Part IV - Special Studies and Perspectives

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part IV

Special Studies and Perspectives

The Paleoepidemiology of Chagas disease

Adauto Araújo • Ana Maria Jansen • Karl Jan Reinhard • Luiz Fernando Ferreira

In 1909, Carlos Chagas first described a case of the disease that now bears his name. The patient was a 2-year-old girl that survived the disease for more than seven decades, eventually dying in 1981, at 74 years of age. She lived with *Trypanosoma cruzi* infection for her entire life. However, for many people, Chagas disease means suffering and death. Many young adults die early from cardiac lesions. Chagas disease is one of the most important parasitic infections, and due to its high morbidity and mortality, many South and Central American countries consider it a serious public health problem.

Even in Brazil, which has succeeded in controlling intradomiciliary vector transmission of *T. cruzi*, Chagas disease is worrying health authorities again, due to the occurrence (mainly in the Amazon) of oral transmission through the ingestion of foods contaminated with the vector's infective forms (metacyclic trypomastigotes) (Dias, 2006).

Such cases occur as outbreaks and involve acute and sometimes severe forms of the disease. Chronic cases are equally important, requiring drug treatment that often results in serious secondary reactions, in addition to hospital or surgical care, beyond the reach of a major portion of the population in endemic areas.

TRYPANOSOMATIDS OF MAMMALS

Trypanosomatids are flagellate protozoa belonging to the Kinetoplastida order, which includes both free-living organisms and parasites. As parasites, the monogenetic and digenetic protozoa are characterized by extranuclear circular DNA in a peculiar array, namely maxicircles and minicircles.

Trypanosomes of mammals provide an excellent example of how molecular paleoparasitology can elucidate epidemiological and biological questions. Trypanosomatids are very ancient eukaryotic organisms, since they probably diverged from the first eukaryotes soon after their association with mitochondria.

Parasites from the order Kinetoplastida are characterized by kDNA, an extranuclear circular DNA arranged in maxicircles and minicircles inside the mitochondrion, close to the flagellar basal body. The Kinetoplastida include the

Trypanosomatidae family, an exclusively parasitic taxon that infects a broad spectrum of animals and plants, and the Bodonidae family, exclusively free-living organisms.

Several biological characteristics are important for understanding *T. cruzi*, a parasite with broad heterogeneity and biological plasticity. The pioneering studies by Carlos Chagas and Brumpt already called attention to these characteristics, which represent an epidemiological maze even to this day.

The heterogeneity of *T. cruzi*, expressed by various biological, biochemical (differences in the infection pattern in mice and growth in axenic culture, differences in the capacity to colonize culture cells, and drug resistance, among others), and molecular markers results in the parasite's wide distribution in nature in distinct epidemiological scenarios. In fact, *T. cruzi* is capable of infecting more than a hundred species of mammals and nearly all their tissues.

Another important aspect of *T. cruzi* biology involves the parasite's multiple transmission mechanisms, e.g., transfusional, congenital, and oral, and by contamination of the skin and mucosa by feces of triatomines carrying the infective forms, called metacyclic trypomastigotes.

T. cruzi is also a generalist parasite in relation to the vector, since it infects dozens of triatomine species from the Reduviidae family. The biological plasticity of *T. cruzi* results in transmission cycles in nature that are characterized as multivariate, complex, and peculiar on a spatial and temporal scale. This complexity means that the human host probably entered into contact with the parasite on different occasions over time and by different routes, depending mainly on interaction with the environment, that is, the ways by which the host was exposed to infection.

Since the pioneering work of Carlos Chagas, researchers have attempted to establish a correlation between some genotypic or phenotypic characteristic of the parasite and the disease profile, local epidemiological characteristic, or association with the vector or animal reservoir. Although many aspects of *T. cruzi* biology and ecology are known, there are still numerous unanswered questions. Scientists have never succeeded in robustly correlating a given type of isolate with the disease or its epidemiology.

T. cruzi is now known to be a protozoan parasite with a multiclonal population structure with high genetic diversity. Molecular tools have grouped different subpopulations of the parasite into two distinct and phylogenetically distant genotypes. The genotypes were named *T. cruzi* I and *T. cruzi* II. The first studies in biochemical characterization, analyzing different isolates of the parasite, identified three types of zymodemes. In Brazil, Z1 and Z3 are associated with the wild transmission cycles (Miles et al., 1977, 1978), while Z2 is associated with the domestic transmission cycle in the region below the Amazon (Anonymous, 1999).

More recently, six subpopulations were described in the taxon: TCI and TCII, with the latter genotype subdivided into TCIIa, TCIIb, TCIIc, TCII, and TCf, originating in part from two hybridization events in the parasite. However, a new proposal for *T. cruzi* denomination has already been presented, during a meeting of experts from the Brazilian Society of Protozoology in 2009 (Zingales et al., 2009).

TRYPANOSOMATIDS OF MAMMALS, THEIR ORIGIN AND DISPERSAL, AND INTRODUCTION OF THE HUMAN HOST INTO THE TRANSMISSION CYCLE

The origin and evolution of trypanosomatids have been discussed since long before the description of *T. cruzi*. Leger (1904) and Minchin (1908) launched the debate, beginning with opposing premises as to whether the ancestral host of Trypanosomatidae was a vertebrate or invertebrate. This debate swayed one way or the other, depending on new evidence produced by new techniques, and no conclusive result has been reached to date.

It has been proposed that the *cruzi* clade originated on the supercontinent formed by South America, Australia, and Antarctica. Based on biogeographical events, it is currently accepted that the trypanosomes from the Salivaria group (*Trypanosoma brucei*) diverged in the Middle Cretaceous approximately 100 million years ago, when South America separated from Africa (Haag, O'Huigin & Overath, 1998).

According to various authors, the human host only entered the *T. cruzi* transmission cycle after the domestication of plants and animals and acquisition of sedentary habits (Rothhammer et al., 1985; Coimbra Jr., 1988; Stevens, Noyes & Gibson, 1998).

Thus, according to the accepted theory on the origin of Chagas disease in humans, some 8,000 to 6,000 years ago the Andean peoples began the domestication of rodents called *cuyes* or *Cavia* sp., known as guinea pigs by Europeans, for food and ritual purposes. The animals were raised indoors and purportedly attracted hematophagous insects, vectors of *T. cruzi*.

The beginning of grain storage and the resulting attraction of granivorous mammals contributed to this process. In human dwellings built of wood and packed earth, triatomine vectors of *T. cruzi* found the ideal conditions for colonization and blood meals, whether from the human inhabitants or their domestic animals. Importantly, triatomines probably adapted to this new habitat, human dwellings, over a very long period, involving irreversible genomic alterations. Interestingly, wild colonies of *Triatoma infestans* have never been reported in Brazil.

Triatoma infestans probably underwent domiciliation 8,000 to 6,000 years ago and later dispersed largely through human migrations to other regions of South America, including to the opposite side of the Andes, after the arrival of Europeans and Africans on the continent. The process accelerated with the dissemination of precariously built mud-and-wattle dwellings, where the vector found the ideal conditions for colonization in the cracks, nooks, and crannies in the walls, so typical of such dwellings, and the intensification of trade. Chagas disease thus originated in the Andean Region in prehistoric populations 8,000 to 6,000 years ago, reaching other regions of the Americas beginning with the European colonial period.

Researchers originally believed that in what is now Brazil, where the indigenous peoples were mostly nomadic and used temporary dwellings to which the triatomines did not adapt well, prehistoric infection with *T. cruzi* would never have been a problem.

PALEOPARASITOLOGY AND THE PARADIGM SHIFT

The paleoparasitological data revealed an opposite situation and changed the classically accepted theory. A hundred years after Carlos Chagas' discovery, another history of Chagas disease began to emerge, having occurred thousands of years before discovery of the disease.

The scenario that has unfolded thus supports the hypothesis that Chagas disease is probably as old as human presence in the Americas. The description of lesions typical of Chagas disease and PCR-positive for *T. cruzi* in pre-Columbian 9,000-year-old mummies from Andean countries proves the antiquity of Chagas infection and disease in humans (Rothhammer et al., 1985; Fornaciari et al., 1992; Guhl et al., 1997, 1999, 2000; Ferreira et al., 2000; Aufderheide et al., 2004).

A similar question relates to *Trypanosoma evansi*, another trypanosome of mammals, widely distributed in nature. This salivary protozoan is included in the *brucei* clade, although mainly transmitted mechanically by horse flies

(Tabanidae). In Brazil, it only occurs in the Pantanal; however, in this swampy biome it is highly prevalent in all the mammalian taxa. Its principal reservoir is the capybara (*Hydrochoeris hydrochoeris*), a caviomorph rodent.

The introduction of *T. evansi* into the American continent has also been attributed to the arrival of infected horses brought by the European colonizers. However, it is unlikely that horses infected with *T. evansi* could have survived extenuating transoceanic voyages under the precarious conditions of the time, followed by the march to the Pantanal. It is far more plausible to assume that the parasite was introduced into the continent with the arrival of primates and caviomorph rodents coming from Africa 35 million years ago. This question can be answered definitively by molecular analysis of organic remains.

The use of molecular tools to trace the origin and dispersal of trypanosomes of mammals, and particularly *T. cruzi*, requires much further research. There are many technical obstacles to overcome and numerous unanswered questions, but molecular paleoparasitology definitely allows tracing the parasite's dispersal since prehistory, plus identifying genomic changes resulting from evolution in the parasite-host-environment system.

THE FIRST HYPOTHESES ON TRANSMISSION OF *TRYPANOSOMA CRUZI* INFECTION IN PREHISTORIC PEOPLES IN NORTHEAST BRAZIL

In 1984, during excavations of the Boqueirão da Pedra Furada archaeological site in the region of the Serra da Capivara National Park in southeast Piauí State, Northeast Brazil, coordinated by Niéde Guidon (Guidon & Delibrias, 1986; Guidon, 1989), we saw kissing bugs (triatomines) constantly attacking the archaeologists who were studying rock paintings on the sandstone walls in the rock shelters of the ancient prehistoric camp.

The paintings cover the entire wall, and at the time, without today's technology, they had to be copied by overlaying them with transparent plastic (Figure 1). Both in the early morning and at the hottest times of the day, with the temperature around 42-45 °C, and at night, when the temperature drops to 8-10 °C, nymphs and adults of *Triatoma brasiliensis* emerged, trying to feed on the blood of archaeologists, students, and laborers in the excavations. Some specimens were infected with *T. cruzi*. On the occasion, we postulated 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).

Interestingly, *Triatoma infestans* never colonized this part of Northeast Brazil (Dias et al., 2000). In 1984 we were unable to test our hypothesis, since the few human remains found by then consisted of bones and coprolites, where the parasite leaves no pathological signs.

Figure 1 – Excavations in Toca do Boqueirão at the Pedra Furada site in the Serra da Capivara National Park, Piauí, Brazil, 1984. Note the archaeologists copying the rock paintings on the sandstone wall

Photograph: Paleoparasitology Laboratory (Ensp/Fiocruz).

Not until several years later, with the introduction of molecular biology techniques for the diagnosis of infectious diseases in archaeological remains, Chagas infection was confirmed in Chilean and Peruvian mummies dated 4,000 BP (Guhl et al., 1999, 2000; Ferreira et al., 2000; Madden et al., 2001).

The first tests were conducted at the Oswaldo Cruz Foundation in Rio de Janeiro. Before applying them to archaeological material, experimental protocols were performed in mice infected with a known parasite burden. After sacrificing the animals, the organs were placed in a drying oven at 40°C until their complete desiccation. Following this experimental “mummification”, molecular biology was used until establishing a standard for use in archaeological material. This was the subject of the PhD dissertation by Otilio Machado Bastos, whose experiment was commented in the *Journal of the Paleopathology Association* (Bastos et al., 1996) and applied successfully to Chilean mummies by Guhl et al. (2000).

According to the findings in Northeast Brazil, the first prehistoric occupations in the region of the Serra da Capivara National Park occurred thousands of years before the 15,000–12,000 years previously assumed for the peopling of South America (Guidon & Arnaud, 1991; Parenti et al., 1998). Although the dates still spark debate (Meltzer, Adovasio & Dillehay, 1994), caves and rock shelters containing numerous rock paintings, some of which dated to 26,000 years, attest to the antiquity of these occupations.

It is thus tempting to suggest that the artists that made these paintings and the other inhabitants of the caves were the first South Americans exposed to Chagas disease, transmitted by *Triatoma brasiliensis*. In fact, to this day, these rock shelters provide a habitat where small mammals, primates, and triatomines find favorable conditions for colonization.

Following recognition of the importance of understanding parasite evolution to better control infectious diseases in modern times (Ewald, 1996; Ewald et al., 1998), paleoparasitology assumed a key role in elucidating the evolutionary process from past to present.

This understanding can and should be achieved by combining paleoparasitology with molecular techniques. Paleoparasitology can document the presence of parasites at a given time in a prehistoric population, and molecular techniques can identify potential changes in the parasites' genome, resulting from evolutionary parasite-host-environment relations. Infectious diseases definitely changed from prehistory to today (Leal & Zanotto, 2000), including modifications in virulence and pathogenicity associated with humankind's historical and social processes.

Based on these assumptions, we began studying *T. cruzi* infection in ancient North and South American peoples using molecular biology techniques to recover the parasite's genetic material in organic remains, both from humans and other animals. Our working hypothesis is that Chagas disease in humans is as ancient as human presence on the American continent, whenever or wherever there were favorable conditions for its transmission. This means a favorable environment, presence of the parasite circulating in vectors, and animals in contact with humans. Chagas disease thus dates back thousands of years, long before guinea pigs were domesticated in the Andes.

We tested human organic remains such as bones and mummified tissues from various North and South American archaeological sites. We also studied animal bones and other remains and attempted to find and identify fragments of arthropod vectors of *T. cruzi* in the archaeological layers. Importantly, human coprolites also need to be examined carefully, since evidence suggests that prehistoric groups in the Americas consumed various arthropods (accidentally or intentionally) (Reinhard, Fink & Skiles, 2003; Johnson et al., 2008).

Results of the extraction and amplification of *T. cruzi* ancient DNA can shed light on key aspects of the parasite's lineages currently circulating in the Americas (Stevens & Gibson, 1999; Stevens et al., 2001). For example, clinical differences were found between individuals infected with the parasite in modern Northeast Brazil, precisely in the region of the Serra da Capivara National Park in Piauí (Borges-Pereira et al., 2002), that can be explained by adaptive processes over time. Paleoparasitology can help trace the origin and evolution of infectious diseases and thus elucidate their emergence and dispersal (Cockburn, 1967).

This work involved researchers from South America and the United States in projects funded by various agencies. The results presented below are based on studies in the Lower Pecos region in Texas, USA, and in archaeological sites in Northeast Brazil and in northern Minas Gerais State, with a semiarid climate and favorable conditions for preservation of organic material. In addition, both these areas of Brazil, the Northeast *caatinga* and the *cerrado* in Minas Gerais, are (or until recently were) endemic for Chagas disease.

Before presenting the results, we will provide a brief review of the findings in the Andean Region, starting with the first descriptions of typical lesions in mummified bodies. The literature also reports the description of megas, histopathological evidence of amastigote nidi, and confirmation of the antiquity of Chagas infection in the oldest known human mummies, the Chinchorro mummies, in the Atacama Desert, dated to 9,000 BP (Aufderheide et al., 2004).

BRIEF REVIEW OF FINDINGS ON CHAGAS INFECTION IN SOUTH AMERICAN MUMMIES

Rothhammer et al. (1985) published one of the first articles on Chagas disease in South American mummies, describing cardiac lesions consistent with chronic Chagas. The evidence was obtained from 35 mummified bodies from the Atacama Desert, but is also based on writings by chroniclers and the adaptation of *Triatoma infestans* to the domicile in past times. The article was a milestone for research on the disease and raises hypotheses that are still being tested, along with others that have already been proven by the paleoparasitological findings.

Fornaciari et al. (1992) autopsied a Peruvian Inca mummy and found evidence of Chagas disease in the lesions, including nidi of amastigotes. The authors used the alcohol rehydration technique proposed by Ruffer (1921) to recover mummified tissues and showed evidence of parasites in the heart muscle.

Bastos et al. (1996) and Guhl et al. (1999) then launched molecular studies in mummies from the Atacama Desert in Northern Chile. These researchers succeeded in isolating DNA from mummified tissues dated 4,000 BP. Less than a month later, Ferreira et al. (2000) published an article on Chilean mummies applying DNA techniques, also in the Atacama Desert, but in the area of the current city of San Pedro de Atacama, an oasis and center of intense trade in the past. This finding, following on the above-mentioned studies by Bastos et al., confirms the extent of Chagas infection in the region during a period of up to 2,000 years.

This served as a point of departure for the interesting article published by Guhl et al. (2000), discussing prehistoric human migrations and Chagas disease. Previously used techniques were discussed by Madden et al. (2001), and Aufderheide et al. (2004) finally published an article on the isolation of *T. cruzi* DNA in Chinchorro mummies dated 9,000 BP, thus several thousand years before the known time of domestication of rodents and camelids (Rothhammer et al., 1985).

There is thus no doubt that Chagas disease was present in pre-Columbian peoples of Chile and Peru. More recently, Orellana (2008) identified Bolivian mummies infected with *T. cruzi*, which backs the hypothesis that transmission by *Triatoma infestans* began in the Bolivian altiplano.

Humans probably entered into contact with the *T. cruzi*-infected vector on different occasions, on the American continent, simultaneously or not, depending on how they acted and interacted with the environment. Interaction with the environment is still known to be an important risk factor (Romaña, Emperaire & Jansen, 2003; Emperaire & Romaña, 2006).

BASIS AND SUPPORT FOR RESEARCH IN OTHER REGIONS: THE CURRENT SITUATION – LOWER PECOS, TEXAS, USA

While Chagas disease is classified as hyperendemic in South America, human infections are rarely described in the United States or along the northern border of Mexico.

However, some *T. cruzi* transmission occurs in the natural environment in North America, mainly in the State of Texas. Seven species of *Triatoma* are known in Texas: *Triatoma gerstaeckeri*, *Triatoma indictiva*, *Triatoma lecticularius* (syn. *Triatoma heidemannii*), *Triatoma neotomae*, *Triatoma protracta*, *Triatoma rubida*, and *Triatoma sanguisuga* (Elkins, 1951a; Lent & Wygodzinsky, 1979). Most are widely distributed in Texas. *Triatoma rubida* is found in western Texas, close to New Mexico, while *Triatoma neotomae* is distributed mainly in the Rio Grande Valley (Elkins, 1951b; Eads, Trevino & Campos, 1963).

These seven triatomine species have been found infected with *T. cruzi* (Wood, 1941a, 1941b; Davis, McGregor & De Shazo, 1943; De Shazo, 1943; Sullivan et al., 1949; Elkins, 1951a; Pippin, Law & Gaylor, 1968). They are all considered potential vectors of Chagas disease. These species have also been found infected in other regions of North America (Wood, 1941a, 1941b, 1942; Usinger, 1944).

Some of the hosts of *T. cruzi* in Texas are the so-called woodrats (*Neotoma micropus*, *Neotoma mexicana*, *Neotoma* spp.) (Packchanian, 1942; Sullivan et al., 1949; Burkholder, Alysson & Kelly, 1980; Ryckman, 1986), but many other rodents and small mammals serve as hosts. Examples include small wild rodents (*Perognathus hispidus*, *Liomys irroratus*, and *Onychomys leucogaster*), mice (*Mus musculus*), North American opossum (*Didelphis virginiana*), nine-banded armadillo (*Dasypus novemcinctus texanus*), and bats (*Pipistrellus hesperus*) (Wood, 1941a; Packchanian, 1942; Burkholder, Allison & Kelly, 1980).

Four detailed studies were conducted in the Rio Grande Valley from 1941 to 1980 (Wood, 1941; Sullivan et al., 1949; Eads, Trevino & Campos, 1963; Burkholder, Allison & Kelly, 1980). Five triatomine species were involved in *Trypanosoma cruzi* transmission, and 25% of 861 captured specimens were infected. Known hosts in the Rio Grande Valley are the rodent species *Neotoma micropus*, *Perognathus hispidus*, *Liomys irroratus*, and *Onychomys leucogaster* (Burkholder, Allison & Kelly, 1980), but infections were also diagnosed in bats (*Pipistrellus hesperus*) (Wood, 1941a).

The possibility of *T. cruzi* causing disease in the human host in Texas was first demonstrated in 1942 by Packchanian (1943). Since then, five cases of Chagas disease in the region have been confirmed by laboratory tests (Woody & Woody, 1955; Betz, 1984; Roberts & Janovy, 2009). Serological surveys in southern Texas, including the Rio Grande Valley, showed at least 24 new cases by the late 1970s (Woody, Dedianous & Woody, 1961; Woody, Hernandez & Suchow, 1965; Kagan, Norman & Allain, 1966; Burkholder, Allison & Kelly, 1980). In addition, *T. cruzi* infection was detected in dogs and non-human primates, as well as in coyotes and other animals (Kasa et al., 1977; Williams et al., 1977; Meurs et al., 1998; Burkholder, Allison & Kelly, 1980; Grögl et al., 1984).

In addition to the cases in Texas, autochthonous cases have been reported in California (Schiffler et al., 1984). Various species of domestic animals were infected experimentally with *T. cruzi* in Maryland (Diamond & Rubin, 1958). It is currently known that *T. cruzi* infects numerous species of animals across a major part of the United States. The main concern among health authorities is that Chagas disease could also spread across the country through migratory movements, transfusions, and/or congenitally (Milei et al., 2009).

THE SEMIARID REGIONS OF BRAZIL

Although Brazil has eliminated intradomiciliary transmission of *T. cruzi* by *Triatoma infestans* throughout the country, transmission by wild species still occurs in some rural areas (Ramos Jr. & Carvalho, 2001). *T. infestans* was found in some villages in southern Piauí, but not in the southeastern part of the State, including the city of São Raimundo Nonato, where only sylvatic species were recorded. In this part of Northeast Brazil, the main vector species identified around or even inside human dwellings are *Triatoma brasiliensis* and *Triatoma sordida*, both considered sylvatic.

Triatoma brasiliensis adapted well to the region's adobe houses and outbuildings, mostly built close to the mountains, near the natural waterholes. This poses a problem for vector control activities, since the insect's sylvatic/domiciliated habits make control and eradication measures inefficient. Some economic development and basic technology have reached the villages and settlements, including electricity. Lighting at night began attracting more insects from the wild environment, thus increasing the risk of transmission. In the small villages surrounding the Serra

da Capivara National Park, *Triatoma brasiliensis* routinely infests human dwellings (Dias et al., 2000). These kissing bugs are also readily found in the rock shelters and niches in the sandstone or limestone hills, the natural habitat of rodents and marsupials, found infected with *T. cruzi* (Gomes, 1993). The parasite's life cycle is thus maintained in both wild and domestic animals. The parasite circulates between two environments, natural and manmade, just a few meters apart (Xavier et al., 2007).

The current population living around the park originally migrated mainly from other States of the Northeast in the late 19th and early 20th centuries, when they fled prolonged droughts and settled in small villages in the region. In the early 20th century, many families lived in caves or rock shelters, like the prehistoric peoples. They tapped rubber from *maniçoba* (*Manihot glaziovii*), for which a heavy demand developed during World War II.

The demand for *maniçoba* sustained these families for years. Many children were born in the *caatinga* and lived there for years without ever meeting any outsiders or experiencing any other way of life. Some of them still live in the villages, infected with the *T. cruzi* they acquired in the past (Ramos Jr. & Carvalho, 2001). There are reports of sudden deaths in young adults, as well as chronic cardiac and/or digestive conditions (Borges-Pereira et al., 2002, 2006, 2007; Sarquis et al., 2006).

These characteristics, plus the fact that these small groups remained isolated for decades and intermarried, make the conditions particularly interesting for studying evolutionary aspects in the history of Chagas disease, related both to the pathogenesis of the disease and genetic traits in the human population. If it proves possible to recover *T. cruzi* genetic material in organic remains from archaeological sites and compare it to modern DNA, the information would certainly be valuable for understanding the evolutionary history of Chagas disease in the region.

REGIONS UNDER STUDY: THE PREHISTORIC PICTURE – LOWER PECOS, TEXAS, USA

In 2003, a case of prehistoric Chagas disease was confirmed in North America. It was the mummified body of an adult male who died with his intestine full of feces, suggestive of Chagas megacolon (Reinhard, Fink & Skiles, 2003). The mummy was dated 1,150 BP, and the diagnosis was confirmed by recovering parasite DNA using molecular biology techniques (Dittmar et al., 2003). It became the most recently notified case and also the most ancient known case in the United States.

Since Chagas disease is rare in North America, documented human cases are important and deserve reporting, whether recent or ancient. Lesions found in a mummified body proved the seventh case of Chagas disease in Texas, providing important data for both paleoepidemiologists and modern-day epidemiologists (Reinhard, Fink & Skiles, 2003).

The Rio Grande region (Rio Bravo) boasts a very rich bioarchaeology, especially between the towns of Ciudad Acuna, Coahuila, and Del Rio in Texas, reaching the tributaries of the Amistad International Recreation Area and both sides of the U.S.-Mexico border. Many mummified bodies have been found in this region with its semiarid climate, offering a prime opportunity for paleoepidemiological studies. This particular mummy, in which Chagas disease was confirmed (Reinhard, Fink & Skiles, 2003), was studied by Turpin, Henneberg & Bement (1986), who compared the results with those of four other mummies. Radiocarbon dating determined the death at 1,150 BP.

The mummy, identified as SMM, was a male individual that died between 35 and 45 years of age. The study of pollen and food remains (Turpin, Henneberg & Bement, 1986) showed that grasses predominated at the time, followed by pollen from Asteraceae and small amounts of *Pinus*, *Cheno* Am, *Celtis*, and some unidentified species. Other taxa included Onagraceae, Umbelliferae, *Juniperus*, Malvaceae (malva), *Dasyllirion*, *Agave*, *Quercus*, *Prosopis*,

and fern spores. Macroscopically, *Opuntia* and grass seeds identified as *Setaria* were found, along with fiber, probably from *Agave*. The coprolites yielded a surprising variety of animal remains, including bone fragments from various fishes, snakes, bats, rodents (*Peromyscus*, *Thomomys*) and 250 fragments of grasshoppers. Analysis of the pollen and animal remains indicates that the individual died in the spring (Turpin, Henneberg & Bement, 1986).

The mummy consists of a nearly complete skeleton, with hair, conjunctive tissues, and a large portion of the digestive tract. The corpse had been laid on its right side and was found with a mass of desiccated organic material, probably the contents of the small intestine, covering the right side of the thorax and abdominal area. These remains are still under study to determine their origin. The large intestine is nearly complete, and its appearance is consistent with megacolon (Reinhard, Fink & Skiles, 2003).

This case of Chagas disease diagnosed in the SMM mummy may have resulted from natural infection by triatomine feces containing trypomastigote forms and by active penetration of the mucosa. Although the species has still not been determined, the evidence points to a limited range of possibilities. The infection rates found in triatomines in the Rio Grande Valley suggest *Triatoma gerstaeckeri* and *Triatoma neotomae* as the most likely species. However, *Triatoma neotomae* prefers to feed on the blood of rodents, while *Triatoma gerstaeckeri* is more opportunistic, feeding on various animals, including humans (Packchanian, 1939; Eads et al., 1963, Lent & Wygodzinsky, 1979). One study in Texas showed that the latter species was involved in all the human cases (Woody, Dedianous & Woody, 1961).

However, infection via triatomine feces is not the parasite's only form of transmission. The food contents of the SMM mummy actually suggest that this individual may have been infected by ingesting contaminated food or the vector itself. Cases of infection with *T. cruzi* can occur when the contaminated insects are swallowed, or animals are eaten raw (Usinger, 1944; Lent & Wygodzinsky, 1979). The intestinal contents of the SMM mummy include fragments of the white-footed mouse (*Peromyscus leucopus*), pocket gopher (*Thomomys* sp.), a bat, and 250 grasshoppers. These gastronomic preferences raise the hypothesis that this individual might have swallowed a triatomine whole, infected with *T. cruzi*.

Analyses of other coprolites from the Southwest United States (Reinhard, 1990, 1992; Reinhard et al., 1992) show that the hunter-gatherers frequently ate a wide variety of insects such as grasshoppers, crickets, and ants. Although at present the ingestion of vectors or vertebrate hosts does not appear epidemiologically important, the case of this mummy suggests such a possibility in societies with different eating habits from those of modern Western society.

Another aspect of the pathoecology of Chagas disease in this area is the consumption of raw or undercooked rodents in prehistory. The consumption of rodents and a high dental abscess rate may have facilitated infection. The oral transmission route is known to be extremely efficient. Besides the fact that the metacyclic forms are perfectly capable of penetrating the intact mucosa, the stomach's acid environment exposes epitopes on the parasite's surface that increase its infectivity (Yoshida, 1997). In addition, 84% of the adults excavated in the Lower Pecos showed dental abscesses that could have served as a portal of entry for the parasite.

Pathological lesions in the intestine are clearly visible in the mummy. As in modern times, Chagas disease was endemic in the region in prehistoric times, although with low endemicity. Cave-dwelling provided ideal conditions for its pathoecology in the Lower Pecos region. Excavations of the caves have recovered large amounts of plant fibers, an ideal habitat for both rodents and triatomines. Humans used straw for bedding, which shows that they actually slept in the caves (Shafer, 1986). Such conditions would have led to the prevalence of Chagas disease in the population at that time.

In an interesting study, Dittmar et al. (2003) recovered *T. cruzi* 18S rDNA from muscle tissue in this mummified body. The resulting sequence was aligned manually with available sequences in GenBank (Sequencer 4.1.1.) and

submitted to phylogenetic analysis using maximum likelihood (ML) analysis (Felsenstein, 1981). The finding confirms the digestive form of Chagas disease described by Reinhard, Fink & Skiles (2003).

THE STUDY OF PREHISTORIC CHAGAS DISEASE IN BRAZIL

In the *cerrado* region of northern Minas Gerais State, in the Peruaçu Valley, Central Brazil, André Prous found the partially mummified body of an individual 35 to 40 years of age, buried in an oval grave under a rock shelter, close to the Peruaçu River (Kipnis, 2008).

Initial examination of coprolite fragments from this mummy yielded eggs of *Echinostoma* sp., an uncommon parasite in the human host, and hookworm eggs (Sianto et al., 2005).

Preservation of organic remains is very good in this part of Central Brazil. Although gallery forests line the rivers, the vegetation is typical of the *cerrado*. The body was placed in the fetal position, protected with leaves and covered with baskets. The individual's head was wrapped in leaves and his body was partially mummified. Arms, legs, abdominal skin, and some muscles are well-preserved. Many personal objects were found in the grave. Although healed fractures were identified in the foot bones, the skeleton shows no signs of violence. There are evident oral lesions such as tooth wear and a dental abscess. A large mass in the abdominal cavity was identified as accumulated feces, obstructing the descending and sigmoid colon. The shape of the fecal mass reproduces the intestine's circumvolutions.

Careful cleaning was performed and the mass was exposed after submitting the body to computed tomography (CT), obtaining detailed images of the inside of the mummy (Fernandes et al., 2008). To confirm the suspicion of Chagas megacolon, since the Peruaçu Valley is now endemic for the disease, PCR was performed to test for *T. cruzi*, with positive results. It was also possible to confirm the parasite's presence in various tissues, showing that the individual presented a high parasite burden, while *T. cruzi* DNA in the tissue adjoining the fecal mass confirms the disease as megacolon. This pathology is associated with the TCII genotype and is typical of the chronic phase of the disease, quite common in the area before control of transmission by *Triatoma infestans*. Nevertheless, characterization of the parasite from one of the samples showed a TCI profile.

In addition, this condition is attributed to human infection with genotype TCII. 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 TCI/ TCII infection. Importantly, the morbidity of infection with TCI or TCII in human species still poses unanswered questions such as the association between these genotypes and greater or lesser degrees of morbidity, or even different clinical manifestations of the disease.

Positive PCR results in nearly all the tissue fragments show that the parasite had spread systemically and that the individual presented a high parasite burden, typical of acute Chagas disease, but which apparently clashes with the presence of megacolon, typical of the chronic phase.

Araújo et al. (2005) studied the same material, with apparently contradictory findings. These authors used as the target 18S rDNA (126bp and 159 bp) and mitochondrial 12S rDNA (148 bp) and confirmed infection with *T. cruzi*, but concluded that the parasites found were genotype TCII. This apparently contradictory result may mean a mixed TCI/ TCII infection in the same individual.

Studying another individual from this archaeological site, Lima et al. (2008) observed infection with genotype TCI in a human bone fragment dated 7000-4500 BP. The bone was described as that of a woman some 35-39 years of age from a hunter-gatherer group.

Genotyping was obtained by amplifying and sequencing a mini-exon (339 bp) gene fragment. Hybridization of this DNA fragment with specific primers (sequences of mini-exon TcI and TcII and total k-DNA) confirmed the presence of *T. cruzi* in the material. The presence of genotype TCI in human bone tissue dated to 7000-4500 BP in Minas Gerais State, a region where this genotype is not reported infecting humans (Fernandes et al., 1998, 1999), shows that the distribution of *T. cruzi* genotypes is dynamic and varies in time and space.

The possibility of genotyping parasites found in archaeological remains opens the fascinating perspective of reconstituting the origin and dispersal of *T. cruzi* and its subpopulations, hopefully solving the question of the ancestral host of *T. cruzi*.

CONCLUSIONS

Many important vestiges of the parasite and host genomes survive during the co-evolutionary process of the parasite-host-environment system (Araújo et al., 2003). The genome transmits these vestiges, revealing important events in the evolutionary process.

Paleoparasitology, especially with the use of molecular biology techniques, offers new ways of studying parasite-host relations, unveiling the ancient genome of parasites and hosts and the ways by which they have changed or remained the same. Paleoparasitology can help elucidate issues pertaining to the antiquity of parasite-host relations, as well as their origin and evolution (Araújo & Ferreira, 2000; Araújo et al., 2003).

Ujvari (2008) comments quite appropriately on these possibilities provided by paleoparasitology, discussing issues ranging from the origin of parasitism in trypanosomatids to the formation of species in the Americas. He comments that *T. cruzi* genetic material can help reconstruct 100 million years of the planet's geographic history, and that it can be used for the geographic history of the American continent. He reaches these conclusions based on studies of African and Australian trypanosomes, comparing them to American ones.

Studies on the paleoparasitology of Chagas disease can shed light on the antiquity of this disease in the Americas through extraction and amplification of *T. cruzi* DNA from human and other animal organic remains, including fragments of vectors and vertebrate hosts found in archaeological sites.

We can compare material extracted from different periods of human occupation, for example hunter-gatherers and agriculturalists, or cave and rock shelter dwellers and villagers. We can thus test and apply evolutionary approaches and cladistic analyses from the pre-Columbian period to modern times. We have begun to paint a paleoepidemiological picture, with interesting possibilities for emergence of the disease in the inhabitants (or occasional occupants) of caves and rock shelters, while it appeared to decrease or disappear completely in the groups that lived in villages and occasionally changed their place of occupation.

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