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# Parasite findings in archeological remains: Diagnosis and interpretation

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# Parasite findings in archeological remains: Diagnosis and interpretation

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**Abstract**

Paleoparasitology has contributed to resolving the debate about the peopling of the Americas and determining the antiquity of human parasite infection. Hookworm (Ancylostomidae) and whipworm (*Trichuris trichiura*) and other exclusive human intestinal parasites have been recorded in pre-Columbian America. These parasite species originated in pre-hominids and have accompanied humans across continents when people went out of Africa. However, for those human populations that crossed the Bering Land Bridge from Siberia to Alaska, cold climate conditions hampered parasite transmission. Alternative migration routes have been proposed to explain the presence of these parasites in pre-Columbian populations in the Americas. Other parasites were established in the New World long before humans entered the American continents.

One such malady is Chagas disease. Chagas disease, caused by *Trypanosoma cruzi*, offers an example of how animals and humans have interacted in the past. Classical theory points to the origin and dispersion of human *T. cruzi* infection among Andean populations, starting with sedentary habits and animal domestication 6000 years ago. However, recent PCR results in mummified bodies outside the Andean region have challenged this theory. Pre-Columbian Brazilian mummies were found positive for *T. cruzi* infection, raising an alternative hypothesis on the antiquity of Chagas disease in the Americas. Paleoparasitology is a new tool to study past events, shedding light on human and other animal behavior, migration routes, diet, and other aspects of host–parasite environment evolution.

## 1. Introduction

Paleoparasitology has contributed to the understanding of events in the past by discoveries of parasite remains in archeological material. Presented herein are two remarkable findings of human and other animal parasites that have added new data to the knowledge of pre-historic populations in the Neotropical region. Data address both human and animal parasites, focusing an migratory, climate influence, and cultural habits in the past.

## 2. Intestinal parasites and the peopling of the Americas

Parasites of humans and other animals can be very specific. Some of the human parasites are considered of having been inherited from human ancestors and would have been shared previously by pre-hominids and modern ape ancestors. On the other hand, humans, along with their biological and social evolution, have continuously acquired other parasites.

*Trichuris trichiura* (whipworm) and ancylostomids (hookworms—*Necator americanus* and *Ancylostoma duodenale*) are intestinal worms that infect humans. They were probably inherited from African ancestors and may have been accompanying humans since *Homo* species appeared on Earth (Araújo *et al.*, 2003). These parasites originated from a phylogenetic pathway, and are very specific to humans and their close relatives, and are not found in other animals in natural conditions.

Today, hookworm and *T. trichiura* infection are commonly found in tropical and subtropical regions. *T. trichiura* adult worms live in the large intestine. Eggs are passed with feces and develop in the soil for 2 weeks until it becomes infective to another human host. Adult hookworms live in the small intestine and eggs are also passed with feces. In the soil, larvae hatch from the eggs and molt three times until it becomes infective after 10–14 days. Both parasites need special environmental conditions to complete their life cycles. Eggs and larvae need soil temperature ranging around 22 °C to evolve (Roberts and Janovy, 2000).

Along with hookworms, *T. trichiura* dispersed out of Africa accompanying its human hosts wherever soil and climate conditions have been favorable. Due to biological necessities to maintain their life cycle and to be transmitted from host to host, proper environmental conditions needed to prevail wherever humans had settled.

Therefore, the first pre-historic migrants who crossed the Bering Land Bridge from Siberia to Alaska to peopling the Americas would have lost these parasites (Araújo *et al*., 1981, 1988). Cold climate conditions prevailed in the high latitudes of Asia and North America during the time humans crossed from one continent to another (Montenegro *et al*., 2006).

However, hookworm and whipworm eggs were found in human coprolites dating up to 7230 years ago in South and North American archeological sites (Ferreira and Araújo, 1996; Gonçalves *et al*., 2003). Paleoparasitological evidence is abundant (Reinhard *et al*., 2001) but human migrations crossing the Bering region could not have introduced these parasites in the Americas. Alternative routes were proposed to explain the infection in the Neotropical regions occupied by pre-Columbian populations (Araújo *et al*., 1988).

An intensive debate arose regarding hookworm egg diagnosis (Kliks, 1982; Fuller, 1997) and possible maintenance of parasite infection in pre-historic migrants during the Bering region passage from Asia to the Americas (Hawdon and Johnston, 1996). Paleoparasitologists offered arguments based on biological and anthropological aspects (Ferreira *et al*., 1983; Ferreira and Araújo, 1996; Faulkner and Patton, 2001; Reinhard *et al*., 2001), and finally Montenegro *et al*. (2006) modeled climate conditions in the Arctic region showing the impossibility for the infection to persist under the low temperatures prevailing at the time accepted for the peopling of the Americas. Transpacific contacts or coastal migrations were proposed to explain the introduction of the parasites (Araújo *et al*., 1981, 1988; Montenegro *et al*., 2006).

Hookworm disease is characterized by anemia and depletion. Symptoms appear and are worse when the individual has a low intake of iron and other nutrients. Because of insufficient data, at this point researchers have not been able to associate hookworm infection with poor health in pre-historic human populations. However, Reinhard (1992) and Reinhard and Bryant (2007) showed a consistent association between porotic hyperostosis and helminth infection in Ancestral Pueblo population in Arizona. Hookworm disease and porotic hyperostosis may have a correlation, but data are still too limited for any conclusion. Therefore, further studies are needed.

Paleoparasitology quantitative techniques have been assayed to estimate parasite loads and the impact of disease in Paleo-Indian North American populations (Reinhard, 1992). Intestinal parasite eggs were found abundantly in agriculturalist pre-historic populations living in agglomerated and sedentary conditions, while they are scarce in hunter-gatherers groups, both in South America (Ferreira *et al*., 1989) and in North America (Hugot *et al*., 1999).

Regarding prevalence differences, in the Neotropical region parasite load in pre-Columbian populations seemed never to have reached the high prevalence rates recorded in Europe during equivalent periods (Bouchet *et al*., 2003; Fernandes *et al*., 2005; Le Bailly *et al*., 2006). Paleoparasitological record showed that primeval inhabitants of the Neotropical region were already parasitized by the common intestinal parasites (Gonçalves *et al*., 2003), but prevalence rates were low. Therefore, it was only after the coming of Europeans and Africans that intestinal parasites became a public health problem, with increasing prevalence rates. Analyses of the earliest colonial sites confirm this hypothesis for North America (Reinhard *et al*.,1986). Data from Spanish and Portuguese colonial period have been collected with the aim of evaluating the epidemiological transition during European occupation (Fugassa *et al*., 2006).

Parasites are biological markers of human–pathogen evolution, and paleoparasitology record can be used to understand human life in the past. Some species of parasites have accompanied their human hosts since they went out of Africa and colonized other continents. However, climate conditions were ever-limiting factors for some parasite species to be transmitted. Therefore, humans lost some intestinal parasites when they went to the high latitudes of Asia and crossed the Bering Land Bridge to North America. As evidenced by paleoparasitology record, hookworm and whipworm eggs were found in human coprolites in different archeological sites in Neotropical region, dated from 1,100 to 7,200 years before present (BP) (Reinhard *et al*., 2001). Consequently, it is suggested that they were introduced with human hosts by migration routes other than the Bering region (Ferreira and Araújo, 1996).

## 3. Chagas disease dispersion in pre-historic America

*Trypanosoma cruzi* is a protozoan that causes an infectious disease in humans. The disease is characterized by intestinal and cardiac symptoms. Known as Chagas disease, the parasite is transmitted to humans by insect vectors and considered to have appeared only after the advent of plant and animal domestication and the establishment of sedentary habits in the Andean region. Hypothetically, it was only after European colonization that infection would have spread to other parts of South America (Coimbra, 1988). However, infection in humans may predate this classical point of view, and seems to be present among Brazilian Lowland ancient inhabitants as evidenced by paleoparasitological findings.

The accepted theory concerning the origin of Chagas disease in humans is that about 6,000 years ago pre-historic people in the Andean region began to raise guinea pigs (*Cavia* sp.) as domestic animals for food, pets, or rituals, as commented by Coimbra (1988). The animals were kept inside the houses. Because of their association with human domestic contexts, these rodents became good reservoirs for *T. cruzi*, a protozoan parasite that infects blood, muscle tissue, and other cells of mammals. The parasite is transmitted from one host to another by triatomines, a blood-sucking insect. Triatomines would have been attracted to the wood and adobe houses and by the presence of rodents and humans. It is believed that *Triatoma infestans*, one of the most effective vector triatomine species, became domiciliated at that time in the Andean region (Dias *et al.*, 2002).

South American Lowlands exhibit a different environmental situation. It is believed that pre-historic South American native people living in the Amazon region and the savannah had nomadic habits, and the kind of dwellings they used hampered triatomine adaptation. This situation persisted up to now, as Chagas disease among present-day South American native populations is very rare (Coimbra, 1988).

However, we are testing the hypothesis that pre-historic hunter-gatherers were exposed to infection due to ecological conditions, different from the South American native population settled in villages. There are triatomine species that can transmit *T. cruzi* living in caves and rock-shelters sucking blood from mammal reservoirs (Borges-Pereira *et al.*, 2006). Therefore, Chagas disease could have been prevalent among ancient populations that used caves or rock-shelters as dwellings or places for rituals.

Triatomines are insects that can adapt to different habitats. Thus, environmental changes caused by anthropic interferences may alter parasite transmission. This is exemplified in the Amazon region, where the inhabitants were considered not exposed to Chagas disease until a new vector species was discovered, transmitting the parasite to humans. Autochthonous cases were recently recorded in the region (Coura *et al.*, 2002).

Although *T. infestans* may have spread only after European, and African's arrival to South American Lowlands during colonial times, when dwellings began to be built with clay and wood, other triatomine species may have been transmitting the parasite to humans living in the caves or in the forest. *T. infestans*, brought from the Andean region by trade routes (Ferreira *et al.*, 2000; Guhl *et al.*, 2000), helped thereafter in infection dispersal in South America, turning the disease into an important public health problem. However, the hypothesis is that the disease in the Lowland South American native population has predated these events.

In 1984, during the excavation of an archeological site in the Brazilian northeast (Guidon and Arnaud, 1991), we observed that archeologists were attacked by triatomines while studying rock art on the big rock-shelters walls. The species was identified as *Triatoma brasiliensis*, and the insects were in activity during the whole day, under daylight warmest temperatures of 42–45 °C, and at night, when the temperature falls to 8–10 °C. Some were found infected with *T. cruzi*. We then postulated that the ancient artists, as well as other inhabitants of the rock-shelters, thousands of years ago, were also attacked by these insects, and some of them became infected by the parasite (Ferreira *et al.*, 2000; Araújo *et al.*, 2003). It is important to note that *T. infestans* never colonized that part of the Brazilian northeast (Dias *et al.*, 2002). At that time it was impossible to test our theory, as the only human remains found were bones and coprolites, where the parasite leaves no pathological signals.

Years later, with the introduction of molecular biology to diagnose infectious diseases in ancient remains, Chagas disease was confirmed in 4000-year-old Chilean and Peruvian mummies (Guhl *et al.*, 1999, 2000; Ferreira *et al.*, 2000; Madden *et al.*, 2001). We tested techniques to be applied in mummified tissues in order to recover *T. cruzi* ancient DNA (Bastos *et al.*, 1996), successfully applied later (Guhl *et al.*, 2000; Aufderheide *et al.*, 2004). We tested protocols in desiccated rodent bones infected with different number of trypomastigote and *T. cruzi* strains, as well as at different stages of the disease, to establish parameters for DNA extraction. After establishing protocols, techniques were applied to archeological material, especially taking into account that bones are most commonly found than any other mummified organic remains. It was possible then to test the hypothesis of an ancient origin of the disease outside the Andean region, and trace a paleoepidemiology of Chagas disease (Araújo *et al.*, 2003).

Recently, a case of Chagas disease was recorded in the Southern part of North America in a mummified body found with the intestines full of coprolites, suggesting a case of megacolon due to Chagas disease (Reinhard *et al.*, 2003). The mummy was dated 1,150 years BP The case was confirmed by biological molecular techniques (Dittmar *et al.*, 2003).

New data appeared recently confirming the possibility of Chagas disease in ancient Brazilian pre-historic populations. A partially mummified body dated 1,200 years BP, excavated in Central Brazil (Prous and Schlobach, 1977), was found positive to *T. cruzi* infection by the PCR technique (Araújo *et al.*, 2005). Evidence of megacolon lesion was also found.

These preliminary data indicate Chagas disease among pre-Hispanic populations in other parts of the Americas outside the region considered as of its origin. Therefore, Chagas disease may have occurred in human populations living in the Neotropical region encompassing the region from Southern North America to the South of South America, including the Brazilian Lowlands. We aim to trace parasite dispersion using an epidemiological approach, and the consequences of human infection among ancient populations.

## 4. Conclusions

Paleoparasitology, combined with zooarchaeology, may bring interesting contributions to understand human life in the past. There are parasites transmitted directly from host to host, but others are transmitted by arthropod vectors. Infectious diseases transmitted by arthropod vectors are restricted to the distribution area where the parasite and the arthropod are in close contact with the host. Therefore, their distribution is limited by favorable environmental conditions for the vector life cycle. Combining zooarchaeology and paleoparasitology findings in archeological sites may shed light on the health and disease aspects of ancient populations (Reinhard, 1992).

Hookworm and whipworm are soil-transmitted helminths. Both require climate conditions in the soil to maintain infection in the human host. Consequently, climate aspects can be inferred when these parasites are found in archeological sites (Reinhard *et al.*, 2001). Therefore, as discussed above, helminth parasite species considered inherited from human ancestors are interesting biological markers to study ancient migrations (Ferreira and Araújo, 1996; Reinhard *et al.*, 2001).

Chagas disease in the Neotropical region is another interesting example. Although common knowledge accepts the origin of infection starting with plant and animal domestication in the Andean region circa 6,000 years ago (Dias *et al.*, 2002), paleoparasitological record showed that Chagas disease affected people in Central Brazil in pre-Columbian times. Further studies are needed, but different aspects can be explored. Insect remains began to be investigated in the search for triatomine fragments, as well as bone and teeth of mammals that are potential reservoirs for *T. cruzi*. Molecular biology techniques are especial tools to be used aiming to recover parasite genome sequences in that kind of material (Dittmar *et al.*, 2006; Pruvost *et al.*, 2007). Therefore, archaeozoologists and paleoparasitologists may cooperate in the study of ancient parasitism and human life in the past (Reinhard, 1987; Reinhard, 2006), as well as in other aspects of paleoenvironment and local fauna.

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