasites (Araújo 1988a; Reinhard 1985) and experimental desiccation of eggs (Confalonieri 1988a; Confalonieri et al. 1985, 1988). Also, considerable time and energy was committed to defining fecal morphology so that human coprolites could be identified with certainty Chame (1992). By 1988, we felt confident enough in our recovery and statistical techniques to summarize our methods in a single paper (Reinhard et al. 1988). In our first summary of the methods of paleoparasitology in context of the goals of the field, we pointed out that although the greatest body of data in our field comes from coprolites and latrine soils, mummies held potential for greatly expanding the list of parasites found in the prehistoric New World (Reinhard et al. 1988).

Despite the empirical basis of our research, our findings continued to be criticized. Mummies had an all important role in calming the disquiet that was caused by our findings. The find of whipworm Pizzi and Schenone (1954), fish tapeworm (Callen and Cameron 1960), and hookworm (Allison et al. 1974) remains in mummies gave absolute proof to the presence of these parasites in the prehistoric New World. However, our data from coprolites was

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disputed. For example, Ferreira et al. (1980) published the discovery of prehistoric worm eggs and larvae from prehistoric coprolites found in the state of Minas Gerais in Brazil. Whipworm eggs and hookworm eggs and larvae were found. Kliks (1982) published a rebuttal to the Minas Gerais finds based partly on his belief that the coprolites came from dogs or bears and also that the egg morphology was not specific to human species of whipworm and hookworm. Thus, the exacting analysis of fecal morphology and egg/larvae morphology did not satisfy Kliks. Subsequently, Filho et al. (1983) repeated the discovery of whipworm and hookworm eggs in a prehistoric mummy from Minas Gerais. The find of the eggs in a mummy demonstrated undeniably that these prehistoric parasites were associated with humans. Ferreira et al. (1983) were then able to respond to Kliks in a positive way, having clearly demonstrated that whipworm and hookworm were without doubt parasites of prehistoric Brazilians.

If data from coprolites are hard to present as convincing evidence of parasitism, parasite eggs from latrines are even more difficult to for skeptics to accept. This is illustrated by Hevly et al. (1979) who discovered five species of parasites in latrine sediments from Elden Pueblo, Arizona. They encountered whipworm, pinworm, taeniid tapeworms, hymenolepidid tapeworms, and *Ascaris* roundworms in prehistoric latrine sediments. Hevly et al. (1979) used a multidisciplinary team to diagnose the eggs and date them to prehistoric times. The team included an archaeologist, a palynologist, a mycologist, a zoologist, and dating experts. Despite the fact that the diagnosis was done by a parasitologist (Anderson), a palynologist (Hevly), and in consultation with a mycologist, some of the species were disputed (Horne 1985). Reinhard et al. (1987) reviewed the data and determined that the identifications were valid.

By the mid-1980s and up until now, enough data were collected to begin developing models which explained the geographic dispersion of parasites in prehistory, especially of whipworm (Confalonieri 1988b) and hookworm (Araújo 1988b, 1988c, 1996; Ferreira and Araújo 1996). Also, the interrelation of human evolution and parasitism were examined (Reinhard 1988). These are still very active areas of intellectual exploration, based on knowledge of parasite ecology and prehistoric distribution of parasite species. Also during this period, a paleoepidemiological approach emerged (Herrmann 1987). This was developed in context of refined archaeological excavations which focus on disease ecology.

The results of these studies elucidated the influence of agriculture on prehistoric parasitism (Reinhard 1988). We have also demonstrated that paleoparasite data show how differences in house construction influence certain parasitic diseases (Araújo et al. 1998). The patterns of subsistence, sanitation, and housing between prehistoric villages has been examined with respect to parasitism (Reinhard 1996). Also, aspects of evolutionary theory have been explored and tested with paleoparasitological data (Aufderheide and Rodriguez-Martin 1998: 224). In addition, attempts have been made to relate paleoparasitological data to the osseous expression of disease such as porotic hyperostosis (Reinhard 1992a).

Mummies have played a role in these studies as well. For paleoepidemiological reconstructions, mummies are a good source in information. A basic epidemiological concept is prevalence. Prevalence is the measure of the number of people infected with a parasite species at a given point in time. To gain an idea of prevalence, one must be able to sample individuals. Although true measures of prevalence may never be achieved in paleoaparasitology, mummies provide a better approximation than coprolites. With mummies, one can be certain that the parasites extracted from coprolites recovered from the intestinal tract represent one individuals infections. This is not true for coprolites recovered from latrines. With coprolites from latrines, one can never be certain of how many individuals are represented by the coprolites (for discussion see Reinhard 1990). Therefore, as we have searched for a measure of prevalence, mummies have become a recent focus for study. The identification of fish tapeworm prevalence was based on analysis of Chinchorro mummies (Arriaza 1995; Arriaza et al. 1998: 191; Reinhard 1998: 379). The prevalence and paleoepidemiology of lice has been defined by the analysis of 146 Chinchorro mummies from Peru (Aufderheide and Rodriguez-Martin 1998; Reinhard 1998). Thus, present and future studies of paleoepidemiology will be dependent on analysis of mummies.

Our latest summary of paleoparasitological technique (Araújo et al. 1998) shows that our science has advanced considerably since our first technique paper (Reinhard et al. 1988). A new aspect of paleoparasitology is the search for molecular evidence of parasites. The first indication that parasite DNA could be recovered from coprolites was by Guhl et al. (1997) who recovered *Trypanosoma cruzi* DNA from Peruvian mummies. Since then, researchers at Fundação Oswaldo Cruz (FIOCRUZ) have been searching for parasite DNA. This involves experimental approaches (Bastos et al. 1995, 1996) and direct examination of prehistoric remains for ancient parasite DNA. A new laboratory, the Laboratorio Paleoparasitologia Molecular, was dedicated in the Escola Nacional de Saúde Pública, FIOCRUZ in 1998 specifically for this purpose. Two significant research projects have been completed (Iñiguez 1998; Cantarino 1998). These projects involved experimental desiccation and recovery of DNA from modern specimens as well as application of PCR techniques to prehistoric remains with the end goal of developing methods that can be applied to prehistoric remains successfully. Iñiguez has explored three areas of molecular methodology. She experimentally desiccated modern feces inoculated with *Vibrio cholerae* and *Bacillus sphaericus*. One of these bacteria, *B. sphaericus* forms spores. Iñiguez was able to recover DNA from both organisms and found that *B. sphaericus* was more conducive to DNA recovery, probably due to its sporulation capabilities. This study suggest that DNA may be readily recoverable from ancient eggs and cysts. Iñiguez also used RAPD-PCR techniques to recover ancient DNA from coprolites. Nucleotide sequencing and hybridization resulted in the recovery of DNA sequences consistent with *Shigella flexneri*. She also found that silica purification was needed with coprolites to eliminate the inhibitory substances in feces that limit a DNA enzymatic reactions.

Cantarino approached the PCR problems inherent in mummified tissue. Specifically she looked for *Leishmania* DNA in preserved rodent museum specimens. The rodents were collected in the 1950s during a search for plague reservoirs in northeast Brazil. Many of the 60,000 rodents in the collection were from areas in which *Leishmania* was endemic. Therefore Cantarino reasoned that some of the rodents were possible infected with *Leishmania*. Skin samples from 20 rodents were analyzed and *Leishmania* DNA was recovered from two. Thus, Cantarino was able to devise a recovery technique that can also applied to human mummies.

The possibilities of molecular analysis are promising. In the future, these techniques will be applied to mummies and coprolites for the recovery of DNA. This is especially useful for tracing the distribution of protozoan parasites.

Thus, as we proceed into the Twenty-first Century, paleoparasitology is a field that deals with the archaeology of parasitism, or archaeoparasitology (Reinhard 1990). We are now expanding our studies to elucidate the prehistoric ecology of disease. We foresee that paleoparasitology in the near future will include excavations of prehistoric sites designed to recover data specific to understanding the proliferation of parasite infections. Such excavations will include sampling house floors for eggs of parasites, sampling of architectural remains for evidence of parasite vectors, examination of irrigation features for evidence of intermediate hosts, and through evaluation of sanitation practices. In fact, we are just beginning such a project in northeastern Brazil. Incorporated in these studies are mummies and other human corporeal remains.

Mummy studies will play an important role in the development of the archaeology of parasitism. The most significant recent mummy research from this perspective is that of Jane Wheeler, Katharina Dittmar and their colleagues who are working with animal mummies from southern Peru (see Dittmar, this volume). These studies have already produced exciting evidence of parasitism. Leguia and Casas (1998) have identified four nematode species and one protozoan species in llamas and alpacas. In addition, ectoparasite studies revealed two species of lice from llamas. The importance of these llama, alpaca, cuy, and dog mummies relates to the evaluation of animal domestication on the evolution of parasitism. This has long been a topic of scholarly polemics, but Wheeler and her colleagues hold the potential of actually evaluating the interrelations between animals and humans in the prehistory of parasitism.

The Future of Paleoparasitology Heresy

It is satisfying for us to see that our heresy of the past is now widely accepted truth. An example of the impact of our work is represented in the perspectives of ancient New World parasitism presented in two books by Robert Desowitz. In 1981, *New Guinea Tapeworms and Jewish Grandmoth-*

ers: *Tails of Parasites and People* was published. In that book, Desowitz acknowledged that only pinworm parasitized New World Indians before Columbus. In 1997, *Who Gave Pinta to the Santa Maria?: Torrid Diseases in a Temperate World* was published. In the later work, Desowitz acknowledges the fact that paleoparasitology, and specifically mummies, had conclusively demonstrated that hookworm and other parasites were present in prehistoric South Americas.

But what other parasites will we find? The heretical hypothesis that first stimulated Ferreira to explore paleoparasitology was presented by Magalhães and Dias (1944). They suggested that *Schistosoma mansoni* had a prehistory in the New World. This was further discussed by Paraense (1959). Ferreira (1988: 8) writes, "Talvez pelo fato de ser herética, a idéia me fascinou. Sempre senti profunda admiração pela coragem dos hereges". (Perhaps for the fact of being heretical, the idea fascinated me. I always felt deep admiration for the heretics' courage). The data we have accumulated over the past twenty years has dispelled the old beliefs that there were no significant parasitic diseases in the prehistoric New World. This revelation allows us to entertain even more heretical thoughts as to what parasites were present in the ancient New World. It even allows us to speculation on the evolutionary origins of human parasites. Could it be that a species of hookworm evolved in the New World? Was the New World really malaria-free before European colonization? Was there an endemic parasite fauna in the New World with sibling species of Old World parasites? These and other heretical questions we can now entertain and address with paleoparasitological analysis of mummies, especially with molecular techniques.

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