

REVIEW

THE PROCESS OF *Leishmania* INFECTION - DISEASE AND NEW PERSPECTIVES OF PALEOPARASITOLOGY

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SUMMARY

Species of the genus *Leishmania* (Kinetoplastida, Trypanosomatidae) are causative agents of leishmaniasis, a complex disease with variable clinical spectrum and epidemiological diversity, constituting, in some countries, a serious public health problem. The origin and evolution of leishmaniasis has been under discussion regarding some clinical and parasitological aspects. After the introduction of paleoparasitology, molecular methods and immunodiagnostic techniques have been applied allowing the recovery of parasite remains, as well as the diagnosis of past infections in humans and other hosts. The dating of archaeological samples has allowed the parasitological analysis in time and space. This manuscript presents the state of the art of leishmaniasis and prospects related to paleoparasitology studies and their contribution to the evolutionary and phylogenetic clarification of parasites belonging to the genus *Leishmania*, and the leishmaniasis caused by them.

KEYWORDS: *Leishmania*; Paleoparasitology; Ancient DNA; Origin and evolution of diseases; Infection and disease in leishmaniasis.

INTRODUCTION

Leishmaniasis at present

Parasites of the genus *Leishmania* are obligatory intracellular protozoa belonging to the order Kinetoplastida, Trypanosomatidae family and are causative agents of leishmaniasis. The main feature of this order is the presence of a single branched mitochondria containing an extended region called kinetoplast. The kinetoplast, located near the basal body of the flagellum, has genetic material, the kDNA. This is an extra-nuclear circular DNA arranged in maxi and mini-circles, widely used in molecular analysis¹. This parasite is transmitted through the bite of infected females sandflies (Diptera: Psychodidae, Phlebotominae), of the genus *Phlebotomus* (Loew, 1845) in the Old World, and *Lutzomyia* (France, 1924) in the Americas².

The disease is manifested in the host in one of four forms: cutaneous disease with skin lesions, ulcerative or not, but limited; mucocutaneous disease that may evolve to destructive lesions of the mucous; cutaneous diffuse form of the disease that is spread on the skin and occurs in anergic individuals or late in patients that have been treated for visceral leishmaniasis or kala-azar, the form of disease in which the parasite has tropism for mononuclear phagocytic system cells of the spleen, liver, bone marrow and lymphoid tissues³.

There are several species of *Leishmania* and at least 13 are associated with human disease^{4,5}. According to the taxonomic classification of the species proposed by Lainson & Shaw (1987)⁶, that is currently the most used, the *Leishmania* species are divided into two subgenera: *Leishmania* and *Viannia*.

Data from the World Health Organization (2014)⁷, consider that leishmaniasis is present in 98 countries and three territories on five continents. About 1.3 million new cases occur annually, of which 300,000 cases are of visceral leishmaniasis, 90% of which occur in Bangladesh, Brazil, Ethiopia, India, Nepal and Sudan; and around 1,000,000 cases of cutaneous leishmaniasis occur mainly in Afghanistan, Algeria, Brazil, Colombia, the Islamic Republic of Iran, Pakistan, Peru, Saudi Arabia, Syrian Arab Republic and Tunisia; and mucocutaneous leishmaniasis occur mainly in Brazil, Peru and the Plurinational State of Bolivia. Unfortunately, of the 1.3 million estimated cases, only about 600,000 are officially reported⁷.

There are species whose presence are described only in reptiles, therefore they are classified in a separate genus called *Sauroleishmania*^{8,9}. *Sauroleishmania* is considered an ancestor subgenus of the *Leishmania* genus⁸. However, molecular analysis revealed the close relationship between these species and the ones that infect mammals^{10,11,12}. This

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subgenus contains a species of considerable importance – *Leishmania tarentolae*.

Initially isolated in *Tarentolae mauritanica*¹³, a Moorish gecko, *L. tarentolae* species was also found in North Africa, France, Malta and Italy^{8,14,15,16,17}. Pozio *et al.* (1983)¹⁶ also found *L. tarentolae* in *Cryptodactylus kotsehyi* Steindacher (1870), in a small lizard cohabiting with *T. mauritanica*. Mohamed Elwasila¹⁸, in 1988, first described this *Leishmania* species in ancient lizards of the species *Tarenrolae annularis* recovered by Geoffroy in the year 1809, in Khartoum, Sudan.

In the Old World, after transmission to the reptile host by means of vector ingestion belonging to the genus *Sergentomyia* (France & Parrot, 1920), the parasite seems to have gained access to the host circulation through the surfaces of the intestinal mucosa, possibly remaining in the reptile blood stream for all its life in the extracellular promastigote form¹². Elwasila (1988)¹⁸, searching for natural infections in reptiles in Sudan, analyzed a total of 16 reptiles, detecting the presence of *L. tarentolae*, in the amastigote form, and three of them belonged to the species *T. annularis*. However, this form of *Leishmania* parasitemia was significantly low, and only 3 to 9 intracellular amastigotes could be found in a few leukocytes. Although not pathogenic to humans, *L. tarentolae* species has all the virulence genes found in the other species that are pathogenic to humans, with the exception of the A2 gene, responsible for the visceralization in species of the *Leishmania donovani* complex and also playing an important role in the transformation of promastigotes into amastigotes forms¹⁹. These forms can also be found in infected reptiles in which transmission is passive cutaneous^{20,21}. Nevertheless, Azizi *et al.* (2009)¹⁹ demonstrated that this gene does not have the same role in the non-pathogenic *L. tarentolae*. Thus, more studies are needed to clarify this issue. It is noteworthy that these genetic characteristics make the *L. tarentolae* species an important model to work on the development of vaccines²².

In the past, experimental studies were conducted in mammals, including a human volunteer who was inoculated with promastigote forms so as to observe the development of the parasite and possible emergence of pathogenicity in this volunteer. The results showed that *L. tarentolae* cycle did not develop in the human volunteer as it takes place with other *Leishmania* species, as there were no amastigote forms and no pathogeny²³.

Leishmaniasis in the Americas

For a long time, the history of leishmaniasis has been studied, requiring further clarifications regarding the origin and the behavior of parasites responsible for this infection, as well as host-parasite relationships along time.

Rabello (1925)²⁴ has suggested the existence of cutaneous leishmaniasis forms in the Americas for many years, and it was considered thereafter an indigenous disease in the Americas, long before the arrival of Europeans. Archaeological studies developed in so-called “Peruvian huacos” analyzed anthropomorphic ceramics constructed by Andean people who reproduced healthy human figures and disease-mutilated ones that could correspond to the occurrence of “uta” and “espundia”, local names for cutaneous and mucous forms of cutaneous leishmaniasis, respectively, among the Incas in the pre-Columbian period²⁵. However, the surest indication, and probably the oldest of the disease’s existence

in regions close to Brazil, was found in Tello’s study (1908) “Antiguedad de la syphilis en el Peru”, that describes the journey of missionary Frey Dom Hipolito Sanchez Rangel Fayas y Quiros, who toured in 1827 by the Amazonian region, crossing the *Solimões-Amazonas* river. According to these records, the mission had the opportunity to observe the existence of people with ulcers in legs and arms, related to insect bites, resulting in destructive lesions of the mouth and nose. On this occasion, Frey Hipolito affirmed: “[...] los mosquitos y demás insectos, son si no se tiene cuidado, um poderoso foment de llagas profundas y fétidas em piernas y brazos, hedor de boca, gallico [...]”.

However, early reports of the disease in South America have been published by the Spanish Balthasar Ramirez in his book “Descripcion del Reyno del Peru” (1580), where the disease was called “Mal de los Andes”. In the same period, Fray Rodrigo de Loayza (1586) noted the presence of nasal lesions not only in Indians but also in people entering the Andes region²⁸. Sometime later, in 1884, in Italy, the dermatologist Achille Breda described the disease in 18 Italians from *São Paulo* State. Nevertheless, it was not possible to identify the nature of the parasite. Breda was the first to refer to the disease as “Bouba Brasileira” and later, in 1910, the dermatologist Edouard Jeanselme considered that Breda was the first to describe the clinical aspects of the American cutaneous leishmaniasis in detail. For this reason, the disease became known by many researchers as “Breda Disease”²⁷.

The Bahian physician Juliano Moreira, in the year 1894, presented at the meeting of the Society of Medicine and Surgery of *Bahia*, the study entitled: “Existe na Bahia o Botão de Birska?”. In this study, Moreira meticulously described the clinical forms encountered and he named it *Bahia* Button. This was the first time that the existence of Birska button was demonstrated in Brazil. In addition, he reported the possibility of a transmitter insect of the disease. According to him: “À picada de um inseto, o muruim, tem sido muitas vezes *atribuido* por alguns doentes o início da *afecção*”²⁸.

During the construction of the Railroad Northwest of Brazil, in 1908, numerous patients arrived from deforestation areas, especially from *Bauru* city in *São Paulo* State, so that the disease has become known as *Bauru* ulcer.

The discovery of the cutaneous leishmaniasis agent in mucosal lesions by Splendore (1911)²⁹ and the confirmation of the disease in several parts of the country, as in the *Vale do Rio Doce*, *Minas Gerais*, *Amazonas* and Southern *Bahia*, among others, represented the most proficient period with respect of the disease studies in Brazil.

Although he has had a short life, Gaspar Vianna was one of the most famous personalities in research related to leishmaniasis³⁰. In 1911, he discovered a new species of the genus *Leishmania*, *Leishmania braziliensis*, now called *L. (Viannia) braziliensis*³¹. In the next year, Vianna developed a treatment for cutaneous leishmaniasis by using intravenous injections of tartar emetic³². This treatment is still used nowadays.

Aragão³³, in 1922, demonstrated for the first time the role of cutaneous leishmaniasis transmission by sandfly bites in the *Rio de Janeiro* city, when analyzing the high density of the sandfly *Lutzomyia intermedia*, with the presence of infection in *Águas Férreas* city, in the *Laranjeiras* neighborhood. The confirmation of *L. intermedia* as a potential vector

of *L. (V.) braziliensis* occurred after the onset of an ulcer in the nose of a dog, containing amastigotes after inoculation of a saline suspension containing *L. intermedia* previously fed with the patient's cutaneous leishmaniasis lesions.

In 1939-1940, the public health physician Samuel Barnsley Pessoa described the cutaneous leishmaniasis as an "occupational disease edge of the forest" and in 1941, it was estimated in more than 30,000 the number of patients only in rural areas, and these areas were considered highly endemic³⁴.

The decades from 1950 to 1980 was a period characterized by the participation of Oswaldo Forattini in studies related to cutaneous leishmaniasis. In this research, Forattini was able to elucidate issues on transmission, the existence of natural *Leishmania* spp. reservoirs, describe the Phlebotominae family taxonomy, as well as aspects of the ecology and behavior of the vector species of this protozoan. Throughout the period, several infection outbreaks took place in some Brazilian states: *São Paulo*, *Mato Grosso do Sul*, *Paraná*, *Rondônia* and *Amapá* enabling pioneering discoveries on possible *Leishmania* animal reservoirs. The results of their research clarified the epidemiological transmission of cutaneous leishmaniasis and also the cycles that may have parasites in nature³⁵.

Until the early 1960s, only to species of *L. (V.) braziliensis* were recognized to cause cutaneous leishmaniasis. Nevertheless, in 1961, Pessoa³⁶ proposed the subdivision of *L. braziliensis* in the varieties *L. braziliensis*, *Leishmania guyanensis*, *Leishmania peruviana*, *Leishmania mexicana* and *Leishmania pifanoi*, according to the differences in the clinical forms of the disease, in distinct geographical regions.

Since then, the classification of the parasite has been based on other criteria such as their behavior in culture, in experimental animals and their vectors, leading to the distinction between the *mexican* and the *braziliensis* complex³⁷. Over time, the advances made by electron microscopy, molecular biology, biochemistry and immunology, have opened new perspectives in the taxonomy of these parasites, and have applied new methods for their characterization³⁸.

Unlike cutaneous leishmaniasis, visceral leishmaniasis, known as Kala-azar (black fever), by the inhabitants of Assam, India, has symptoms that are similar to the ones of other tropical diseases¹. Fever is one of the main symptoms, but there are no visible lesions, hindering the finding of ancient descriptions in the medical records.

Historical data are very important in helping the laboratory tests to detect *Leishmania* DNA in archaeological remains. Historical documents are essential since they can, in many cases, help to unveil the space-time of leishmaniasis. Furusawa & Borges (2014)³⁹, for example, based on the analysis of postmortem inventories of slaves found in the Documentation Historical Center, demonstrated the possible existence of cutaneous leishmaniasis in the *Vassouras* city, *Rio de Janeiro* State, Brazil, in the early nineteenth century, before the period considered as the arrival of disease in Southeastern Brazil.

Hypotheses for the origin of the genus *Leishmania* and subgenera *Leishmania* and *Viannia*

Several possible origins of the genus *Leishmania* and subgenera

Leishmania and *Viannia* are proposed. Some authors suggest that the genus *Leishmania* has had its origin in the Neotropics (biogeographic region that includes Central America, from the southern part of Mexico and the peninsula below California, southern Florida, and all the Caribbean islands, to South America, while others have proposed the Palearctic, the zoogeographic region that includes Europe, North Africa, large part of Arabia and Asia to the north of the Himalayas, or the Nearctic (geographic region including the North America)^{40,41,42}. Through the use of molecular biology techniques Noyes *et al.* (2000)⁴³ obtained significant results that support the hypothesis of origin in the Neotropics. However, studies by Kerr *et al.* (2000)⁴⁰, based on the interpretation of molecular data supported by biochemical, molecular, biogeographic, ecological and entomological factors, proved that *Leishmania* has originated in the Palearctic. This hypothesis suggests that the genus *Leishmania* has entered the Nearctic across the Bering Strait during the Oligocene, rapidly dispersing during the Pliocene until reaching the Neotropics. Considering the genetic polymorphism and clonality of the genus *Leishmania*, parasites belonging to this genus may have made its way to the area where today the Americas are located, even before the separation of Gondwana, about a hundred million years ago⁴⁰.

However, regardless of their origin, the spread of this parasite occurred together with the migration of their vectors and hosts²⁵. The description of a new genus and species, *Paleoleishmania proterus*, found in remains of blood on the proboscis and digestive tract of a sandfly belonging to the Cretaceous period that was found in amber, shows that the parasite-vector relationship has existed since ancient times, suggesting that the definitive host of this parasite has been a reptile or a primitive mammal⁴⁴.

Initially it was suggested that the subgenus *Leishmania* had appeared in the Paleocene, shortly after the extinction of dinosaurs and the emergence of the first placental mammals⁴⁵. Currently, it is suggested that there has been a common ancestor of *Leishmania* which originated two distinct clades: the *Sauroleishmania* genus that infected reptiles, and *Leishmania* that currently infects mammals⁴⁵.

Considering the subgenera *L. (Leishmania)* and *L. (Viannia)* and a possible origin in the Palearctic, the current distribution of species and their molecular trees, the subgenus *L. (Leishmania)* would have been the first to emerge. But there is controversy with respect to the origin of the subgenus *L. (Viannia)*⁹. Studies prove that this subgenus only occurs in the Neotropics, while the subgenus *Leishmania* occurs both in the New World (Neotropical regions and South Nearctic), as in the Old World (Palearctic regions, African and Eastern)⁴⁶.

However, currently the most accepted theory is that the subgenus *L. (Viannia)* was originated after the separation of Gondwana and the subgenus *L. (Leishmania)* has its origin in the Palearctic, coming to the Americas by the Bering Strait⁴⁰.

Speculations about the origin of some *Leishmania* species and infection by *Leishmania* spp.

Discussions are not restricted to the possible origin the genus and subgenera. Some authors suggest, for example, that in the Old World, species responsible for visceral leishmaniasis have been separated in *L.*

donovani and *Leishmania infantum* about 1 million years ago⁴⁰. This theory was also proposed by Mauricio *et al.* (2007)⁴⁷ based on analyzes of molecular trees of these species. The *L. donovani* species, therefore, appears to have originated in East Africa, the same region of the human origin.

For *Leishmania (Leishmania) major*, another species that causes cutaneous leishmaniasis in several regions of the world, it is suggested that its origin was in North Africa, where the Saharan region had humid climate and was covered by forest⁴⁷.

It is still unclear if humans have led the infection to the Middle East, or if it was acquired from other hosts. However, the sandfly vectors have existed long before the arrival of humans and other animals that today serve as hosts in Europe and Asia. It has also been suggested that the infection has originated in Central Asia in zoonotic reservoirs, dispersing to India, to the Mediterranean and to West Africa around the fourteenth century⁴⁸.

Nevertheless, the dynamics of the population in South America between the Amazon and the inter-Andean regions was important to clarify the spread of infection and if this theory is confirmed, we will be able to say that the Andes would be the cradle of cutaneous leishmaniasis in the New World⁴⁹.

Until now studies have failed to confirm the existence of visceral leishmaniasis before the arrival of Europeans to South America⁵⁰. The studies of SHAW (2006)⁵¹ suggest that *Leishmania (Leishmania) chagasi* is originally from Europe and that it is very similar to the species *L. (L.) infantum*. Taxonomically *L. (L.) infantum* (synonym *L. (L.) chagasi*) belongs to the complex of species *L. (L.) donovani*. According to Abramson *et al.* (1995)⁵², the formation of the isthmus between North America and South America may have occurred before the spread of *Leishmania* in the Amazon region and the Northeast of Brazil, a highly endemic area of *L. (L.) chagasi*. Thus, the idea that the species *L. (L.) chagasi* has been brought by the Europeans during the fifteenth century^{53,54} is reinforced. Another hypothesis is that this parasite has existed among indigenous of the Americas^{51,55,56,57,58,59}.

Direct evidence of DNA fragments of *Leishmania* spp. in archaeological remains by different techniques

The Paleoparasitology provides new tools for the study of parasitic diseases, in which the host-parasite-environment complex cannot be separated, since it offers the only possibility of working with ancient parasites⁶⁰ found in remnants of the human species and other animals recovered from archaeological sites, paleontological, or any other source that has maintained them preserved⁶¹. Thus, infections have been diagnosed with *Leishmania* spp. in ancient populations by the application of different techniques.

Poinar & Poinar, in 2004, described a new genus and species of trypanosomatid (*Paleoleishmania proterus*) found in remains of blood on the proboscis and in the gastrointestinal tract of a sandfly found in amber from the Cretaceous period, by light microscopy. Therefore, they demonstrated that the parasite-vector relationship has existed since ancient times, suggesting that the definitive host of this parasite has been a reptile or a primitive mammal⁴⁴.

In certain high altitude areas, as in the Atacama Desert, Chile, where the disease is not normally found, the presence of these parasites suggests a pattern of mobility in endemic areas^{62,63} dating from 1,000 BC. This has been discussed in studies by Costa *et al.* (2009)⁶² who identified bone disfiguring facial injuries with leishmaniasis characteristics in four women who died in a community located in a high altitude desert where there was no leishmaniasis. To confirm the etiology of injuries, authors have used molecular methods that have identified *Leishmania* DNA in three of the individuals analyzed.

Zink *et al.* (2006)⁶⁴ used molecular techniques, mitochondrial DNA (mtDNA) to detect *Leishmania* DNA in samples of more than 90 mummies originated from the pre-Egyptian Abydos Dynasty (3,500-2,800 BC) and the Western Empire (2,050-1,650 BC). The analysis of parasite DNA found in 13 of these mummies was consistent with the DNA of *Leishmania (Leishmania) donovani*, suggesting the visceral form of infection.

Nerlich *et al.* (2012)⁶⁵ and Bianucci *et al.* (2012)⁶⁶ used molecular and immunodiagnostic tests to detect the presence of *L. (L.) infantum* in bone fragments of Eleonora from Toledo (1,522-1,562), wife of Cosimo I de' Medici and member of one of the major political Italian families during the Renaissance. The positive identification of *Leishmania* infection in bone samples was obtained using two independent approaches. The first one was the molecular analysis of ancient DNA (aDNA) by the amplification of a specific fragment of 123 bp (base pairs) from a conserved region of the mini-circle molecule of the *Leishmania* kinetoplastid mitochondrial DNA (kDNA)^{67,68} and the results were compatible with *L. (L.) infantum*. This result is achieved through the technique of Polymerase Chain Reaction (PCR) that was replicated in two independent laboratories. The second approach was a protein assay that showed a positive reaction, i.e., recognized a *L. infantum* protein, and at the same time IgG against *L. infantum* was detected by a Western blot technique.

Guillen & Allison (2005)⁴⁹, examining a peruvian mummy of a six years old girl, dated 800 BC, observed the presence of macrophages containing *Leishmania* as revealed by immune-histological examination.

As noted, the different methodologies used have been productive, providing considerable information that helps us in paleoparasitological studies of this protozoan. Small aDNA fragments detected in tissues and bones of mummies, as well as immunodiagnostic techniques used to assist molecular methods have shown that infection by this parasite were present in mummies from different regions and different periods, clarifying questions about the host-parasite relationship and providing evidence for the studies of evolution and phylogeny of these parasites.

Leishmania - infection and disease

Parasitic infections and parasitic diseases are two distinct situations originated from a single process. The parasite is a necessary condition but not sufficient to start a parasitic disease⁶⁹. The parasitic disease results from the presence of a certain parasite in a certain host, in a given population, in a given environment, and over a period of particular life coevolution of both protagonists⁶⁹.

The first attempts to classify clinical forms of leishmaniasis

date from the early twentieth century. The different clinical forms in patients affected by cutaneous leishmaniasis induce many specialists to an erroneous diagnosis⁷⁰. One of the forms resembles very much the manifestations of hanseniasis; at that time called leprosy. The first atypical and rare cases, one in Brazil, one in Bolivia and two in Venezuela presented maculo-papular and nodular lesions and the spread of the disease, its chronicity and resistance to treatment, the wealth of parasites in the lesions and the great similarity with leprosy led this clinical manifestation of leishmaniasis to be called "lepromatoid form"^{71,72}. Destombs⁷³, in 1960, comparing leishmaniasis with the polar forms of leprosy, considered the primary diffuse cutaneous and the cutaneous forms of leishmaniasis as the benign and malignant poles of the disease. In 1970, Padilha-Gonçalves⁷⁴ described the concept of cutaneous leishmaniasis polarity, considering the cutaneous form as the benign pole, the primary diffuse cutaneous as the malignant pole and the mucosa as part of the spectrum border. Thus, Padilha-Gonçalves referred to an immune-resistant pole and immunosuppressed pole⁷⁴.

The historic and classical classification of leishmaniasis is: visceral (Kalazar) produced by the *L. donovani* species (Laveran and Mesnil, 1903), cutaneous (the Orient Button) produced by *Leishmania tropica* (Wright, 1903) and mucocutaneous form (cutaneous) produced by *L. braziliensis* (Gaspar Vianna, 1911). However, this classification was made exclusively by observing the clinical and evolutionary behavior of the disease⁷⁵. However, according to Azulay (1977)⁷⁶ who had already found clinical and immune-pathological differences among leishmaniasis infections, he related the differences to defensive reactions of the human organism. Most of the times, the clinical descriptions of American cutaneous leishmaniasis (ACL) were attributed to infections by *L. braziliensis*³¹; nevertheless clinical characteristics of infections by different species of *Leishmania* have been demonstrated, leading to the proposal of various classifications^{77,78,79}. In addition, there is a close relationship among the occurrence of different clinical forms of the disease and factors related to species and parasite strains, vector habits, factors related to the host immune response and to the host-parasite interaction. Regional differences have also been observed in the occurrence of geographical areas of disease^{6,38,80}.

Considering the original description of species and proposed classifications based on extrinsic criteria and on results obtained by biochemical and molecular methods, it is currently accepted that there are several *Leishmania* species, of which more than 20 are associated with human disease in the Old and the New World⁷.

Regardless of the limitations of molecular paleoparasitology studies in which only small fragments can be analyzed, molecular techniques have recently provided the first evidence of aDNA sequences of *L. tarentolae* species found in the bone marrow and the intestines of an adult male mummy, coming from the Itacambira's municipality, Minas Gerais State, Brazil, dated from the colonial period. Although, more studies are needed in an attempt to recover *L. tarentolae* DNA from other regions so as to prove that the infection in the mummy was not an isolated finding, this result at least opens up some prospects for the understanding of the health-disease process. This finding reopens the debate about the potential existence of a *L. tarentolae* strain in human macrophages, as well as its capacity to systematically disseminate⁸¹. Nevertheless, as has been discussed in the case of sauroleishmaniasis, until the moment, no pathogenic effect caused by *L. tarentolae* has been proved in humans.

The finding of this *Leishmania* species in the Brazilian mummy raises an important question that could be related to epidemiological investigations performed in endemic areas in which positive serological results were found, but there was no evidence of injury. A similar situation was demonstrated by Marzochi *et al.* (1980)⁸², after analyzing the frequency of antibody titers through an indirect immunofluorescence test and the Montenegro test in 47 individuals in Jacarepagua neighborhood, Rio de Janeiro State, Brazil suspected of having for American cutaneous leishmaniasis. The study found positive antibody titers in 57.2% of individuals without lesions or scars, examined in 1974 and reexamined in 1978. This discordance between the serological results and clinical manifestations had already been demonstrated by Pessoa & Barreto (1948)²⁶ in the Brazilian territory and by Aston & Thorley (1970)⁸⁴, analyzing Montenegro test results performed among Amerindians of the Xingu National Park region.

Would those be cases of *Leishmania* infection without illness? However, the detection of a DNA fragment compatible with *L. tarentolae* in a human host, although mummified and dated of 300 years ago, brings the possibility of infection by closely related species incapable of causing injury, but with the potential to stimulate the host defense and immunity.

Unfortunately, questions related to parasites of the genus *Leishmania* still remain, especially regarding the origin of these parasites. The paleoparasitology can clarify events that took place in ancient times among the species of *Leishmania* and their hosts, contributing to the understanding of the origin and evolution of the parasite⁸⁴. Although the immunodiagnostic techniques continue to be applied in paleoparasitologic studies for the diagnosis of *Leishmania* spp.⁶⁶, the emergence of molecular techniques that have higher sensitivities have significantly opened this field of study. The technical adaptation of PCR to detect aDNA made the diagnosis of various infections in prehistoric populations possible. However, there is always the need to develop new methods and procedures adjustments according to the process and the status of preservation of the archaeological material^{85,86,87}.

The paleoparasitological steps are promising to clarify the gaps that still exist in relation to leishmaniasis. The most encouraging is that there are many possibilities for the paleoparasitology research that could bring new data on the antiquity and developmental stages of the host-parasite relationship, and also epidemiological data which could suggest strategies applicable today.

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