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One hundred years since the discovery of Chagas disease associated with Trypanosoma cruzi *infection, growing attention has focused on understanding the evolution in parasite-human host interaction. This interest has featured studies and results from paleoparasitology, not only the description of lesions in mummified bodies, but also the recovery of genetic material from the parasite and the possibility of analyzing such material over time. The present study reviews the evidence of Chagas disease in organic remains excavated from archeological sites and discusses two findings in greater detail, both with lesions suggestive of chagasic megacolon and confirmed by molecular biology techniques. One of these sites is located in the United States, on the border between Texas and Mexico and the other in state of Minas Gerais, in the Brazilian* cerrado *(savannah). Dated prior to contact with Europeans, these results confirm that Chagas disease affected prehistoric human groups in other regions outside the Andean* altiplanos *and other transmission areas on the Pacific Coast, previously considered the origin of* T. cruzi *infection in the human host.*

Key words: paleoparasitology - paleoepidemiology - Chagas disease - ancient DNA - mummies - *Trypanosoma cruzi*

Although Chagas disease transmission by *Triatoma infestans* has been eliminated from Brazil, the remaining chronic cases still pose a serious problem, especially in regions of the country where access to health care is more difficult. Chagas disease brings suffering and death for many people in Latin America, where many young adults still die early from cardiac lesions. Brazilian health authorities have been alert to the occurrence (mainly in the Amazon) of oral transmission through the ingestion of foods contaminated with vector-derived infective forms (metacyclic forms) (Dias & Coura 1997, Roque et al. 2008). These cases of oral transmission, occurring as outbreaks, are characterized by the appearance of acute and sometimes quite severe forms of the disease.

Chagas disease must also have been a serious problem among prehistoric populations, especially if the possibility of sudden death in young adults and the consequences of the chronic phase, whether intestinal or cardiac is considered. Several paleoparasitological findings point to Chagas infection in various regions of the Americas, as well as cases of severe cardiac and megacolon lesions. This article discusses the evolutionary aspects of trypanosomatids and their transmission to humans in prehistoric times in the Americas.

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Trypanosomatids in mammals

Trypanosomatids are flagellate protozoa of the Kinetoplastida order, which includes both free-living organisms and parasites. The latter comprise monogenetic and digenetic organisms with extranuclear circular DNA peculiarly arranged as maxi and minicircles. Trypanosomatids of mammals provide an excellent example of how molecular paleoparasitology can shed light on epidemiological and biological questions. Trypanosomatids are very old eukaryotic organisms that probably diverged from the first eukaryotes soon after their association with the mitochondrion.

Kinetoplastida are characterized by the presence of kDNA or kinetoplast DNA, an extranuclear DNA network formed by circular molecules, maxi and minicircles and that correspond to the parasite mitochondrial genome, localize near the flagellate's basal body.

The Kinetoplastida include the Trypanosomatidae family, an exclusively parasitic taxon that infects a wide range of animals and plants, and the Bodonidae family, that includes parasitic and exclusively free-living organisms.

Trypanosoma cruzi is characterized by important heterogeneity and biological plasticity. These peculiarities were already observed in the pioneering studies by Carlos Chagas and Brumpt and represent an unresolved epidemiological conundrum to this day. The heterogeneity of *T. cruzi* that is expressed by various markers, as biological (differences in the infection pattern in mice and growth in axenic culture, differences in competence when colonizing culture cells and resistance to chemotherapeutic agents, among others), biochemical and molecular, result in the wide distribution in nature and in distinct epidemiological scenarios. In fact, *T. cruzi* is capable of infecting more than a hundred species of mam-

mals and, within these, nearly all the tissues. Another important aspect of *T. cruzi* biology relates to the parasite's multiple transmission mechanisms: transfusional, congenital, oral and by contamination of the skin and mucous membranes with triatomine feces carrying the infective metacyclic trypomastigotes forms. *T. cruzi* is also a generalist parasite in relation to the vector, since the protozoan infects dozens of triatomine species from the Reduviidae family. The parasite's biological plasticity results in transmission cycles in nature that are characterized as multivariable, complex and peculiar on a temporal and spatial scale. This complexity means that over time, humans have probably contacted the parasite on different occasions and through different routes, depending mainly on how they interacted with the environment, i.e., how they were exposed to infection.

Since the pioneering work by Carlos Chagas, researchers have attempted to establish a correlation between some genotypic or phenotypic characteristic in the parasite and the disease's profile and characteristics related to local epidemiology, the vector, or animal reservoirs. Although many aspects of *T. cruzi* biology and ecology have been elucidated, numerous questions still remain unanswered and no one has ever consistently associated a given type of isolate to the disease or its epidemiology.

We do know that *T. cruzi* presents a multiclonal population structure, with high genetic diversity. The first biochemical characterization studies analyzing different isolates of the parasite identified three types of zymodemes, two of which (Z1 and Z3) were associated in Brazil with the sylvatic transmission cycles, while Z2 was associated with the domestic transmission cycle below the Amazon (Miles et al. 1977, 1978). Molecular tools grouped different sub-populations of the parasite into two distinct and phylogenetically distant genotypes. These genotypes were termed *T. cruzi* I and *T. cruzi* II and correspond, respectively, to Z1 and Z2. More recently, six sub-populations were described in the taxon: TCI and TCII, with the latter genotype subdivided into TCIIa, TCIIb, TCIIc, TCIIe and TCd that excepting TCIIb, having originated in part from two hybridization events in the parasite (Fernandes et al. 1999, Llewellyn et al. 2009).

Origin and dispersal of trypanosomatids of mammals and the introduction of humans into the transmission cycle

The origin and evolution of the trypanosomatids have been discussed since before the description of *T. cruzi*. Leger (1904) and Minchin (1908) began the debate, starting from opposite premises as to whether the ancestral host of Trypanosomatidae was vertebrate or invertebrate. This debate has tended one way or the other, depending on new evidence provided by new techniques, but without ever reaching a definitive conclusion.

It has been proposed that the cruzi clade originated on the super-continent formed by South America, Australia and Antarctica. Currently, and based on biogeographic events, it is agreed that trypanosomes from the Salivaria group (*Trypanosoma brucei*) diverged in the Middle Cretaceous, approximately 100 million years ago, when South America separated from Africa (Haag et al. 1998). According to several authors (Rothhammer et al. 1985, Coimbra Jr 1988, Stevens & Gibson 1999), humans were introduced into the *T. cruzi* transmission cycle after domestication of plants and animals and acquisition of sedentary habits. Thus, according to the widely accepted theory on the origin of Chagas disease in humans, approximately 8,000-6,000 years ago the Andean peoples began domesticating small rodents (*Cavia* sp.) for consumption and for rituals. The animals were raised inside the houses, where they attracted hematophagous insects, specifically *T. cruzi* vectors. The beginning of grain storage and the resulting attraction of grain-eating mammals also presumably played an important role in this process. The triatomine vectors of *T. cruzi*, especially the *T. infestans* species, found optimum conditions in the mud and daub human dwellings for colonization and blood meals from both humans and their domestic animals. Importantly, adaptation by a triatomine to a new habitat, in this case human dwellings, is and probably was a very long process that includes genetic simplification (Schofield et al. 1994) - it is interesting to recall that sylvatic colonies of *T. infestans* have never been described in Brazil.

The *T. infestans* species probably became domiciliated at that time, spreading to a major extent with the help of human migrations to other parts of South America, including the opposite side of the Andean Cordillera. The process intensified after the arrival of Europeans and Africans on the continent, with the spread of precarious human dwellings made of "mud and daub", where the vector found prime conditions for colonization in the cracks and crannies in the walls (typical of this kind of construction). According to this theory, Chagas disease thus originated in the Andean Region, in prehistoric populations 8,000-6,000 BP, reaching other regions of the Americas beginning with the European colonial period (Dias & Coura 1997).

It was thought that in what is now Brazil, *T. cruzi* would never have been a problem for the indigenous populations, since the latter were mostly nomad and lived in dwellings to which triatomines did not easily adapt (Coimbra 1988).

Paleoparasitology and the paradigm shift

The data from paleoparasitology told a different story and changed the classically accepted theory. A hundred years after the discovery by Carlos Chagas, another story of Chagas disease began to emerge and which took place thousands of years before the disease was discovered.

Thus, the scenario that has emerged corroborates the hypothesis that Chagas disease is probably as old as man's presence in the Americas: the description of lesions typical of Chagas disease that are PCR-positive for *T. cruzi* in mummies from pre-Colombian Andean countries 9,000 years ago demonstrates the antiquity of the infection and disease in humans (Rothammer et al. 1985, Fornaciari et al. 1992, Guhl et al. 1997, 1999, 2000, Ferreira et al. 2000, Aufderheide et al. 2004).

The use of molecular tools to trace the origin and dispersal of trypanosomes in mammals, and particularly that of *T. cruzi*, is still wrestling with numerous technical difficulties and unanswered questions, but molecular paleoparasitology is certainly a useful tool for tracking the parasite's dispersal since prehistory and for identifying changes in the genome that resulted from the evolution in the host-parasite-environment system.

The first hypotheses on transmission of *T. cruzi* infection in prehistoric populations in Northeast Brazil

In 1984, during excavations in the Boqueirão da Pedra Furada, archeological site in the Serra da Capivara National Park in southeast state of Piauí (PI), Northeast Brazil, coordinated by Niéde Guidon (Guidon & Delibrias 1986, Guidon 1989), we witnessed the archeologists constantly being attacked by triatomines while they studied the rock paintings on the sandstone walls of the rock shelter in the prehistoric camp. The paintings were all over the wall and, at that time, without the current technologies, it was necessary to copy them for subsequent study. Both in the early morning and in the hotter hours of the day, with temperatures reaching 42-45°C, as well as at night, when the temperature drops to 8-10°C, *Triatoma brasiliensis* nymphs and adults emerged, attempting to feed on the blood of the archeologists, students and workers. Some of the captured specimens were infected with *T. cruzi*. We postulated then that the ancient artists and other inhabitants of the rock shelters were also attacked by the vector and infected with the parasite (Ferreira et al. 2000). Importantly, *T. infestans* never colonized this part of Northeast Brazil (Dias et al. 2000). At the time of the excavations, it was impossible to test our hypothesis, since the few human remains uncovered there consisted of bones and coprolites, where the parasite leaves no pathological signs.

It was not until a few years later, with the introduction of molecular biology techniques for the diagnosis of infectious diseases in archeological remains, that Chagas infection was confirmed in Chilean and Peruvian mummies from 4,000 BP (Guhl et al. 1999, 2000, Ferreira et al. 2000, Madden et al. 2001).

The first tests were performed at the Oswaldo Cruz Foundation, Rio de Janeiro, Brazil, before applying them to the archeological material. Tentative protocols were conducted in mice infected with a known parasite load. After sacrificing the animals, the organs were placed in a drying chamber at 40°C until their complete desiccation. Following this experimental "mummification", molecular biology techniques were used to obtain a test standard that was subsequently applied to the archeological material (Bastos et al. 1996).

According to findings in Northeast Brazil, the first prehistoric occupations in the Serra da Capivara National Park area occurred thousands of years before the 15,000-12,000 BP accepted for the peopling of South America (Guidon & Arnaud 1991, Parenti et al. 1998). Caves and rock shelters containing numerous rock paintings, some of them dated to 26,000 BP, attest to the antiquity of this occupation. It is thus tempting to suggest that the first artists, the authors of these paintings, and the other cave dwellers, were the first South Americans exposed to Chagas disease, with transmission of the parasite by *T. brasiliensis*. In fact, to this day these rock shelters provide a habitat where small mammals, primates and triatomines find prime conditions for colonization.

With recognition of the importance of understanding parasite evolution in order to better control infectious diseases at present (Ewald 1996, Ewald et al. 1998), paleoparasitology has become the best approach for unraveling the evolutionary process from the past to present days. This understanding can and should be achieved by combining paleoparasitology and molecular techniques. With paleoparasitology, we can document the presence of parasites at a given time in the prehistoric population, and with molecular techniques we can identify the potential changes in the parasites' genome, resulting from evolutionary relations in the host-parasite-environment. There is no doubt that infectious diseases have changed from prehistory to the present (Leal & Zanotto 2000), including changes in virulence and pathogenicity associated with humankind's historical and social processes.

Paleoparasitology of *T. cruzi* infection

Based on these assumptions, we began to study *T. cruzi* infection in ancient populations from North and South America, using molecular biology techniques to recover genetic material from the parasite in organic remains of both human and animal origin. Our working hypothesis was that Chagas disease in humans is as old as human presence on the American Continent, providing there were conditions for transmission of the infection, i.e., a favorable environment, presence of the parasite circulating in vectors and animals living with humans. In this sense, Chagas disease dates to thousand of years ago, long before small rodents were domesticated in the Andean Region. We thus tested human organic remains from various North American and South American archeological sites, including bones and other mummified tissues. Animal bones and other remains were also tested and we also sought to find and identify fragments of *T. cruzi* arthropod vectors in the archeological layers. Curiously, human coprolites also needed to be carefully examined, based on evidence that prehistoric groups in the Americas accidentally or intentionally consumed various arthropods (Reinhard et al. 2003, Johnson et al. 2008).

The results of ancient *T. cruzi* DNA extraction and amplification can shed light on key aspects of the known parasite strains circulating today in the Americas (Stevens & Gibson 1999, Stevens et al. 2001). For example, differences have been found in the clinical characteristics of persons infected by the parasite in present-day Northeast Brazil, exactly in the Serra da Capivara National Park area in PI (Borges-Pereira et al. 2002), which can be explained by adaptive processes over time. Paleoparasitology allows us to trace the origin and evolution of infectious diseases and thus to better understand their emergence and dispersal (Cockburn 1967).

This study involved researchers from South America and the United States (US) in research projects funded by various agencies. The findings presented below were

from studies in the Lower Pecos Region in Texas, United Sates, and archeological sites from the northeastern and northern region of state of Minas Gerais (MG), Brazil, both with a semi-arid climate with favorable conditions for the preservation of organic material. In addition, until recently both regions were endemic for Chagas disease.

Before presenting these results, we will provide a brief review of the findings from the Andean Region, starting with the first descriptions of lesions consistent with Chagas disease in mummified bodies with a description of mega syndromes. We also included a description of the histopathological detection of amastigote nests, until confirmation of the antiquity of Chagas infection in the oldest human mummies, the Chinchorro mummies, from the Atacama Desert, which date to 9,000 years BP (Aufderheide et al. 2004).

Brief review of Chagas infection findings in South American mummies

One of the first articles on Chagas disease in South American mummies was by Rothhammer et al. (1985), describing cardiac lesions consistent with the chronic clinical stage of the disease. The evidence was obtained from 35 mummified bodies from the Atacama Desert, but was also based on news from ancient chroniclers and the domiciliation of *T. infestans* in past times. The article was a milestone for research on the disease and raises hypotheses that are still being tested, although some have already been confirmed by the paleparasitological findings.

One Peruvian Inca mummy was autopsied by Fornaciari et al. (1992), who found evidence of Chagas disease in the lesions, including the presence of amastigote nests. They used a serial alcohol rehydration technique proposed by Ruffer (1921) to recover mummified tissues and succeeded in demonstrating the parasites in the heart muscle.

Guhl et al. (1997, 1999) began molecular studies in mummies from the Atacama Desert in Northern Chile. They were able to isolate *T. cruzi* DNA from mummified tissues dating to 4,000 years BP. Less than a month apart, Ferreira et al. (2000) published an article on Chilean mummies, also from the Atacama Desert, but from the area of the present-day city of San Pedro de Atacama, an oasis in the desert and a busy trade center in the past. This finding, launched by the research of Bastos et al. (1996), confirms the extent of Chagas infection in the region for periods of up to 2,000 years. This was one of the starting points for the interesting article published by Guhl et al. (2000), discussing prehistoric human migrations and Chagas disease. The techniques used then are discussed by Madden et al. (2001), and Aufderheide et al. (2004) finally published the isolation of *T. cruzi* DNA from Chinchorro mummies dated to 9,000 BP, therefore several thousand years before the known time when rodents and camelids were domesticated (Rothhammer et al. 1985).

There is thus no doubt that Chagas disease was present in pre-Colombian populations in what is now Chile and Peru. Prehistoric human groups on the American Continent probably made contact with the parasite on several occasions, depending on the way they acted and interacted with the environment. Even to the present, environmental management is known to correlate importantly with the risk of Chagas disease (Emperaire & Romaña 2006).

Target regions: the prehistoric context - Lower Pecos, Texas, US

A case of prehistoric Chagas disease was recently confirmed in North America. It was the mummified body of an adult male who died with his large intestine full of feces, suggestive of Chagasic megacolon (Reinhard et al. 2003). The mummy was dated to 1,150 BP and the diagnosis was confirmed by recovering parasite DNA using molecular biology techniques (Dittmar et al. 2003). This was both the most recently detected prehistoric case and the oldest known case in the US. This single case in a mummy is important for both paleoepidemiologists and present-day epidemiologists (Reinhard et al. 2003).

The Rio Grande region (Rio Bravo) has a very rich bioarcheology, especially between the border towns of Ciudad Acuna, Coahuila, and Del Rio, in Texas, reaching the tributaries of the Amistad International Recreation Area and both sides of the international border. Many mummified bodies have been found in this semi-arid region, and they provide an excellent opportunity for paleoepidemiological studies. Reinhard et al. (2003) confirmed Chagas disease in this particular mummy, studied previously by Turpin et al. (1986), who compared the findings to four other mummies. Radiocarbon dating showed that the individual died 1,150 years ago. The mummy, identified as SMM, is that of a male who died between 35 and 45 years of age. Palynological and dietary investigation (Turpin et al. 1986) showed that grasses predominated at the time, followed by Asteraceae pollen and small amounts of *Pinus*, *Cheno* Am, *Celtis*, and some unidentified species. Other taxa that were present included Onagraceae, Umbelliferae, *Juniperus*, Malvaceae (malva), *Dasylirion*, *Agave*, *Quercus*, *Prosopis*, and fern spores. Macroscopically, *Opuntia* and grass seeds identified as *Setaria* were found, as well as fibers, probably *Agave*. Based on analysis of pollen and animal remains, the individual probably died in the spring (Turpin et al. 1986).

The mummy consists of a nearly complete skeleton, with hair, connective tissues, and a large portion of the digestive tract. The body was placed in the lying position on the right side, and was found with a mass of desiccated organic material, probably the content of the small bowel, covering the right thoracic and abdominal area. These remains are still being studied to determine their anatomical origin. The large intestine is almost complete, and its appearance is consistent with megacolon (Reinhard et al. 2003).

This case of Chagas disease diagnosed in the SMM mummy may have resulted from natural infection through triatomine feces containing trypomastigote forms and active penetration through the mucous mem-

branes. Although it is not possible to determine the species involved, evidence indicates a limited range of possibilities. The infection rates found in triatomines in the Rio Grande valley suggest *Triatoma gerstaekeri* and *Triatoma neotomae* as the most probable species. However, *T. neotomae* prefers to feed on rodent blood, while *T. gerstaekeri* is more opportunistic, feeding on various animals, including humans (Packchanian 1939, Eads et al. 1963, Lent & Wygodzinsky 1979). One of the studies in Texas found that the latter species was involved in all the human cases (Woody et al. 1961).

However, infection with triatomine feces is not the only form of infection. In fact, the digestive contents from the SMM mummy suggest that the individual may have been infected by ingesting either contaminated food or the vector itself. It is notorious that *T.cruzi* transmission can occur when infected insects are ingested or when the flesh of infected animals is eaten raw (Usinger 1944, Lent & Wygodzinsky 1979).

The coprolites yielded a surprising variety of animals, including skeletal fragments from fish, snakes, bats, and rodents (*Peromyscus*, *Thomomys*) and 250 fragments of grasshoppers. Moreover, the SMM mummy's digestive contents included fragments of white-footed mouse (*Peromyscus leucopus*), pocket gopher (*Thomomys* sp.), a bat, and 250 grasshoppers.These gastronomical preferences raise the possibility that some triatomine infected with *T. cruzi* may have been ingested whole. These gastronomical preferences raise the possibility that some triatomine infected with *T. cruzi* may have been ingested whole. Analyses of other coprolites from the Southwest US (Reinhard 1990, 1992, Reinhard et al. 1992) show that the hunter-gatherers frequently ate a wide variety of insects, like grasshoppers, crickets and ants. Although the ingestion of vectors or vertebrate hosts does not appear to be epidemiologically important at present, the case of this mummy raises this possibility in societies with different eating habits from those of modern Western society.

Another important aspect of Chagas disease pathoecology in this area is the consumption of raw rodents in prehistory. The oral route is known to be highly efficient for *T. cruzi* transmission. Besides the fact that the metacyclic forms are perfectly capable of invading intact mucous membranes, the stomach's acid environment exposes epitopes on the parasite's surface that increase their infectivity (Yoshida 2008). Furthermore, 84% of the adult individuals excavated in Lower Pecos showed dental abscesses that may also have served as a portal of entry for the parasite.

Pathological lesions are clearly visible in the SMM mummy's intestines. As in modern history, Chagas disease was endemic in the region in prehistoric times, although it is believed to have displayed low endemicity. The prehistoric way of life, especially the use of caves and rock shelters as dwellings, provided the ideal conditions for Chagas pathoecology in the Lower Pecos Region. Excavations in the rock shelters retrieved a large amount of plant fibers, the ideal habitat for rodents and triatomines. People used straw as bedding, showing that they in fact slept in the rock shelters (Shafer 1986). These conditions must have been common at the time and determined the Chagas disease prevalence in the population.

To complete the diagnosis, an interesting study was presented by Dittmar et al. (2003), who recovered *T. cruzi* 18S rDNA from muscle tissue in the SMM mummy. The resulting sequence was manually aligned with the available sequences in GenBank (Sequencher 4.1.1.). The phylogenetic analysis using maximum likelihood (Felsenstein 1981) showed that the recovered *T.cruzi* rDNA was in the TCII genotype. The finding confirms the digestive form of Chagas disease described by Reinhard et al. (2003).

The study of Chagas disease in Brazilian prehistory

An excavation by André Prous, in the *cerrado* (savannah) region of northern state of MG, in the Peruaçu Valley, in Central Brazil, unearthed the partially mummified body of an individual 35-40 years old. The individual had been buried in an oval grave inside a rock shelter near the Peruaçu River (Kipnis 2008). Initial examination of fragments of coprolites from this mummy showed *Echinostoma* sp. eggs, an uncommon parasite in the human host, and hookworm eggs (Sianto et al. 2005).

Interestingly, in this part of Central Brazil, the preservation of organic remains is quite good. Although there are gallery forests along the rivers, the overall vegetation is characteristic of the *cerrado*. This particular body was placed in the fetal position, protected by leaves and covered with baskets. The individual's head was wrapped in leaves and his body was partially mummified. The arms, legs, abdominal skin and musculature of some body parts are well preserved. Numerous personal objects were found in the grave. Although consolidated fractures were found in the foot bones, the skeleton shows no signs of violence. There are important signs of oral lesions, like dental wear, caries and tooth abscess. A large mass in the abdominal cavity was identified as accumulated feces, obstructing the descending and sigmoid colons. The shape of the fecal mass reproduces the intestinal convolutions. This mass was carefully cleaned and exposed, but only after performing a computed tomography scan of the body, obtaining detailed images of the mummy's interior (Fernandes et al. 2008). To confirm the suspicion of a case of Chagas megacolon, since the Peruaçu Valley is now endemic for the disease, PCR was used to test for the presence of *T. cruzi* and produced a positive result. Further, it was possible to demonstrate the presence of *T. cruzi* in the tissue adjoining the fecal mass, thereby confirming the disease as Chagas megacolon. This presentation is associated with the TCII genotype and is typical of the chronic phase of the disease, which was quite common in the area before the control of vector transmission by *T. infestans*. However, characterization of the parasite in one of the samples showed a TCI profile. The presence of TCI in an individual with megacolon could be explained by a change in the epidemiological profile of the disease or by an undetected mixed infection (TCI/TCII).

Importantly, the morbidity of infection with TCI or TCII in humans still poses some unanswered questions. A mixed infection is supported by the studies of Araújo et al. (2005), who examined the same material. These authors, using mitochondrial 18S rDNA (126bp and 159 bp) and 12S rDNA (148 bp) as the target, confirmed the *T. cruzi* infection, but concluded that the parasites found in the body belonged to genotype TCII. Positive PCR in nearly all of the fragments tested showed that the parasite was disseminated throughout the body and that the individual presented a high parasite load, typical of the acute phase of the disease and apparently inconsistent with the megacolon, typical of the chronic phase (Fernandes et al. 2008). The high parasitic load found in this mummy may also be explained be sequential reinfections: in fact, the worsening of infection with increase of parasitic load has recently been described as the result of reinfection in experimental conditions (Andrade et al. 2006).

In the study of another individual from this same archeological site, Lima et al. (2008) observed infection with the TCI genotype of *T. cruzi* in a human bone fragment dated to 7,000-4,500 BP. The authors described this fragment as belonging to a female approximately 35-39 years old belonging to a population of hunter-gatherers. Genotyping was obtained by amplifying and sequencing a fragment of the mini-exon (339 bp) gene. Hybridization with specificprobes for this DNA fragment (sequences from mini-exon TcI and TcII) andtotal k-DNA for the amplified products derived from kinetoplast minicircles, confirmed the presence of *T.cruzi* in the material.

The presence of the TCI genotype in human bone tissue dated to 7,000-4,500 BP in the state of MG, a region where this genotype has not been recorded as infecting the human host in the present (Fernandes et al. 1999), shows that distribution of the *T. cruzi* genotypes is dynamic and varies in different temporal and spatial segments. The possibility of genotyping the parasite found in archeological remains opens up the fascinating possibility of reconstructing the origin and dispersal of *T. cruzi* and its subpopulations and could hopefully resolve the question concerning the parasite's ancestral host.

In conclusion, paleoparasitology, especially using molecular biology techniques, offers a new and powerful tool in the study of host-parasite interfaces, since it allows investigating the ancient genome of both parasites and hosts in distinct time scales. Paleoparasitology can elucidate aspects pertaining to the origin and evolution and antiquity of host-parasite relations (Araújo & Ferreira 2000, Araújo et al. 2003). Ujvari (2008) comments very appropriately on these possibilities in paleoparasitology, ranging from the study of the origin and evolution of trypanosomatids to the development of vertebrate species in the Americas. He comments that the study of the genetic material from *T. cruzi* can be helpful in clarifying the geographic history of the Americas. He reaches these conclusions based on the possibility of comparing African and Australian versus American trypanosomes DNA. Studies on the paleoparasitology of Chagas disease can shed light on the origin and spreading of this disease in the Americas through the obtention, sequencing and comparison of *T. cruzi* DNA derived from organic remains of humans and other mammals, including fragments of vectors found in archeological sites. The comparative study of DNA from different periods of human occupation, as in the periods of hunter-gatherers and farmers, cave or rock shelter dwellers and village groups, offers a unique possibility of rebuilding the evolutionary history of humans and their parasites. Concerning Chagas disease, a new paleoepidemiological picture begins to take shape, with data pointing to a cyclic emergence and vanishing of Chagas disease according to the human life style ie from the inhabitants or occasional occupants of caves and rock shelters to the of the village groups that periodically moved their dwelling sites.

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