

Reprinted from: Ortner, D. J. and A. C. Aufderheide, 1991 Human Paleopathology: Current Syntheses and Future Options. Washington: Smithsonian Institution Press.

## Trends and perspectives in paleoparasitological research

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This report presents some methodological questions involved with our research on parasites from archeological material in Brazil. Our investigations deal mainly with parasitological findings in human and animal coprolites from South American archeological sites and rarely with mummies, since, for paleoecological and paleoanthropological reasons, mummies are not common in Brazil. A review of helminths in mummified human remains has been presented recently (Horne 1986:4-5).

The first finding of parasites in archeological material was in 1910, when Ruffer found *Schistosoma haematobium* eggs in renal tissue of Egyptian mummies, and paleoparasitology has been growing ever since as a scientific discipline. The term "paleoparasitology" was first used by Jean Baer (1971:317), although he mentioned it only parenthetically, commenting on the study of the coevolution of hosts and parasites. It acquired its definitive meaning after the first paper of Ferreira et al. (1979) and is widely used today to characterize the study of parasitic forms in archeological material. After some decades of research, interesting findings have been obtained, and new questions arise concerning the interpretation of these findings. In this report we relate our experience regarding the use of some methods in paleoparasitological investigation as well as difficulties in the interpretation of the data.

In a recent review, Reinhard et al. (1988) discussed the principal techniques for isolation of parasitic forms from coprolites in soil and fecal deposits from archeological sites, and thus these will not be commented upon here.

In paleoparasitology as well as in paleopathology sensu stricto, the main methodological question is the reliability of the diagnosis of the material. Our experience primarily involves the study of eggs and larvae of intestinal parasitic helminths found in archeological material from South America. These differ to some extent from those in the Old World material (see below).

The methodological issues with which we deal involve three main aspects: (1) identification of the zoological origin

of the material found (human or animal?); (2) recognition of the possible morphological alterations in the parasitic forms resulting from the desiccation process in archeological deposits or from other physical and biological events during many centuries; (3) better techniques for studying parasite morphology, aimed toward their specific identification.

It is necessary to stress that the approach to these questions is based on knowledge from zoological and morphological sciences, biometrics, electron microscopy, and biochemistry. We will comment only on analysis of helminths, since the other common intestinal parasites, the protozoans, are poorly preserved and can rarely be found.

The first problem faced by paleoparasitologists is the identification of the origins of coprolite material found free in archeological sites, that is, outside mummified bodies. It must be stressed there is an important difference regarding the contents of parasite-containing archeological sediments from the New World and the Old World. Because the latter sites are mostly historical and urban, the possibility of misdiagnosis lies between human coprolites and fecal material produced by domestic animals. In the American sites, at least those from South America, human coprolites have been found in places which could have been also occupied only by wild animals, since the South American Paleo-Indian did not domesticate animals. Therefore we are doing surveys at the archeological sites in the semi-arid regions of Brazil to increase our knowledge of the morphological aspects and contents of the feces of recent local fauna, basically the same animals as from the prehistoric Holocene. This approach was initiated by Fry (1977:7) and is being used to describe the size, form and contents of fresh animal feces as well as feces naturally and artificially desiccated. With this we intend to prepare a catalogue to serve as a guide for the identification of coprolites. So far the results are encouraging because of the peculiarities of South American fauna in general, particularly at Brazilian excavation sites where there are few large omnivorous or carnivorous animals whose feces could be more easily misidentified as those of human origin.

However, for the assessment of the origin of coprolites we must not forget other parameters, such as their food content as well as parasite composition known to be typical for the human host. Such parameters also include biochemical studies which, although still in their infancy, certainly became an important option after the identification of steroids from 2000-year-old North American coprolites (Lin et al. 1978).

A second problem relates to the possible structural modifications found in parasites contained in fecal material from archeological sites. Under the influence of environmental factors, parasite remains could undergo deformities and/or size modifications making their identification difficult or even impossible. Such physicochemical processes could destroy parasitic forms in the fecal mass; these phenomena seem to be responsible for the scarcity of findings of protozoan cysts in coprolites. The first attempt to solve this problem came from the experimental approach inaugurated by Adamson (1976) when he assessed the persistence of eggs of *Schistosoma* sp. in artificially desiccated tissues in order to evaluate the actual frequency of these findings in Egyptian mummies. This approach was then extended to several normal tissues as well as to soft tissue lesions (tumors, for example) by Zimmerman (1972, 1977, 1978) in an effort to assess the possibilities of histopathological diagnosis in mummified bodies. More recently we started to study the morphological modifications which occurred in helminth eggs and larvae after artificial desiccation and rehydration of fresh feces using different techniques. We have tested whether helminth eggs, such as those of the nematode genus *Trichuris* sp. (Confalonieri et al. 1985) and ancylostomids (Araújo 1987), whose diagnosis depends not only on qualitative morphological characteristics but also on size variations, would undergo significant alterations in their dimensions.

So far these experiments have demonstrated that the desiccation process does not cause deformities in these biological structures that would hinder an adequate identification.

Finally, detailed morphologic study of parasites found in ancient material should be considered in qualitative as well as in quantitative aspects. In the former case, the differential diagnosis rests on detectable microscopic differences of closely related taxa. For this purpose the best technique is scanning electron microscopy, which can reveal variations in the surface relief of eggs and larvae of helminths. A comparative morphological study using this technique is presently underway in our laboratory. It focuses on larvae of *Ancylostoma duodenale* and *Necator americanus*, the most common human hookworms whose desiccated forms cannot be easily separated with the light microscope.

Diagnosis of some parasites depends on biometric evaluation. This includes the ova of *Trichuris*, the helminth most commonly found in South American coprolites, but also very common in European archeological deposits. These are being studied with some taxonomic techniques, such as the Student t-test, for small samples (Sokal and Rohlf 1969:223).

The test was applied to the identification of eggs of this genus from small fragments of coprolites from South American sites (Confalonieri 1988) under circumstances in which morphological criteria to the identification of the fecal material cannot be used. In such cases in which several species of *Trichuris* have overlapping size ranges, including the human *T. trichiura*, the statistical procedure is useful since it indicates in probabilistic terms the possibility for human origin of the material.

We are using, again with *Trichuris* eggs, a new biometric parameter for a better discrimination of the different species. This is the linear regression coefficient between length and width of the eggs, already used by Joyner and Norton (1980) in the specific diagnosis of protozoan oocysts. Thus we can add another variable for a more complete morphological evaluation of the egg of each species. This parameter was shown to be especially useful for the differential diagnosis of eggs of *T. trichiura* and *T. suis*, two sister species commonly associated in archeological material from Europe (Jones 1982).

In summary, advances in techniques and methods applied to paleoparasitological investigation are the result of new approaches from biomedical and zoological sciences. In the future these will provide a greater reliability for identification of parasitic diseases in pre- and protohistorical populations.

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