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Leishmania chagasi Cunha & Chagas, 1937: nativa ou introduzida? Uma breve revisão

Leishmania chagasi Cunha & Chagas, 1937: ¿nativa o introducida? Una breve revisión

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ABSTRACT

This review focused on the etiology of American visceral leishmaniasis due to a recent polemic regarding the origin of its etiological agent, *Leishmania chagasi* Cunha & Chagas, 1937. This parasite was described as a new *Leishmania* species in light of its inability to produce experimentally visceral disease in domestic dogs; this characteristic distinguished it from the other prior known etiologic agent of visceral leishmaniasis in the Mediterranean Basin of Europe, *Leishmania infantum* Nicolle, 1908. After 50 years of *Leishmania chagasi* investigation, the genus *Leishmania* was reviewed and the parasite was reclassified as a member of the subgenus *Leishmania*, species *Leishmania* (*Leishmania*) *chagasi*. Recently, after molecular analysis using the random amplified polymorphic DNA (RAPD) technique that compared *L.* (*L.*) *chagasi* with *L.* (*L.*) *infantum*, it was concluded that these species were genetically indistinguishable and, therefore, *L.* (*L.*) *chagasi* was regarded as synonymous with *L.* (*L.*) *infantum*. For this reason, this review has evaluated all knowledge concerning the eco-epidemiology of *L.* (*L.*) *chagasi* in the Brazilian Amazon, principally in regard to the sylvatic habits of its phlebotomine sandfly vector, *Lutzomyia longipalpis*, and its vertebrate reservoir, the wild fox *Cerdocyon thous*, with the aim of showing that *L.* (*L.*) *chagasi* cannot be neglected from the parasitological investigation of visceral leishmaniasis in the New World; it must be considered, at least, at the subspecific level as *Leishmania* (*L.*) *infantum chagasi*.

Keywords: *Leishmania*; Leishmaniasis, Visceral; *Leishmania chagasi*.

HISTORICAL BACKGROUND ON THE TAXONOMIC STATUS OF THE ETIOLOGIC AGENT OF AMERICAN VISCERAL LEISHMANIASIS

The etiologic agent of American visceral leishmaniasis (AVL) was first described by Cunha and Chagas[3] with the specific name of *Leishmania chagasi*, a protozoal parasite that belongs to the family Trypanosomatidae Doflein, 1901 emended Grobben, 1905 and the genus *Leishmania* Ross, 1903. The decision to regard it as a new *Leishmania* species was principally due to the repeated negative results that followed from attempts to experimentally infect dogs with the parasite, which distinguished it from the other previously known etiologic agent of visceral leishmaniasis in some European countries of the Mediterranean Basin, *Leishmania infantum* Nicolle, 1908. However, only 50 years after the description of *Leishmania chagasi*, Lainson and Shaw[10] performed a wide review of the genus *Leishmania* and reclassified the parasite as a member of the subgenus *Leishmania* Saf'Janova, 1982 (= Ross, 1903), species *Leishmania* (*Leishmania*) *chagasi*.

Recently, after a large comparison of several strains of *L.* (*L.*) *chagasi* that have been isolated from different origins and countries in South America, especially in Brazil (from humans, domestic dogs, and the wild fox *Cerdocyon thous*), and of strains of *L.* (*L.*) *infantum* that originated from the endemic area of visceral leishmaniasis in some countries from the Mediterranean Basin in Europe, such as Portugal and Spain, the random amplified polymorphic DNA (RAPD) technique illustrated that the DNA sequences of both species of parasites were identical. For this reason, the authors of this analysis concluded that *L.* (*L.*) *chagasi* is synonymous with *L.* (*L.*) *infantum* and therefore proposed that *L.* (*L.*) *chagasi* should not be considered as a valid species[15].

More recently, however, Lainson and Shaw[11] defended the maintenance of the parasitological entity at the subspecific level as *Leishmania* (*L.*) *infantum chagasi* based on its ecological characteristics, such as the sylvatic habitat of its phlebotomine sandfly vector, *Lutzomyia longipalpis*[7], and its natural vertebrate reservoir, the wild fox *Cerdocyon thous*[20], in addition to previously established distinct

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differences between the kDNA fragment profiles of *L.* (*L.*) *chagasi* and *L.* (*L.*) *infantum* demonstrated by the use of a restriction endonuclease digestion technique[6,5], radioiodinated surface proteins of their promastigotes, monoclonal antibodies generated against promastigote surfaces[18], and comparative radiorespirometry studies[4].

THE CURRENT DILEMMA ON THE ORIGIN OF *LEISHMANIA CHAGASI*: IS IT INDIGENOUS OR INTRODUCED?

Despite the reasonable argument presented by Lainson and Shaw[11] that aimed to maintain the scientific name *L.* (*L.*) *i. chagasi* at the subspecific level, which appears absolutely justifiable in view of the long scientific history built on more than 50 years of published works on the eco-epidemiology, clinical manifestations and immunopathology of the disease caused by this parasite in America, some authors[15,16,14] have made attempts to disqualify any effort towards the maintenance of the parasitological entity either at the specific level [*L.* (*L.*) *chagasi* Lainson & Shaw, 1987] or at the subspecific level [*L.* (*L.*) *i. chagasi* Lainson & Shaw, 2005]. For these authors, only *L.* (*L.*) *infantum* and *L.* (*L.*) *donovani* are recognized as etiologic agents of visceral leishmaniasis, with the former occurring in the endemic areas of the Mediterranean Basin in Europe, North Africa and Central and South America and the latter in the endemic areas of India, East-Africa and the Middle East.

Taking this issue into account, we consider opportune to express our opinion concerning the taxonomic proposal by Mauricio et al[15,16], who used DNA molecular analysis of the parasite as the unique and definitive criterion for deciding the originality of *Leishmania* species under study. Furthermore, it should also be stressed that a species definition might not only be based on its phylogenetic background but also on its biological, phenetic and phenotypic characteristics that are the result of the parasite-genomic background and environment interactions. Thus, modern genetic concepts and technologies should be used to utilize these concepts[24]. In this respect, it appears clear that Mauricio et al[15,16] have neglected the phenotypic features that are typically used for classifying *Leishmania* parasites, such as the following: the morphology of amastigote and promastigote forms; the experimental biological behavior of the parasite in domestic and wild animals and in the phlebotomine sandfly vectors; and immunology, biochemistry, eco-epidemiology and pathogeny, i.e., the clinical manifestations of infection[10,19].

In contradiction to these authors[15,16,14] who asserted that *L.* (*L.*) *chagasi* (synonymous with *L.* (*L.*) *infantum*) was introduced in America during its colonization, two issues must be addressed before any affirmation. First, considering that the first AVL cases in Brazil were diagnosed in 1934[17], how to explain that in a small time interval, little more than 400 years after colonization, *L.* (*L.*) *infantum* was promptly capable of surviving in *Cerdocyon thous*, which, differently from domestic dog, does not develop any signals or symptoms due to a pathogenic action induced by the parasite. This type of adaptation reflects a stage of coexistence between the parasite and the vertebrate host, which is generally accepted only after a long interactive process between the parasite and the immune response of the vertebrate host that occurs over thousands of years. Second, *L.* (*L.*) *infantum*, which was well adapted to its phlebotomine sandfly vector, *Phlebotomus dubosqi*, in endemic countries in Europe, would have needed to promptly adapt to another species of phlebotomine sandfly vector, *Lutzomyia longipalpis*, from a continent that presents completely different weather and ecology from those in Europe. Additionally, it is also important to emphasize recent evidence suggesting that this speciation process between the parasite and its phlebotomine sandfly vector is strongly influenced by a specific interaction between the ligand glycoconjugate molecules, principally lipophosphoglycan (LPG), present on the plasmatic membrane surface of the metacyclic promastigote forms of *Leishmania* species and their receptors on the epithelial cell membrane of the midgut wall of the phlebotomine sandfly vector[2,22,21]. LPG has been implicated as a specific adhesion molecule that mediates the interaction of *Leishmania* with the midgut epithelium of the phlebotomine sandfly vector. Thus, it might be expected that *L.* (*L.*) *infantum*, which is naturally transmitted by *Phlebotomus dubosqi*, would promptly be capable of adapting to the midgut epithelium of *Lutzomyia longipalpis*, a phlebotomine sandfly vector for a completely different species and genus of *L.* (*L.*) *infantum* in an endemic area in Europe.

As previously established, this speciation process among the parasite, its biological vector and its wild reservoir, which characterizes a natural enzootic cycle of *Leishmania* species, was strongly supported in the case of a *Leishmania* parasite that is very closely related to that *L.* (*L.*) *chagasi* that was isolated from the viscera (liver and spleen) of three healthy *Cerdocyon thous* wild foxes that were captured in a forested peri-urban area called "Utinga Environmental Park" (Figure 1), near Belém, the capital city of Pará State, Brazilian Amazon, where there is no evidence of human or canine visceral disease to date. However, this *Leishmania* parasite has proved to cause lethal visceralizing infection in golden hamsters after three months of intraperitoneal inoculation[12]. Thus, considering that this area is still uninhabited by humans and dogs, this finding represents a strong evidence on the existence of an indigenous enzootic cycle of *L.* (*L.*) *chagasi*-like parasites in the Brazilian Amazon, which was certainly originated much before the recent history of America colonization.

a: The water treatment station of Belém water supplying company; b: Água Preta Lake, the natural source of Belém water supplying company.

Figure 1 – The Utinga Environmental Park situated in a forested peri-urban area close to Belém, the capital city of Pará State, Brazilian Amazon

Another point that contradicts the evolutionary hypothesis[15,16,14], which agrees that the genus *Leishmania* originated in South America in the Paleocene or Eocene with subsequent diversification after migration into Asia, concerns the interpretation on what has occurred with the ancestral *Leishmania* that migrated to Asia. The evolutionary hypothesis asserts that no ancestral *Leishmania* remained in South America, however, how to explain the origin of a large number of *Leishmania* species existing in this region; actually, there are more than 20 well-recognized *Leishmania* species belonging to subgenera *Leishmania* and *Viannia*, including *L.* (*L.*) *chagasi*, the only leishmanial parasite responsible for AVL[11].

CONCLUDING REMARKS

Considering the aforementioned aspects, two major features should be addressed. The first feature refers to the considerable speculation over the evolutionary hypothesis proposed by Lukes et al[14] based on the phylogenetic analysis between *L.* (*L.*) *chagasi* and *L.* (*L.*) *infantum*. As we have commented, although a great phylogenetic similarity between these two parasites has been demonstrated, it was proposed by these authors that the genus *Leishmania* originated in South America in the Paleocene or Eocene with subsequent diversification after its migration into Asia. This yielded two main branches: *Leishmania donovani*, in the Indian subcontinent, and *Leishmania infantum*, in Europe. More recently, it was also suggested that *Leishmania infantum* would have been introduced in America by settlers. This evolutionary hypothesis appears both speculative and contradictory; it is impossible to believe that a *Leishmania* ancestor would migrate into Asia without leaving in its original birth place a descending parasite. Recently, in the Utinga Environmental Park, the same area where Lainson et al[12] found a *L.* (*L.*) *chagasi*-like parasite from natural visceral-infection in *Cerdocyon thous*, a *Leishmania* parasite was identified in *Lutzomyia tuberculata*, *Leishmania* (*Viannia*) *utingensis* Braga, Lainson, Ishikawa & Shaw[1]. This parasite has never been found out of the local-type where it was firstly isolated, clearly demonstrating, together with *L.* (*L.*) *chagasi*-like parasites, the existence of closed indigenous enzootic cycles of *Leishmania* parasites in the New World that undoubtedly have directly descended from an indigenous *Leishmania* ancestor. In accordance with this finding, it should also been emphasized the recent finding of *Lutzomyia longipalpis* in a forested area in the Instituto Evandro Chagas field in Ananindeua Municipality, Pará State, Brazil[23], which is contiguous with the Utinga Environmental Park. This further confirms the existence of an indigenous enzootic cycle of *L.* (*L.*) *chagasi* in this area where there is no evidence of human or canine visceral disease.

The second feature to be addressed is the criteria (morphology, experimental biology, eco-epidemiology, immunology, biochemistry, and pathogenicity) used for identification and classification of *Leishmania* parasites, which were largely discussed by Lainson and Shaw[10] in the last review of the genus *Leishmania*. As it was recently observed by Tibayrenc[24], although the great advantages of *Leishmania*-DNA analysis by molecular biology techniques for different aims have been acknowledged, DNA analysis should be used as a complementary tool for the precise identification of a *Leishmania* species and not as the determining factor for its identification. This way, we cannot omit the importance of some works regarding, principally, the originality of the biology and eco-epidemiology of *L.* (*L.*) *chagasi* in America[7,20,12,13,9,8].

In conclusion, we cannot agree with the proposal of disqualifying *L.* (*L.*) *chagasi*, which has been recognized for over 50 years as the etiologic agent of AVL, in view of its DNA similarity to *L.* (*L.*) *infantum*. This finding alone does not confirm its taxonomic characteristics sufficiently enough to negate all scientific knowledge that has been accumulated concerning this Leishmania parasite in the last 50 years. Thus, we believe the best decision to be made regarding this issue is to maintain the subspecific status of the etiologic agent of AVL as *L.* (*L.*) *i. chagasi*, as it was proposed by Lainson and Shaw[11] in their last review of the neotropical *Leishmania* parasites with medical importance.

Leishmania chagasi Cunha & Chagas, 1937: nativa ou introduzida? Uma breve revisão

RESUMO

Esta revisão aborda a etiologia da leishmaniose visceral americana devido a uma recente polêmica sobre a origem do seu agente etiológico, a *Leishmania chagasi* Cunha & Chagas, 1937. Conforme é sabido, este parasito foi descrito como uma nova espécie de *Leishmania* em razão da sua incapacidade de produzir, experimentalmente, a leishmaniose visceral no cão doméstico; este caráter a diferenciou de outro agente etiológico já conhecido da leishmaniose visceral na Bacia do Mediterrâneo na Europa: *Leishmania infantum* Nicolle, 1908. Após 50 anos da descrição da *Leishmania chagasi*, o gênero *Leishmania* sofreu ampla revisão e o parasito foi reclassificado como um membro do subgênero *Leishmania*, espécie *Leishmania* (*Leishmania*) *chagasi*. Recentemente, em seguida a uma análise molecular usando a técnica da amplificação aleatória polimórfica do DNA (RAPD), que comparou a *L.* (*L.*) *chagasi* com a *L.* (*L.*) *infantum*, foi concluído que ambos os parasitos eram geneticamente indistinguíveis e, portanto, que a *L.* (*L.*) *chagasi* era sinônimo de *L.* (*L.*) *infantum*. Por esse motivo, esta revisão procurou agregar todo o conhecimento sobre a eco-epidemiologia da *L.* (*L.*) *chagasi* na Amazônia brasileira, principalmente acerca dos hábitos silvestres de seu flebotomíneo vetor, *Lutzomyia longipalis*, e seu reservatório vertebrado, a raposa do campo *Cerdocyon thous*, com o propósito de demonstrar que a *L.* (*L.*) *chagasi* não pode ser negligenciada do cenário parasitológico da leishmaniose visceral no Novo Mundo; ela deve ser considerada, pelo menos em um nível subespecífico, como *Leishmania* (*L.*) *infantum chagasi*.

Palavras-chave: *Leishmania*; Leishmaniose Visceral; *Leishmania chagasi*.

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RESUMEN

Esta revisión aborda la etiología de la leishmaniasis visceral americana debido a una reciente polémica sobre el origen de su agente etiológico, la *Leishmania chagasi* Cunha & Chagas, 1937. Como se sabe, este parásito fue destrito como una nueva especie de *Leishmania* en razón de su incapacidad de producir, experimentalmente, la leishmaniasis visceral en el perro doméstico; este carácter la diferenció de otro agente etiológico ya conocido de leishmaniasis visceral en la Cuenca del Mediterráneo en Europa: *Leishmania infantum* Nicolle, 1908. Luego de 50 años de la descripción de la *Leishmania chagasi*, el género *Leishmania* ha sufrido amplia revisión y el parásito fue reclasificado como un miembro del subgénero *Leishmania*, especie *Leishmania* (*Leishmania*) *chagasi*. Recientemente, en seguida a un análisis molecular usando la técnica de amplificación aleatoria polimórfica del DNA (RAPD), que comparó *L.* (*L.*) *chagasi* con *L.* (*L.*) *infantum*, se concluye que ambos parásitos eran genéticamente indistinguibles y, por lo tanto, que *L.* (*L.*) *chagasi* era sinónimo de *L.* (*L.*) *infantum*. Por ese motivo, esta revisión buscó agregar todo el conocimiento sobre la ecoepidemiología de *L.* (*L.*) *chagasi* en la Amazonía brasileña, principalmente acerca de los hábitos silvestres de su flebótomo vector, *Lutzomyia longipalis*, y su reservorio vertebrado, el zorro de campo *Cerdocyon thous*, con el propósito de demostrar que no se debe ser negligente con *L.* (*L.*) *chagasi* en el escenario parasitológico de la leishmaniasis visceral en el Nuevo Mundo; debe ser considerada, al menos en un nivel subespecífico, como *Leishmania* (*L.*) *infantum chagasi*.

Palabras clave: *Leishmania*; Leishmaniasis Visceral; *Leishmania chagasi*.

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