



The Paleoparasitology in Brazil and Findings in Human Remains from South America: A Review

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Abstract: The review article presents some of the history of how paleoparasitology started in Brazil, making highlight the great responsible Dr. Luiz Fernando Ferreira and Dr. Adauto Araújo, the trajectory of paleoparasitology in Brazil since 1978 and its performance in science to the present day. In sequence, it is made a presentation of parasitological findings on human remains found in archaeological sites in South America, highlighting Brazil, Argentina, Chile, and Peru, where major discoveries have occurred. Many of the parasites found in archaeological material and mentioned in this review went out of Africa with the peopling of Europe and from there they dispersed around the world, where climatic conditions allow the transmission. However, humans have acquired other parasites of animals, since humans invaded new habitats or creating new habits adopting new technologies, thus expanding its range of influence on the environment. Thus, this review article is finalized with information that explain the importance of these findings in the interaction between parasites, human host, and ambient.

Key words: Paleoparasitology, paleoparasitology in Brazil, South America, human remains, review

INTRODUCTION

The paleoparasitology in Brazil – How it all began?

As a branch of paleopathology, aimed at meeting parasitic forms in archaeological material, the paleoparasitology is gaining more space in the scientific world. Not failing to mention the pioneering work of Sir Marc Armand Ruffer, to publish, in 1910, the finding of *Schistosoma hematobium* eggs in kidney tissue of Egyptian mummies [1]. Subsequently, Lothar Szidat [2] studying 2 bodies discovered in bogs of Prussia, found eggs of *Trichuris trichiura* and *Ascaris lumbricoides*.

In South America, paleoparasitology developed with the first studies conducted in Brazil. It was in the 1970s, specifically in 1978, with Dr. Luiz Fernando Rocha Ferreira da Silva, at the Laboratory of Paleoparasitology Eduardo Marques, located in the Department of Biological Sciences, DCB, the National School of Public Health Sergio Arouca, ENSP, Oswaldo Cruz

Foundation, Fiocruz, that paleoparasitology in Brazil, including this term was created. The great interest of this paleoparasitologist was whether parasitic infections had arrived in the Americas with the colonizers and slaves or would already be on the American continent before colonization [3]. The union with Dr. Adauto José Gonçalves de Araújo, in 1978, made the paleoparasitology in Brazil take flight. An article by Professor Benjamin Caio Dias who defended the autochthonous character of *Schistosomiasis mansoni* in the national territory, against all orthodoxy, was the runway for takeoff of the paleoparasitology in Brazil. There were no eggs of *S. mansoni* in coprolites analyzed. However, there were found hookworm eggs, which were also considered import. This allowed us to consider the question of humans' entry into America. In 1972, Fonseca [4] already discussed the possibility of infection by hookworms in America in pre-Columbian times, based on chroniclers' documents in the 17th century and parasitological tests in isolated indigenous tribes without contact with colonizers. Thus, under the orientation of Dr. Luiz Fernando Ferreira, Adauto Araújo received his master's degree in parasite biology in 1980, by Oswaldo Cruz Foundation with the study "Contribution to the helminth study of archaeological material in Brazil."

The passage through the Bering Strait as the only alternative

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for humans' entry in America as well as hookworm, was the big question. Being a geohelminth needed maturation in the soil, cold temperatures would have destroyed the parasite eggs. That was the idea defended by Dr. Aduino Araújo in his doctoral thesis in public health at the Oswaldo Cruz Foundation in 1987, with the study "Paleoepidemiology of the ancylostomiasis" under the orientation of Dr. Luiz Fernando Ferreira. These findings suggest, in fact, alternative routes, by sea, as a possibility for human migrations in prehistoric America, making questionable the absolute exclusivity of the Bering Strait as a gateway [5]. This is a line, whose results are accumulated until the present day, creating consistent data for discussions about the origin of host populations in new territories [6].

Shortly thereafter, Dr. Aduino Araújo made contact with Canadians studying paleoclimate and confirmed his hypothesis. As a famous book for the time: "The KonTiki expedition", where the author after consultation with Indians on Lake Titicaca built a boat with methodology of prehistory and managed to reach Polynesia leaving America. Thus, it was confirmed the possibility of crossing also by sea.

Although much of the studies of parasites in archaeological material in South America has been made with mummies of the Andean region [7], the interest also came by parasites of animals from coprolite studies found in archaeological layers [8]. Associating with Dr. Niéde Guidon on studies in the Serra da Capivara National Park, Piauí, Brazil, a series of observations came. One of the thatched houses was considered at the time the basis of Chagas disease. Dr. Aduino Araújo noted that when copying the cave pictures archeologists were attacked by triatomine bugs. Then, he raised the possibility that these insects could also attack the ancient inhabitants of these caves. Nailed it, managing to highlight the *Trypanosoma cruzi* infection, there are 3,500 years [9]. When man came in America, he was exposed to infection. Long before the thatched house, the man was infected. It was a very important work.

Collaborations have grown within Brazil, with several institutions of research, as well as the number of samples to be analyzed. The paleoparasitology in the country continues to grow with Dr. Aduino Araújo and Dr. Luiz Fernando Ferreira. The contributions go beyond the country's frontiers. In 2002 and 2009, Dr. Aduino Araújo held 2 postdocs at the University of Nebraska in Lincoln, USA. From there emerged the great partnership with Dr. Karl Reinhard. Research projects have continued yielding partnerships in several places, such as the National University of Mar del Plata, Argentina; University of

Reims, France; University of Tarapaca, Arica, Chile; University of Turin, Italy; Natural History Museum, Paris, France; and many others.

Until today, the techniques of optical microscopy after rehydration and spontaneous sedimentation of organic material and sediments for the diagnosis of eggs and larvae of parasites [10,11] are often used in the Laboratory of Paleoparasitology Eduardo Marques, DCB, ENSP, Fiocruz. Samples of bone and visceral tissues are also analyzed in search of parasitic DNA fragments in these samples. For this, molecular biology techniques are applied, being the most used, i.e., PCR [12-16].

The first tests with the application of molecular biology to confirm the infection by *T. cruzi* in Chilean and Peruvian mummies were made in the Oswaldo Cruz Foundation, Rio de Janeiro, Brazil, in studies conducted by the team of paleoparasitology. Before the tests were applied in the archaeological material, experimental protocols were made in mice infected with known parasite load. After sacrificing the animals, the bodies were placed in oven at 40°C until complete desiccation. After this experimental "mummification", molecular biology techniques were applied until they reached a standard to be used in the archaeological material. That was the doctoral thesis of Otilio Machado Bastos, whose experiment was reported in the journal, Paleopathology Association [17], and applied by Guhl et al. [18] successfully in Chilean mummies in 2000.

Molecular biology techniques also allow the study of genetic evolution of parasites and the time of its introduction in human populations [19]. Retrieving ancient DNA (aDNA) of the parasite sequences and genotyping of these parasites increases the possibility of reconstruction of these current dispersion patterns [20]. Molecular biology techniques are also applied in the diagnosis of intestinal parasites, when the diagnosis is negative by optical microscopy or for confirmation of findings by this technique.

However, immunodiagnostic techniques have been successfully applied in some studies for the identification of protozoa, such as *Giardia duodenalis* and *Entamoeba histolytica*, as far outside Brazil as in our laboratory [21-23]. Currently, the Laboratory of Paleoparasitology Eduardo Marques follows with projects for the standardization of immunodiagnostic techniques for identification of specific parasites, such as the genus *Leishmania*, as well as the standardization of serological techniques for detection of parasite antigens in archaeological remains.

Concomitantly with analysis of parasites, the macro- and

microscopic analyses of coprolites are carried out in our laboratory since the creation of paleoparasitology in Brazil, with the collaboration of Dr. Sergio Augusto de Miranda Chaves. This analysis may reveal important information about the dietary pattern, paleoclimate, and paleoecological adaptations [24]. The types of organic residues found in coprolites, such as pollen, fiber, starch grains, and bone fragments, in addition to the local archaeological context where coprolites were found, allow inferences about cultural information of ancient peoples. Thus, the practice of agriculture can be identified from the meeting of some plant varieties.

The paleoparasitology in Brazil also collects interesting and funny stories, such as transport of corpses and coprolites for studies in the Laboratory of Paleoparasitology when it was allowed to do this without so many bureaucracies (personal communication, Prof. Dr. Luiz Fernando Ferreira).

Today, the paleoparasitology is without a star, Dr. Aduino Araújo. However, this star continues to shine. He has left all of us many lessons, a wealth of knowledge. He is greatly missed by all who lived and worked with him. He contributed only way to the paleoparasitology worldwide. Thus, the Laboratory of Paleoparasitology Eduardo Marques, Department of Biological Sciences, DCB, continues to collect stories, carry out research, train students, and enrich paleoparasitology in Brazil, together with Dr. Luiz Fernando Ferreira, largely responsible for its existence! After all, "we do not want to reconstruct the past. What we want is to find data to make the present better and more intelligible" (Aduino Araújo). Lots of students, today's professionals were formed by Dr. Aduino Araújo and Dr. Luiz Fernando Ferreira and now have the role of seeds of paleoparasitology. Many of them continue to develop their work of master's, doctoral, and post-doctoral inside and outside Brazil. Partnerships continue. The paleoparasitology continues to grow.

The paleoparasitology studies not only in Brazil but by institutions outside the country cover the analysis of archaeological remains originating not only from humans, but also from animals. However, this review consists of an overview of studies related to parasites found in human remains.

This article starts counting as paleoparasitology was born in Brazil and does a review of some paleoparasitological findings of human remains in regions of South America, ending with the importance of these findings in the interaction between parasites, human hosts, and ambient [25].

THE PALEOPARASITOLOGY IN SOUTH AMERICA

Initially, paleoparasitology in South America was characterized by isolated studies, especially when the material was sent by archaeologists to any known parasitologist, who then described the results [7]. Gradually, research groups were forming and growing in Peru [26], Chile [27], and Argentina [28]. Without much pretension, it can be said that the birth of paleoparasitology in Brazil strongly influenced the organization of other groups dedicated to it in various parts of the world, making it evolve and change knowledges about diseases and parasitic infections in ancient times of humanity [29].

The paleoparasitological data in South America indicate intestinal parasitosis in prehistoric times, and thus, change the classic concept that would come from the European colonizers or trafficking in African slaves, going against the pre-established concepts that said most tropical diseases they would have been brought from Africa to the Americas [30,31].

One of the first paleoparasitology findings was obtained in South America by Pizzi and Schenone [32] in 1954, to find *Enterobius vermicularis* in pre-Columbian Chilean mummy dated 4,000-2,000 years B.P. (before present). Since then, several studies have been conducted. Many parasites found in archaeological material from South America are from Africa and are parasites of humans, since pre-hominid ancestors and humans reached the Americas long before the Iberian navigators, with this important biological markers of prehistoric migrations [8,33].

Below are mentioned as some of the paleoparasitological studies in samples from archaeological sites in Brazil, Argentina, Chile, and Peru.

PALEOPARASITOLOGICAL FINDINGS IN SAMPLES FROM BRAZIL

The hookworm eggs found in human coprolites originating from archaeological sites Boqueirão da Pedra Furada and Sítio do Meio, located in the Serra da Capivara National Park, Piauí, Brazil, demonstrate the oldest dating to the presence of hookworm infections in pre-Columbian populations. The oldest is the site Boqueirão da Pedra Furada, dated $7,230 \pm 80$ years, where were found eggs of *Trichuris trichiura* [34], followed by the Sítio do Meio, with datings between 7,000 and 9,000 years [35].

Hookworms and *T. trichiura* eggs were also found in human coprolites originating from the archaeological site Boqueirão Soberbo, dated $4,905 \pm 85$ to $1,325 \pm 60$ B.P. Acantocephala eggs were found in samples originating from the same archaeological site and with the same dating [36]. In 1980 and 1983, have been published the findings of Ferreira et al. [36,37] with diagnosis of the presence of hookworm eggs and *T. trichiura* in coprolites dated to $3,490 \pm 120$ - 430 ± 70 B.P. found at the archaeological site Gruta do Gentio II, Unaí, Minas Gerais. In samples of the same dating site $3,490 \pm 120$ - 430 ± 70 B.P., *Ascaris lumbricoides* and acanthocephala were found, and samples dated up to 3,500 years have revealed the presence of the larvae of 3 developmental stages of the intestinal worm, *Strongyloides stercoralis*, thus explaining that the climatic conditions to which specimens are subjected can facilitate hatching of the eggs and larval development [38].

Eggs of *T. trichiura* and *A. lumbricoides* were found in coprolites from the archaeological site Lapa Pequena, Minas Gerais (8,000-7,000 BP) [35]. From the Municipality of Itacambira, north of Minas Gerais state, were found eggs of Trichostrongylidae and *T. trichiura* in a mummified body of the Brazilian colonial period [39,40]. Years later, with the application of molecular biology techniques in soft and bone tissues from the same mummy, it was possible to diagnose DNA fragments of *Leishmania tarentolae* [16].

Ferreira et al. [41] in 1989 analyzed samples (2,000 B.P.) from the archaeological site of Furna do Estrago, in Pernambuco. They found *T. trichiura* eggs. Eggs of the same parasites, of *A. lumbricoides* and *Hymenolepis nana*, were found in samples dating from 4,000-2,000 B.P. originating from Santa Elina, Mato Grosso [35]. Coprolite samples belonging to a mummy found in the archaeological site Lapa do Boquete, Minas Gerais, dated 600-1,200 B.P. revealed the presence of *Echinostoma* spp. eggs [42].

Some samples of coprolites previously analyzed by optical microscopy and presenting a negative result for the presence of *A. lumbricoides*, were submitted to molecular biology technique. It was possible to give the diagnosis of DNA fragments of this helminth in samples of human coprolites originating from archaeological sites Toca do Meio, Serra da Capivara National Park, Piauí (8,800 \pm 60 B.P.) and Lapa Pequena, Minas Gerais (8,000-7,000 B.P.) [38]. The molecular technique of PCR was used by Fernandes et al. [13] in 2008 for diagnosing DNA fragments of *T. cruzi* I in soft and bone tissues, as well as fecal mass collected from a mummy found on the site Vale do

Peruaçu, Minas Gerais, dated 560 ± 40 years ago. In the same year, Lima et al. [43] found DNA fragments of *T. cruzi* in bone tissue of a mummy 7,000-4,500 B.P. from the same locality.

As mentioned before, more recent studies with the application of PCR technique was performed in soft tissue samples and collected bone in a mummy (colonial period) adult male, found in the basement of the Church of Santo Antonio Aparecido, in the Minas Gerais state. Viscera and bone tissues, including bone marrow samples, revealed the presence of DNA fragments of *L. tarentolae* [16]. A species of *Leishmania* not pathogenic to humans, belonging to the Old World and which has lizard reptiles as the hosts, was found [44-50]. All findings are listed in Table 1.

PALEOPARASITOLOGICAL FINDINGS IN SAMPLES FROM ARGENTINA

Human coprolites (1,000-500 B.P.) found in the archaeological site Valle Encantado, Neuquén, Argentina were analyzed with encountering of eggs of hookworms and *Trichostrongylus* spp. [33]. While, *E. vermicularis* eggs were found in coprolites from the pre-Columbian period in the site Pie de Palo [51].

In 2006, Fugassa et al. [52] reported the presence of *T. trichiura* eggs in coprolites found in the archaeological site Nombre de Jesús, in Cabo Virgenes, a Spanish settlement in Patagonia. Samples of the same archaeological site presented eggs of *A. lumbricoides* [52]. Eggs of *Ascaris* sp. were found in samples from the site Orejas de Burro, Santa Cruz, dated 3,720-3,978 B.P. and *T. trichiura* eggs in samples of the site Parador Nativo, dated $1,513 \pm 48$ B.P. and Centro Minero, dated 689 ± 44 B.P. Both sites are located in the Rio Negro region [52].

Fugassa [53] in 2006 recorded a larva and hookworm eggs in the Patagonia samples dating from $8,920 \pm 200$ years B.P. In 2008, Fugassa et al. [54] analyzed samples from the site Perito Moreno, Santa Cruz, dated $6,540 \pm 110$ B.P., finding eggs of *E. vermicularis* and *Trichuris* sp. and a rhabditoid larva. In samples of sites Las Mandibulas ($6,540 \pm 110$ B.P.) and Caleta Falsa, in Tierra del Fuego (850 B.P.), the samples presented eggs of *Capillaria* spp. [55].

Not only in human coprolites were recorded the presence of hookworms, as well as in animals coprolites. In the same region of Argentina, Fugassa et al. [52] in 2006 also found eggs of *Uncinaria* genus in canid coprolites, dated to $6,540 \pm 110$ years. All findings are listed in Table 1.

Table 1. Paleoparasitological findings from South America

Country	Locality	Archaeological site	Parasites found	
Brazil	Piauí (National Park Serra da Capivara)	Boqueirão da Pedra Furada	ancylostomids, <i>Trichuris trichiura</i>	
		Sítio do Meio	ancylostomids, <i>Ascaris lumbricoides</i> ^a	
	Minas Gerais	Boqueirão Soberbo	ancylostomids, <i>T. trichiura</i> , acanthocephala	
		Gruta do Gentio II, Unai	<i>T. trichiura</i> , <i>A. lumbricoides</i> , acanthocephala, <i>Strongyloides stercoralis</i> (larvae)	
		Lapa Pequena	<i>T. trichiura</i> , <i>A. lumbricoides</i> ^a	
		Vale do Peruaçu	<i>Trypanosoma cruzi</i> (DNA analysis)	
	Itacambira	Trichostrongylidae, <i>T. trichiura</i> , <i>Leishmania tarentolae</i> (DNA analysis)		
	Lapa do Boquete	<i>Echinostoma</i> spp.		
	Pernambuco	Furna do Estrago	<i>T. trichiura</i>	
	Mato Grosso	Santa Elina	<i>T. trichiura</i> , <i>A. lumbricoides</i> , <i>Hymenolepis nana</i>	
Argentina	Neuquén	Valle Encantado	ancylostomids, <i>Trichostrongylus</i> spp.	
		Pie de Palo	<i>Enterobius vermicularis</i>	
	Cabo Virgenes	Nombre de Jesús	<i>T. trichiura</i> , <i>A. lumbricoides</i>	
	Santa Cruz	Orejas de Burro	<i>Ascaris</i> sp.	
		Perito Moreno	<i>E. vermicularis</i> , <i>Trichuris</i> sp., rabditoid larva	
	Rio Negro	Parador Nativo	<i>T. trichiura</i>	
	Centro Minero	<i>T. trichiura</i>		
	Patagonia	-	ancylostomids (larvae and eggs)	
	Tierra del Fuego	Las Mandibulas	<i>Capillaria</i> spp.	
		Caleta Falsa	<i>Capillaria</i> spp.	
Chile	Santiago	Cerro El Plomo	<i>T. trichiura</i> , <i>Entamoeba</i> spp.	
	-	Atacama	<i>Entamoeba</i> sp.	
	-	Cabuza		
	-	Tihuanaco		
	Atacama Desert	-		<i>Paragonimus</i> sp.
		Northern Chile		<i>T. cruzi</i> (DNA analysis)
		Skulls found in the Atacama Desert.		<i>Leishmania</i> spp. (DNA analysis)
		Chinchorros mummies (Found in Illo, A region of Peru to Antofagasta)		<i>T. cruzi</i> (DNA analysis)
	Iquique	Tiliviche	<i>Diphyllobothrium</i> spp., ancylostomids, <i>E. vermicularis</i>	
	San Pedro de Atacama	Toconao Oriente	ancylostomids	
		Tulan	<i>E. vermicularis</i> , <i>T. trichiura</i> , <i>Trichostrongylus</i> spp.	
		Catarpe 2	<i>T. trichiura</i> , <i>Trichostrongylus</i> spp.	
	Bodies stored in the Archaeological Museum of San Pedro de Acatamarca	<i>T. cruzi</i> (DNA analysis)		
Tarapaca Calley	Caserones	<i>E. vermicularis</i>		
North Chile	-	<i>Diphyllobothrium pacificum</i>		
Arica	San Miguel de Azapa	<i>Diphyllobothrium</i> sp.		
	Lluta Valley	<i>T. trichiura</i> , <i>H. nana</i>		
	Alto Ramirez	<i>Entamoeba</i> sp.		
Peru	Chicama Valley	Huaca Prieta	<i>Diphyllobothrium</i> spp.	
	Huarmey Valley	Los Gavilanes	<i>D. pacificum</i>	
		A mummy of the Murga culture	<i>T. trichiura</i>	
		-	<i>A. lumbricoides</i>	
			<i>E. vermicularis</i>	
	Coast of Peru	-	<i>D. pacificum</i>	
	Osmore	-	<i>D. pacificum</i>	
	Coastal Southern Peru	A mummy Tihuanaco (Found in a gallery burial)	ancylostomids	
		Huari	<i>Entamoeba</i> spp.	
	Cuzco Valley	Mummy of the Inca Civilization (Part of the collection of National Museum of Anthropology and Ethnology, Florence).	<i>T. cruzi</i> (histological analysis)	
-	A child peruvian mummy	<i>Leishmania</i> spp. (immunohistochemical technique)		

^aNegative results by optical microscopy but positive by PCR.

PALEOPARASITOLOGICAL FINDINGS IN SAMPLES FROM CHILE

One of the first paleoparasitological studies in samples from archaeological sites in Chile was done by Pizzi and Schenone [32] in 1954, and they diagnosed *T. trichiura* and *Entamoeba* spp. in coprolites found on the site Cerro El Plomo in Santiago, dating from the pre-Columbian period. Records of *Entamoeba* sp. were made years later in coprolites from the pre-Columbian period originating from the archaeological sites Alto Ramirez, Atacama, Cabuza and Tihuanaco [56]. In 1976, in coprolites of an archaeological site located in the Atacama Desert, Hall [57] found eggs of *Paragonimus* sp.

Samples dating from 4,110-1,950 B.C. (before Christ) found in the archaeological site Tiliviche in Iquique, Chile showed the presence of *Diphyllobothrium* spp. eggs, suggesting a specific type of food for individuals of that population [58]. More recent studies on samples from the same location and the same period showed the presence of hookworm eggs and *E. vermicularis* [35]. Hookworm eggs were also found in coprolites originating from the archaeological site Toconao Oriente, located in San Pedro de Atacama, dating of 2,500-2,100 B.P. [35]. Reinhard and Aufderheide [59] in 1990 diagnosed samples dated 4,000 B.P., found in archaeological site in northern Chile, the presence of *Diphyllobothrium pacificum* eggs, and in 2003, studies were published by Reinhard and Urban [60] who diagnosed *Diphyllobothrium* sp. eggs in samples of the archaeological site of San Miguel de Azapa, dated 4,000-5,000 A.P. (after present) [60].

Analyses performed in coprolites found at the site Tulan also located in San Pedro de Atacama, dated 4,100 B.C.-800 A.D. (*Anno Domini*), and at the site Caserones, Tarapaca Valley, diagnosed the presence of *E. vermicularis* eggs [61,62]. In other samples originating from Tulan site, San Pedro de Atacama, dating from 1,000 B.C. were found *T. trichiura* eggs [63] and in those dated 1,080-950 B.C., were found *T. trichiura* and *Trichostrongylus* spp. eggs [35]. The latter was also found in samples of archaeological site Catarpe 2, San Pedro de Atacama, dating from 1,450-1,525 A.D. In samples from the site Lluta Valley, in Arica belonging to the Inca period, dated 1,200-1,500 A.D., were found eggs of *T. trichiura* and *H. nana* [64].

In 1985, Rothhammer et al. [65] described in mummies found in the Atacama Desert (470 B.C. and 600 A.D.) cardiac lesions consistent with chronic Chagas' disease. Suspicions for the presence of *T. cruzi* infection in mummy 4,000 B.P. from

the Atacama Desert, northern Chile, have led to the analysis by PCR technique, confirming the presence of DNA fragments of the parasite in the heart, esophagus, and colon tissues [66,67]. Mummified tissues were collected from bodies stored in the Archaeological Museum of San Pedro de Atacama, northern Chile, dated to 2,000 B.P.-1,400 A.D. In 4 of 6 samples analyzed, it was possible to recover *T. cruzi* DNA through the PCR technique [9]. Madden et al. [68] in 2001 and Aufderheide et al. [69] in 2004 diagnosed DNA fragments of *T. cruzi* in mummies (9,000 B.P.) belonging to the Chinchorros culture.

Samples originating from Chilean mummies were also analyzed in search for the presence of *Leishmania* parasites. Costa et al. [70] in 2009 identified disfiguring bone lesions, mucosal leishmaniasis characteristics in the skulls of 4 women found in the Atacama Desert. The application of molecular PCR technique diagnosed the presence of *Leishmania* DNA fragments in 3 analyzed individuals. All findings are listed in Table 1.

PALEOPARASITOLOGICAL FINDINGS IN SAMPLES FROM PERU

Analyses conducted in human coprolites of the site Huaca Prieta, Peru, from 3,000 B.C. showed the presence of *Diphyllobothrium* spp. eggs [71]. In 1983, Patrucco et al. [72] found eggs of *D. pacificum* in samples of the site Huamey Valley, dated 2,700-2,850 B.C. In archaeological site located in the region of the coast of Peru, Patrucco et al. [72] in 1983 found eggs of the same parasite dating 10,000-4,000 B.P. In Osmore, Peru, the same authors found eggs of *D. pacificum* in samples dating in 1,020-1,476 B.P. [72].

Coprolite samples (890-950 A.D.) collected from cavity of a mummy Tihuanaco revealed the presence of hookworm eggs [73]. Samples of the site Huamey Valley, 1,000 A.D. [72] and a mummy belonging to Murga culture, Colonial period [56], presented *T. trichiura* eggs. While the analysis of another sample of the site huamey Valley, dated 2,277 ± 181 B.C. revealed eggs of *A. lumbricoides* and *E. vermicularis* [72]. From the archaeological site Huari, a sample of pre-Columbian period was diagnosed with the presence of *Entamoeba* spp. [56].

Fornaciari et al. [74] in 1992, analyzing a Peruvian mummy belonging to the Inca civilization, noted lesions with evidence of Chagas disease. The histological analysis showed the presence of amastigote nests in the heart of this mummy muscle tissue. Studies in a Peruvian mummy of 6 years old, dated 800 B.C. revealed the presence of macrophages with *Leishmania* by

immunohistochemical technique [75]. All findings are listed in Table 1.

INTERACTION BETWEEN SOME PARASITES, HUMANS, AND ENVIRONMENT

Many of the parasites found in archaeological material and mentioned in this review originated in human populations of the Old World [76]. They went out of Africa with the peopling of Europe and from there they dispersed around the world where climatic conditions allow the transmission [77]. However, humans have acquired other parasites of animals, since humans invaded new habitats or creating new habits adopting new technologies, thus expanding its range of influence on the environment. Cockburn [78] in 1980 discussed on the association of humans with their parasites since its formation as a species, considering data from the paleoparasitology and their cultural behavior to explain the acquisition of parasites via phylogenetic and ecological [78].

Humans have adopted alimentary habits according to occupied areas. This is evidenced by paleoparasitological analysis in coprolites with findings of *D. pacificum* eggs. Intestinal infection by this parasite was common in the past as it is today in the Pacific coast region. The consumption of raw fish is a common habit in many countries and cultures around the world. Baer et al. [79] in 1967 reported the presence of *D. pacificum* in modern population of the Peruvian coast, afterward the infection by this parasite was confirmed in Peru and Chile through paleoparasitological analysis in coprolites of pre-Columbian origin [60,63,72,80]. Human transmission of *D. pacificum* occurs through raw fish consumption containing this parasite.

Regarding eggs *Echinostoma* sp. found in a Brazilian mummy [42] and the fact that this parasite has not been recorded in modern population of South America, the discovery was carefully researched before publication. It is interesting that in an article published by Fernandes et al. [13] in 2008, cases of human *Echinostoma* sp. infection in prehistoric population of South America was associated with a case of Chagas disease with intestinal lesions characterized by megacolon. In this case, 2 important points should be emphasized. First, the description of injuries caused by Chagas disease in a mummy out of the Andean region was confirmed by studies of molecular paleoparasitology [13]. Second, infection by *Echinostoma* spp. in the South American human population has not yet

been described [42]. These findings pointed toward the need for more detailed studies on the archaeological sites where the samples were analyzed [81].

As mentioned earlier in this paper, hookworm findings revealed a possible entry of this parasite and human by a transpacific route. For being a geohelminth, this parasite needs the soil for their maturity, and cold temperatures have destroyed their eggs [82]. The idea defended by Araújo and Ferreira [82] in 1996 is also valid for the introduction of *T. trichiura* and *S. stercoralis* infections [83]. Even though considering the durability of whipworm eggs, cold Arctic conditions would not have allowed their survival and development on the soil. The possibility of autoinfection in *S. stercoralis* contributes to the permanence of this disease for a few years in the human host after the initial infection, but it mostly occurs in severe forms and immunodeficient host. Thus, the natural cycle of the parasite would not explain its persistence during the migration process of peopling of the Americas [84]. The same idea of a transpacific route of infection by *A. lumbricoides* would be compatible.

In contrast to the hookworm, the pinworm infection caused by *E. vermicularis* does not need to pass through the soil to transmit from one host to another, so their persistence in a given human population depend on climatic conditions. It could therefore have been brought from the old to the new continent, including by human migrations across the Bering Strait. This may explain the greater geographic dispersion of the findings and its spread in North America, from 10,000 years to the present time [82].

The origin and spread of human infections with *T. cruzi* is discussed in many works, as well as the approach of molecular paleoparasitology, and which may represent in an evolutionary perspective [9]. Findings of genetic material from *T. cruzi* in mummies of the Chinchorro culture presented a static epidemiological pattern of the disease with a prevalence rate (40.6%) approximately constant throughout this period of time. These observations suggest a sylvatic cycle of Chagas disease. It was probably well established at the time with the first humans (members of the Chinchorro culture) when they established the first settlement in the Andean coast for nearly 9,000 years. Thus, they began to participate in this sylvatic cycle, probably aggravated by the various forms of ingestion of the trypanosomatid, including the ingestion of contaminated food. Gradually the domestic cycle began to evolve, leading to its current status [69].

The description of typical lesions of Chagas disease and a

positive PCR for *T. cruzi* in mummies from pre-Columbian countries, dating 9,000 years ago, showed the antiquity of the infection and disease in humans [9,65-67,69,74]. Moreover, the observations made by Dr. Adauto Araújo about the entrance of *T. cruzi* in America, specifically in Southeast region of Piauí, Brazil, showed a scenario that supports the hypothesis that Chagas disease is as old as the presence of humans in the Americas. In 1984, during the excavations of the archaeological site Boqueirão da Pedra Furada, Serra da Capivara National Park, Piauí, Northeastern Brazil, coordinated by Dr. Niède Guidon [85,86], Dr. Adauto Araújo and other researchers saw that archaeologists were attacked by triatomine while they were studying the cave paintings of sandstone walls that make up the rock shelter under the old prehistoric camp.

The paintings are all over the wall and, at the period, it was necessary to copy them covered with a transparent plastic. Early in the morning as in the hottest hours of the day, and at night when the temperature drops, emerged nymphs and adults of *Triatoma brasiliensis* trying to feed on the blood of archaeologists, students, and workers of the excavations. Some specimens were infected with *T. cruzi*. Thus, it was postulated that the ancient artists, as well as inhabitants of the rock shelters, have also been attacked and infected by the parasite [9]. According to the findings in the Brazilian Northeast, the first prehistoric occupations in the region of Serra da Capivara National Park occurred thousands of years before 15,000-12,000 years accepted for the peopling of South America [87,88]. Although the dates still raise controversy [89], caves and rock shelters containing numerous cave paintings, some dating back 26,000 years attest to the antiquity of this occupation.

The finding of macrophages with *Leishmania* in Peruvian mummy of a girl of 6 years old, dating from 800 B.C. was of great importance because it shows that one of the forms of leishmaniasis (cutaneous leishmaniasis) existed in the Americas long before the coming of the European colonizers [75]. The first evidence of the encounter of *L. tarentolae* DNA fragments of the mummy from the colonial period found in the Minas Gerais state, Brazil induces numerous questions. Despite current knowledge of this *Leishmania* species, as being non-pathogenic to humans, here the authors extend backwards to the Brazilian Colonial Period (end of 18th century/beginning of the 19th century) new evidence for *L. tarentolae* molecular signatures in soft and hard tissue biopsies, bone marrow included, from this mummy. The finding implies that a systemic spread of the parasite occurred and led us to speculate that a *L.*

tarentolae strain, possibly now extinct, had visceralization ability. The findings re-opened the discussion concerning the potential survival of an ancient *L. tarentolae* strain within human macrophages and its ability to spread systemically [16].

Many studies still need to be made in the field of paleoparasitology to uncover the issues of parasite-host-environment relationships. The techniques used for the diagnosis of parasites in archaeological origin materials constantly improve aiming to be more accessible the parasitological diagnosis and explanations about the peopling of the Americas and the entry of the parasites in our continent.

The data acquired by paleoparasitological studies is extremely important for experts and interested in the peopling of the Americas [90], since the parasites found in archaeological material are excellent markers to trace human migrations in the past [33].

CONFLICT OF INTEREST

We have no conflict of interest related to this study.

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