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A CONTRIBUTION TO THE STUDY OF HELMINTH FINDINGS IN ARCHAEOLOGICAL MATERIAL IN BRAZIL[1]

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(With 8 text-figures)

The study of ancient faeces or coprolites is particularly interesting as it is performed presently aiming the knowledge of ancient people habits and their parasitic diseases.

The coprolite analysis is not so recent and in the past century, Harshberger (1896) pointed that seeds that were found in human coprolites could give some informations about food habits of prehistoric populations.

We must distinguish two kinds of coprolites: the mineralized, of long geological age and those preserved in organic state (Heizer & Napton, 1969).

Since the development of Callen & Cameron's (1960) rehydration technique, the microscopic analysis of coprolites has allowed the finding, in increased numbers, of parasitic forms.

Thus, paleoparasitology emmerged as a branch of paleopathology in the moment that parasitic forms were sought in archaeological material.

We can refer some reviews and annotated bibliographies about the subject. Häntzchel *et al.* (1968) reviewed a long list of papers, mainly mineralized coprolites of animal origin; Pike (1967), Gooch (1972), Wilke & Hall (1975), Fry (1977) and Araújo (1980) made also reviews about the study of parasites in archaeological remains, but the list of publications are only about coprolites preserved in organic state and the basic subject are the human coprolites.

The presence of parasitic diseases among ancient populations was also inferred by examining mummified tissues (eg. ex Ruffer, 1910 and Cockburn *et al.*, 1977) or the intestinal contents of mummies of the old and New World (Szidat, 1944; Pizzi & Schenone, 1954; Helbaek, 1968; Ou Wei, 1973 and Allison *et al.*, 1974).

This line of research is only in its first steps in our country (Ferreira *et al.*, 1979; Araújo, 1980) and in this paper we report the oportunity we had to examinate coprolites collected in caves of the State of Minas Gerais. The samples are of human and animal origin and the parasitic eggs found stimulated some considerations about their introduction as well as of their hosts, in South America, before the coming of Europeans and Africans.

MATERIAL AND METHODOS

The coprolites analysed were collected in caves of Minas Gerais by archaeologists and sent to us with the identification, strata and datation already done.

The description of the archaeological sites and samples are as follows:

Gruta do Gentio II (site MG RP6) – At Unaí, on the northwest of the State of Minas Gerais, Central Brazil. The climate of the region

[1] Received August 26, 1980.

is dry, with the rainy season from October to April. The vegetation is predominantly that of a savannah type.

The cave is located in a calcareous bank 2.5 km long; the entrance is about 6 m above the soil level and its main chamber measures 10 m x 14 m and about 3 m high, close to the entrance. On the roof and walls paintings of animals, anthropomorphic figures and simbols in red, black and yellow can be observed.

The archaeological studies performed up to the present time detected two moments of the cave occupation: the oldest one is represented by hunter-gatherers that left stone, bone and wooden artifacts., That layer is dated by the ^{14}C method of 8,125 ± 120 BP (Smithsonian Radiation Laboratory-SI-2373).

The findings of the more recent horizonts suggests an occupation by ceramists-agriculturists that left artifacts such as wooden art objects, feathers, snail shells and food remains (peanuts and maize). The site was used also as a burial place and thus the datations obtained for this period were disturbed by the soil remanegement. The radiocarbon analysed from this strata revealed an occupation period ranging from 3,490 ± 120 BP to 430 ± 70 BP (SI – 3521).

The coprolites were collected from this last period and sent to us by the professors Ondemar Dias and Eliana Carvalho from the Instituto de Arqueologia Brasileira, Rio de Janeiro.

We received 38 samples with 50 units that, during excavations, were identified as coprolites. Of these 50 units, 36 proved to be coprolites. The others had only mineral contents, although their shape and size were similar to the coprolites.

The coprolites had not odour and some of them were cilyndrical (Fig. 1) and others, with variable sizes (3 to 10 cm) were amorfs, mixed with earth.

Grande abrigo de Santana do Riacho – At Santana do Riacho, Central region of the State of Minas Gerais. It is located in a calcareous bank and was used by indian groups as a site for habitation and burial place. There have been identified two levels of occupation where several bonfires and painted stones were distributed.

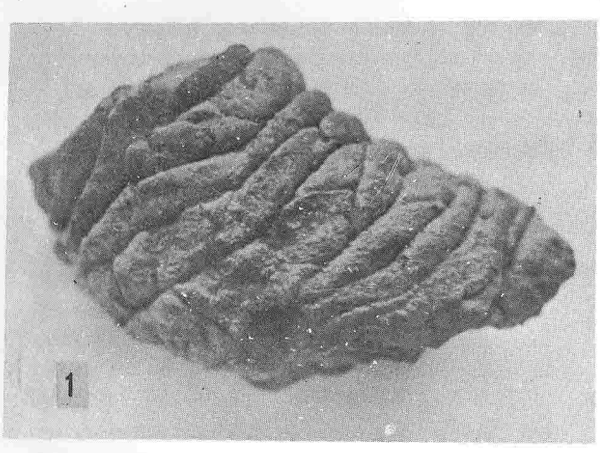
The layer I is dated from 3,990 ± 70 BP to 4,340 ± 70 BP by the radiocarbon. The layer III is composed by 3 levels of burials and one of them is dated of 9,640 ± 110 BP. The occupation of the site can be divided in 3 period: a) Recent period: from the present to 5,000 BP b) Ancient period: composed by 3 levels of burial and lithical industry, dated from 5,000 to 10,00 BP; c) A more ancient period where only fir remains were found (Junqueira *et al.*, 1979).

The coprolites of Santana do Riacho wer collected from the ancient period level in clos association with the burials. The coprolites wer well preserved and is noticeable the remanegemen of the earth in little tunnels (galleries) that lea to "nests" where the coprolites were grouped i great numbers.

We received 10 samples from Dr. Andr Prous, from the Museu de História Natural d Universidade Federal de Minas Gerais, 4 of whicl were not coprolites; the other 6 were compose of 50 to 200 units of cilyndrical form, dark with brilliant spots on a smooth surface, 1 to 3 cm long and with thin extremities (Fig. 2). We received also form Dr. Prous suspected materia from Lapa Vermelha IV, Lagoa Santa and Lap Pequena, Montes Claros, both in the State o Minas Gerais, but the material after analysis proved not to be coprolites or, if so, could not be identified.

In the laboratory each sample received a number and if composed by more than one unit, the letters A, B, etc were added to the number. After that, from each coprolite was separated one fragment of about 3 cm and, in the case of little coprolites, the whole sample was used. The remaining was replaced in the original jar. Each fragment was placed in a transparent glass jar with a lid to close it hermetically and, following Callen & Cameron's (1960) technique, poured the 0,5% aqueous solution of trissodium phosphate ($Na_3PO_4 \cdot 12\ H_2O$), sufficiently to cover the material. The minimum time for rehydration is 72 hours but if longer, it is possible to grow fungal and bacterial contaminants. To prevent this it is advisable to add formol-acetic solution. After 72 hours part of the material was separated and the remaining preserved.

The bigger fragments were then separated under an entomological microscope and the parasitological examination was performed by the spontaneous sedimentation method developed by Lutz (1919). Slides were made at regular intervals and the last one 24 hours after the beginning of sedimentation. The remaining sediment was



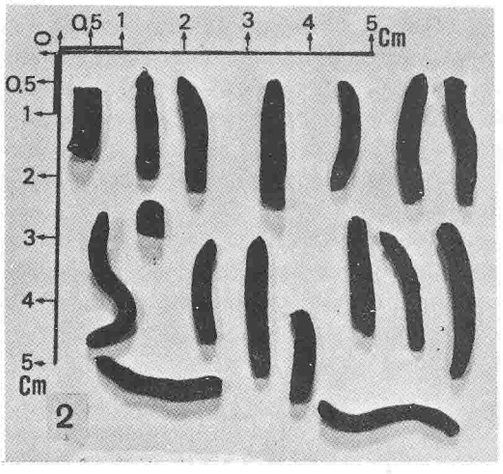


Fig. 1 – Human coprolite from Unaí Cave. Fig. 2 – Coprolites from Santana do Riacho.

aspirated and preserved in formol-acetic solution. The slides are still preserved for microscopic analysis by using the following technique: 1) Add to the sediment drop some fragments of triturated cover slip. 2) Cover it with a cover slip. 3) Using sealing-wax prepared with bee wax (one part) and tar (9 parts), carefully close all the edge of the cover slip with an iron-lute handle (Langeron, 1942). The slides must be placed in horizontal position and protected from light and dust.

RESULTS

The coprolites were separated from lumps that looked like excrements on the basis of its form, size, colour and odour of the trissodium phosphate solution after rehydration, as was postulated by Callen & Cameron (1960). The coprolite contents were also observed, like food ramains and parasite evolutive forms (Callen, 1967). Thus, we obtained:

Gruta do Gentio II: 36 coprolites; 14 lumps.

Of 36 coprolites, 10 showed evolutive forms of parasites:

a) Small larvae of yellow brown colour, blunt anterior extremity and thin end. The internal structures were not well visible, but it was possible to see the oral orifice, the beginning of the intestinal tube and an helix structure that is, probably, its continuation (Fig. 3).

b) Larvae of intermediate size with form and structures like the first described. Its size is aproximately twice that of the anterior (Fig. 4).

c) Sheathed larvae: in this one, which is apparently better preserved, when examined with more attention it is impossible to distinguish the internal structures. They are all rolled up and covered by a sheath that in some places in wrinkled. The anterior extremity is round and the tail very thin (Fig. 5).

d) Eggs of ellipsoid shape and thin membrane. Inside some of them there is a larva similar to those described firstly (*a*) (Fig. 6). Inside others there is something like an embrionary mass. Eight eggs were found in only one coprolite and they measured 73,20 ± 9,16 μm x 40,20 ± 5,09 μm. They were identified as Ancylostomid eggs for the reasons we will discuss later.

e) Brown dark eggs, with double membrane and translucent polar plugs. These eggs, found in two coprolites, measured 55,33 ± 2,22 μm x 28,85 ± 0,09 μm and were identified as Trichurid eggs (Fig. 7).

All the 36 coprolites turned the trissodium phosphate solution to dark brown colour, nearly black, opaque. They showed vegetable remains, pollen, bone fragments, coat and briliant black fragments which were identified as charcoal. Nothing of this occurred with the lumps and although the solution turned brown it remained translucent and smelled like wet earth.

One of the samples, number 24, was of bright colour and, when rehydrated, the colour of the solution was also bright, although opaque. The contents were identical to the other samples including the Trichurid eggs that were also pale.

Grande abrigo de Santana do Riacho: 4 samples, each with one unit, were not coprolites. The other 6, each one with 50 to 200 coprolites, showed under microscopy, several insect fragments and helminth eggs of a dark-brown colour (Fig. 8). The eggs measured 80,57 ± 4,14 μm x 39,21 ± 3,67 μm (10).

Rounded forms like protozoal cysts, were noted in some slides, but is was impossible to confirm this diagnosis for the supposed cysts did not appear again, although we used Ritchie's method and iron-hematoxylin.

DISCUSSION

The rehydration technique by trissodium phosphate proposed by Callen & Cameron (1960) permits the reconstitution of dried fecal material, changing it to the consistence of recent faece and making possible the microscopic examinatio for parasite research using well-known methods.

Callen (1967) stated that no test i suficiently accurate to stablish the human origi of a coprolite. However, when the rehydratio technique by trissodium phosphate is used an turns the solution to opaque dark-brown or black with an offensive odour after 72 hours, accordin to the author, this suggests human coprolites.

Heizer (1970) compared the analysis mad in dry and rehydrated coprolites by trissodiun phosphate and concluded to the superiority o the last method.

According to Fry (1977) there is no absolut test to determinate the human origin of coprolites only when they came from mummified bodie or exclusively human parasites were found. He

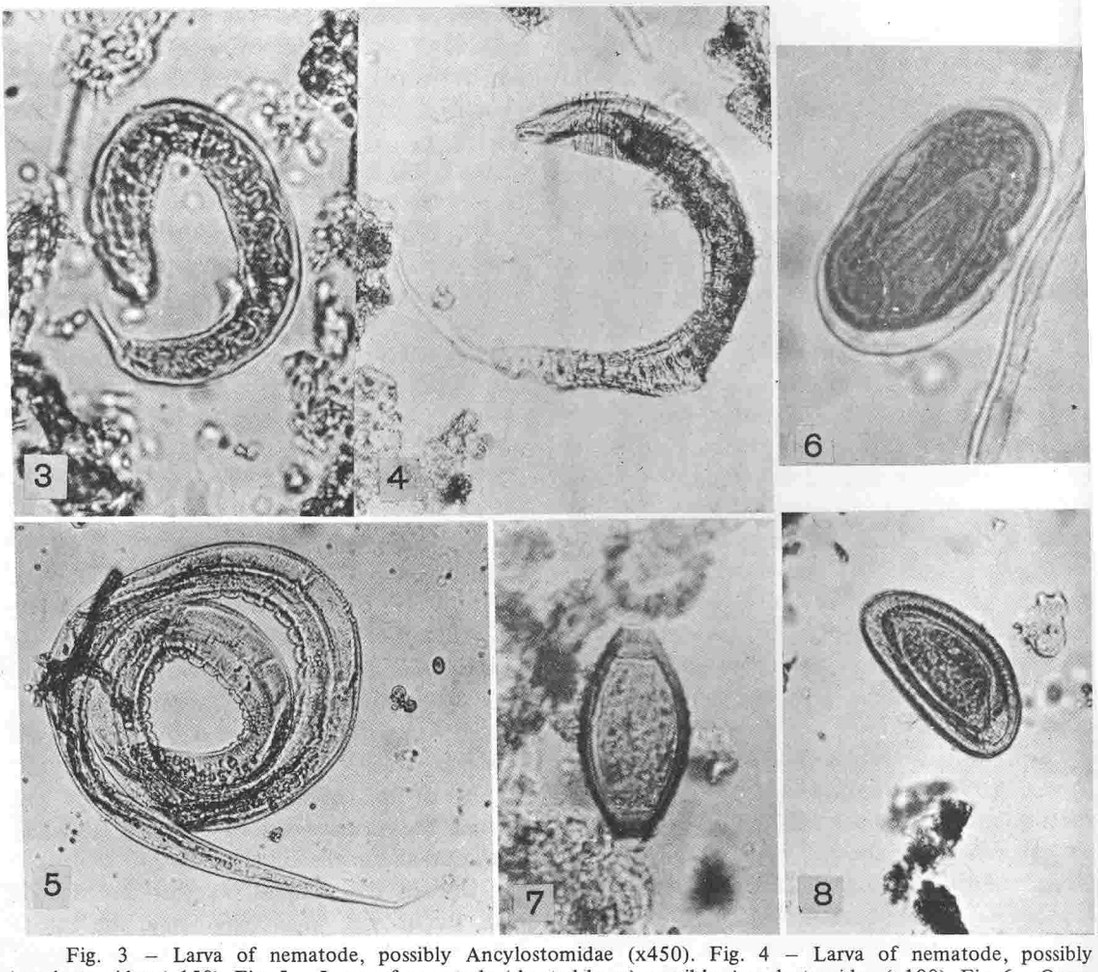


Fig. 3 – Larva of nematode, possibly Ancylostomidae (x450). Fig. 4 – Larva of nematode, possibly Ancylostomidae (x150). Fig. 5 – Larva of nematode (sheated larva) possibly Ancylostomidae (x100). Fig. 6 – Ovum of Ancylostomidae (x150). Fig. 7 – Ovum of *Trichuris trichiura* (x150). Fig. 8 – Ovum of *Parapharyngodon* sp. (x150).

examined one of parameters stablished by Callen (1967) and studied the effects of recent faeces of zoological garden animals and man on the rehydration solution. He concluded that the only animal with faeces that can turn the solution opaque and dark-brown like the human faeces is the coati (*Nasua nasua*).

The 36 coprolites from *Gruta do Gentio II* were considered of human origin for the following reasons:

a) Shape and size: Only two coprolites had the aspect of moulded faeces. The others were amorphous with earth on the surface, looking like agglomerated faeces. For this reason, the shape is not a safe parameter for the slugs were similar in shape to the coprolites.

b) The colour of trissodium phosphate solution: All the 36 coprolites turned the solution into the colour atributed to human faeces except one. The contents, however, were similar to the others (vegetable, pollen, bone fragments, charcoal) as it also contained parasite eggs as is discussed below. This appearance, that is, the lack of colouring, could be explained by a possible decrease of biliar secretion due to some pathology.

c) Smell: All 36 coprolites, after 72 hours in the solution had a foetid odour, as opposed to the 14 slugs that presented the characteristic smell of wet earth. In addition, the solution with coprolites, if not added acetic formalin, presented fungal and bacterial proliferation.

d) Contents: Beyond the food remains and pollen that are preserved for examination, 27,7% of the coprolites showed evolutive forms of parasites.

In 9 of 10 positive we found larval forms of 3 different sizes whose characteristics, mainly the sheathed larvae, made us consider them as evolutive stages of a nematode. The finding of eggs with compatible morphology to that of the Ancylostomids and the presence, inside them of larvae identical to that interpreted as the first stage, reinforced this hypothesis.

The measurements of the eggs are compatible with those of the *Necator americanus* eggs, although the deformation suffered by the majority do not assure a positive identification of the species involved.

In two coprolites, including one with Ancylostomid eggs and larvae, we found *Trichuris trichiura* eggs. The diagnosis was made based on its shape and size and the occurrence of the human whipworm in the same coprolite where suspected Ancylostomid eggs were also present conffirms its human origin.

e) The coprolites were found in human occupation layers as the cultural ramains collected showed.

It is important, when considering the parameters above, the interelation among them. Thus, if we could not be certain of the human origin of one coprolite by its shape, colour of the solution and smell, the microscopic examination will be more elucidative. The presence of human parasites will add a fundamental basis to the analysis.

The coprolites of the Gruta do Gentio II were considered of human origin for they possessed, beyond the dark colour, opacity and foetid odour, helminth eggs whose characteristics were similar to the human whipworm and hookworm, and the fact that they were collected in an indian occupation area dated of a period ranging from 3,500 to 500 BP.

The presence of a human hookworm, probably *Necator americanus*, among brazilian indians in ages before the European and African penetration, make us discuss the introduction of human parasites in our continent.

Schistosomiasis and Ancylostomiasis are commonly referred as parasitic diseases imported from Africa with the slave trade.

The African origin of *Schistosoma mansoni* is categoricaly affirmed by Freitas (1935) who dated its penetration in our country in the XVII century, based in a text of Piso (1648). However, Magalhães & Dias (1944) argued about the possible existence of Schistosomiasis among the indians, before the discovery of Brazil by the Portuguese.

Paraense (1959) commented on the African origin of Schistosomiasis, concluding that it is not so evident and the actual distribution of the 3 human species of *Schistosoma* suggests the possibility of the existence of an evolution center separated for each one in the past, and thus *Schistosoma mansoni* could be originally from South America.

We think that the finding of parasitological proofs in archaeological material will bring the definite answer to this discussion.

The origin of Ancylostomiasis in Brazil has also been discussed and Darling (1921), based on

geographical distribution of human hookworms, suggested that the American man could come from: a) A population without Ancylostomids derived from Asiatic ancestors, coming by the Behring strait. b) A population with mixed infections of *Necator americanus* and *Ancylostoma duodenale.* c) A population with a pure infection of *Necator americanus* derived from Polynesian or Indonesian ancestors, coming from transpacific migrations. Smilie (1922) situated the coming of hookworms to South America, more specificaly to Brazil, by conclusions of the prevalence of *N. americanus* in Africans and *A. duodenale* in Europeans. Thus, a great dissemination of *N. americanus* was made by the African slaves on an insignificant population of *A. duodenale* spreaded by the Portuguese. With imigrations from Japan and Italy the last nematode reached the rate *N. americanus.*

Darling (1921) and Soper (1927) were interested on the presence or absence of human hookworms in South America in prehistoric times and also on the possible routes of migration that their hosts followed.

Manter (1967), based on archaeological findings that indicate a transpacific contact in 3000 BC, between Japanese fishermen and South America and of an Asiatic culture with Equador in 200 BC, said that the two species of Ancylostomids probably had come separated from Asia to America in ancient times.

Fonseca (1972) discussed parasitism and prehistoric human migrations and, among the parasites studied are the Ancylostomids. He used to reinforce his impressions the chronicle of Gabriel Soares de Sousa (1587) who observed a typical clinical picture of Ancylostomosis among the indians.

To this annotation we can add another, of del Castillo (1927) when this chronist visited, in 1519, the city of Tenochtitlaú, the Aztec central Empire, when he noted the custom of the indians to make "breads" of certain special argila that they found in the lake and was "similar" to cheese.

This is very interesting for we can see still today, in Brazil's interior, the practice of selling little clay bricks to be consumed by people with anaemia.

The confirmation of the presence of human hookworms in prehistoric times was made by Allison *et al.* (1974) when they found adults of *A. duodenale* in the small intestine of a Peruvian mummy, and here in Brazil, by Ferreira *et al.* (1979) when they described Ancylostomide eggs in human coprolites of 3,500 to 500 years old.

If Ancylostomosis were already in South America among indians, the parasite came with their hosts by another route than that of the Behring strait, for, as Darling (1921), Soper (1927) and Fonseca (1972) pointed out, the Ancylostomid larvae would not resist the low temperature of the soil.

Fonseca (1972) when referred to Smilie (1922) observed that this author found a rate of one *Ancylostoma* to 54 *Necator* in an indian population and as he examined the neobrazilian population of the area, the relation increased to 1 *Ancylostoma* to 194 *Necator.* Fonseca (1972) concluded that first there was a penetration of *A. duodenale* in South America in prehistoric times, and after, an introduction of *N. americanus* by African slaves in historical times.

When we found Ancylostomid eggs whose measurements are compatible to that of *N. americanus*, in 3,500 to 500 years old human coprolites in our country, we stablish another origin, not African, for this parasite.

It is necessary to note that the slave and the colonization by Europeans and Japanese increased and contributed to the dispersion of the Ancylostomids in our territory, but is was the transpacific migrations that introduced them here.

The Asiatic migrations and the origins of South American man are discussed by Meggers & Evans (1966) and Meggers (1979), who compared the archaeological findings in South and Central America with those of India and Japan.

It is important to add to this discussion a negative finding, the importance of which is noted by Fonseca (1972): the lack, in our samples, of *Ascaris lumbricoides* eggs. Beaver (1964) in Oceania and Soper (1927) in Paraguay also noted this in isolated tribes. This can be used as a counter proof of oceanic origin of certain South American populations.

The numerous coprolites from Grande Abrigo de Santana do Riacho are of the same animal species, since they had the same shape and

Parapharyngodon sp. (Nematoda, Oxyuroidea) were also found in lizars's coprolites from Grande Abrigo de Santana do Riacho, dated nearly 10,000 years.

The presence of eggs of human kookworms in coprolites of a period dated before the penetration of Europeans and Africans to the country's interior, confirms a transpacific origin of certain south-american populations.

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