Acta Tropica 221 (2021) 106018

Contents lists available at ScienceDirect

Acta Tropica

journal homepage: www.elsevier.com/locate/actatropica

Phenotypical and genotypical differences among *Leishmania (Leishmania) amazonensis* isolates that caused different clinical frames in humans and dogs: A systematic review

Taylon Felipe Silva [a,*], Fernanda Tomiotto-Pellissier [a,b], Aline Kuhn Sbruzzi Pasquali [c], Fernanda Pinto-Ferreira [d], Wander Rogério Pavanelli [a], Ivete Conchon-Costa [a], Italmar Teodorico Navarro [d], Eloiza Teles Caldart [d]

[a] *Laboratório de Imunoparasitologia de Doenças Negligências e Câncer – LIDNC, Departamento de Ciências Patológicas, Centro de Ciências Biológicas, Universidade Estadual de Londrina, Londrina, Brazil*
[b] *Instituto Carlos Chagas, Fiocruz Paraná, Curitiba, Brazil*
[c] *Universidade do Oeste de Santa Catarina, Campos Novos, Brazil*
[d] *Departamento de Medicina Veterinária Preventiva, Centro de Ciências Agrárias, Universidade Estadual de Londrina, Londrina, Brazil*

ARTICLE INFO

Keywords:
Cutaneous leishmaniasis
Leishmania amazonensis
Visceral leishmaniasis

ABSTRACT

Leishmania (Leishmania) amazonensis is an important etiological agent of American cutaneous leishmaniasis (ACL) in Brazil. The species causes a large spectrum of clinical manifestations in humans and dogs, ranging from cutaneous, cutaneous diffuse, mucocutaneous, and visceral involvement, however, the factors that drive the development of different disease forms by the same species are not yet fully known. In the present work, it was systematically reviewed the studies addressing phenotypic and genotypic characteristics of *Leishmania (L.) amazonensis* isolates causing cutaneous and visceral clinical frames in humans and dogs, comparing the results observed. For this, four research databases were searched for the following keywords: (*Leishmania amazonensis* AND visceral leishmaniasis) AND (tropism OR virulence OR visceralization OR adaptations OR mutation OR clinical presentation OR resistance OR survival OR wide spectrum). The results revealed that the complexity disease seems to involve the combination of genetic factors of the parasite (as modifications in molecules related to the virulence and metabolism) and also of the host's immune background and status. Nonetheless, the exact mechanism that leads to different clinical manifestations between strains of the same species is still uncertain and future studies must be developed to better elucidate this phenomenon.

Impacts

- *L. amazonensis* can cause differents clinical manifestations in humans and dogs.
- Single Nucleotide Polymorphisms (SNP) and chromosomic alterations in the *L. amazonensis* genome could favor parasite adaptation to the vertebrate infection, survival, and proliferation.
- Strains isolated from CL induce a higher inflammatory condition than strains from VL.

1. Introduction

American cutaneous leishmaniasis (ACL) is the most ommon form of leishmaniasis and causes skin lesions, mainly ulcers, on exposed parts of the body, leaving life-long scars and serious disability or stigma. The World Health Organization (WHO) estimates that almost 1 million of new cases of ACL happens annually. Around 95% of this, occurs in Americas, the Mediterranean basin, the Middle East and Central Asia (WHO, 2021). The *Leishmania (Leishmania) amazonensis* species has been found as an etiological agent in 8-12% of cases of ACL in South America (Burza et al., 2018; Coelho et al., 2011; Gonçalves et al., 2020). The spectrum of clinical manifestations caused by *L. (L.) amazonensis* in

* Corresponding author: Celso Garcia Cid Highway, PR-445, Km 380, Londrina, PR, 86057-970. Laboratório de Imunoparasitologia de Doenças Negligências e Câncer -LIDNC, Departamento de Ciências Patológicas, Universidade Estadual de Londrina, Londrina, Brazil.
E-mail address: taylonfelipe@hotmail.com (T.F. Silva).

https://doi.org/10.1016/j.actatropica.2021.106018
Received 3 May 2021; Received in revised form 15 June 2021; Accepted 15 June 2021
Available online 19 June 2021
0001-706X/

humans and dogs is very large, ranging from cutaneous, cutaneous diffuse, mucocutaneous, and visceral involvement (Oliveira et al., 2007), however the exact mechanism that lead to different clinical frames has not yet been fully described . Barral et al. (1991), reported 11 humans with visceral leishmaniasis (VL) caused by *L (L.) amazonensis* in Bahia. In dogs, similar cases have also been reported in the city of Araçatuba, São Paulo state; Paracatu, Minas Gerais state; Londrina, Paraná state and Governador Valadares, Minas Gerais state (Dias et al., 2011; Hoffmann et al., 2012; Tolezano et al., 2007; Valdivia et al., 2017), however, epidemiological data on visceral involvement caused by *L. (L.) amazonensis* are scarce.

The genomes of *Leishmania* species have a high degree of synteny, which means that the order of genes within chromosomes is conserved in different species, this degree of synteny is used to assess the phylogenetic proximity between species (Downing et al., 2011; Peacock et al., 2007; Raymond et al., 2012). *Leishmania* spp. are considered diploids microorganisms, as they carry two copies of most of their chromosomes (Bastien et al., 1992; Downing et al., 2011; Peacock et al., 2007); however, aneuploidy and variation in the number of chromosomal copies between species and even between isolates of the same species can occur (Downing et al., 2011; Mannaert et al., 2012; Sterkers et al., 2012). This variation in the number of chromosomal copies may be responsible for phenotypic variations in terms of pathogenesis and virulence in the different isolates (Paletta-Silva et al., 2011) for altering the level of expression of proteins (Santos, 2014; Zhang and Matlashewski, 2010). Comparisons between the genomes of the different *Leishmania* species show a reduced number of species-specific genes, the function of most of them is not known and it is believed that they may be related to the tropism of the parasite or clinical picture (Peacock et al., 2007; Smith et al., 2007).

An important characteristic of the genomic structure of the *Leishmania* genus is the existence of tandem matrices of duplicated genes (Peacock et al., 2007). In the absence of a regulated transcriptional control, as found in other eukaryotes, it is believed that these duplications of genes allow the increase of the gene expression of specific proteins, a good example is the A2 gene/protein family, first discovered in *L. (L.) infantum* (Charest and Matlashewski, 1994). Evidence indicates that it is one of the most eligible candidates for virulence and visceralization factor in leishmaniasis (Fernandes et al., 2014; Zhang et al., 2008).

When compared to other species of *Leishmania, L. (L.) amazonensis* has a greater capacity to resist macrophages, neutrophils, and leishmanicidal drugs. In addition, amastigotes are effective in establishing silent infection, preventing the activation of dendritic and natural killer cells. The lesion mediated by *L. (L.) amazonensis* occurs due to the generation of pathogenic CD4 + T lymphocytes and macrophages that produce low levels of IFN-γ, IL-10, and IL-17 and an imbalance between Th1 and Th2 response patterns, culminating in host susceptibility to the parasite (Cardoso et al., 2020; Soong et al., 2012; Wanderley et al., 2019).

Little is known about parasitic factors influencing the outcome of infection, but the activities of ecto-ATPase, ecto-ADPase and 5'-nucleotidase enzymes involved in the hydrolysis of extracellular nucleotides have been thought to play a role in parasite survival and proliferation (Arora and Rai, 2019; Berrêdo-Pinho et al., 2001; Carvalho et al., 2019; Pinheiro et al., 2006).

Furthermore, arginase is an enzyme expressed by parasites and is required for the production of ornithine, which generates polyamines (Aoki et al., 2019a) and it has been associated with the persistence of the parasite in macrophages and neutrophils, and also with the suppression or exhaustion of T cells, impairing the development of an effective immune response (Pessenda and Silva, 2020).

The objective of this systematic review was to search for scientific studies that have compared phenotypic and genotypic characteristics of *Leishmania (L.) amazonensis* isolates causing cutaneous and visceral clinical frames in humans and dogs and to compare the results observed.

2. Material and methods

The systematic review was carried out to answer the following questions: are there any phenotypic and/or genotypic differences between the isolates of *L. (L.) amazonensis* that caused cutaneous or visceral clinical frames in dogs and humans? If so, what are they?

To this, from April 20 to 25, 2020, two investigators searched in four research databases for the following keywords: (*Leishmania amazonensis* AND visceral leishmaniasis) AND (tropism OR virulence OR visceralization OR adaptations OR mutation OR clinical presentation OR resistance OR survival OR wide spectrum). The criterion for article inclusion was papers that compared isolates obtained from visceral and/or cutaneous clinical frames presented by humans or dogs infected with *L. (L.) amazonensis*, besides that, articles need to assess phenotypic and/or genotypic differences among them. Just papers in English or Portuguese were included. The exclusion criterion was papers that deal with other *Leishmania* species or that deal with *L. (L.) amazonensis* causing only cutaneous clinical frames.

The researchers downloaded the libraries resulting from research in PubMed, Scielo, Web of Science, Scopus and, using Mendeley® program (Elsevier, Amsterdam), generated a combined database, excluding duplicates and empty files. Considering the articles from the combined database, two researchers performed the screening by titles and abstracts. After the first article selection, the files were converted into a BibTex format (.bib), grouped into a single folder in the Mendeley® program and their full texts were analyzed according to the inclusion and exclusion criteria mentioned above. In an Excel software spreadsheet, the following information extracted from the selected articles were typed: the geographic origin of the isolate, species from which the isolate was obtained, which tool was used for the phenotypic and/or genotypic comparison of the isolates, how it was carried out and the results and conclusions that were obtained by the authors.

3. Results

A total of 217 articles were identified from the database queries, in duplicate article analysis, 33 articles were removed. Subsequently, a manual analysis of the articles was carried out, eleven papers were read and evaluated for the presence of the inclusion and exclusion criteria (Fig. 1). Four articles remained: Almeida et al. (1996), Oliveira et al. (2007), Souza et al. (2011), and Valdivia et al. (2017). By reading the full texts, we verified that two papers deal with genotypic (Oliveira et al., 2007; Valdivia et al., 2017) and two (Almeida et al., 1996; Souza et al., 2011) worked with phenotypic differences among *L. (L.) amazonensis* isolates that caused visceral and cutaneous clinical frames in humans and dogs. All of them were conducted in Brazil, three with isolates of *L. (L.) amazonensis* from Bahia state (Almeida et al., 1996; Oliveira et al., 2007; Souza et al., 2011) and one with isolates of *L. (L.) amazonensis* from Minas Gerais state (Valdivia et al., 2017), published between 1996 and 2017.

Taking into account papers that evaluated phenotypic profiles, the authors inoculated 5×10^6 promastigotes subcutaneous in the left paw of the mice by the lineage BALB/c (Almeida et al., 1996) or CBA (Souza et al., 2011). The differences between cutaneous and visceral isolates are described in Tables 1 and 2. Considering papers that evaluated genotypic profiles, Oliveira et al. (2007) found differences between isolates, however, they did not allow differentiating specifically cutaneous from visceral isolates. Valdivia et al. (2017) observed different results between cutaneous and visceral isolates that are described in Table 3.

4. Discussion

There are several gaps to be filled for a complete understanding of the relationship between the genotype of the parasite and the clinical manifestation of the disease. Genetic variations in *Leishmania* parasites are fundamentally important because they are associated with emergent

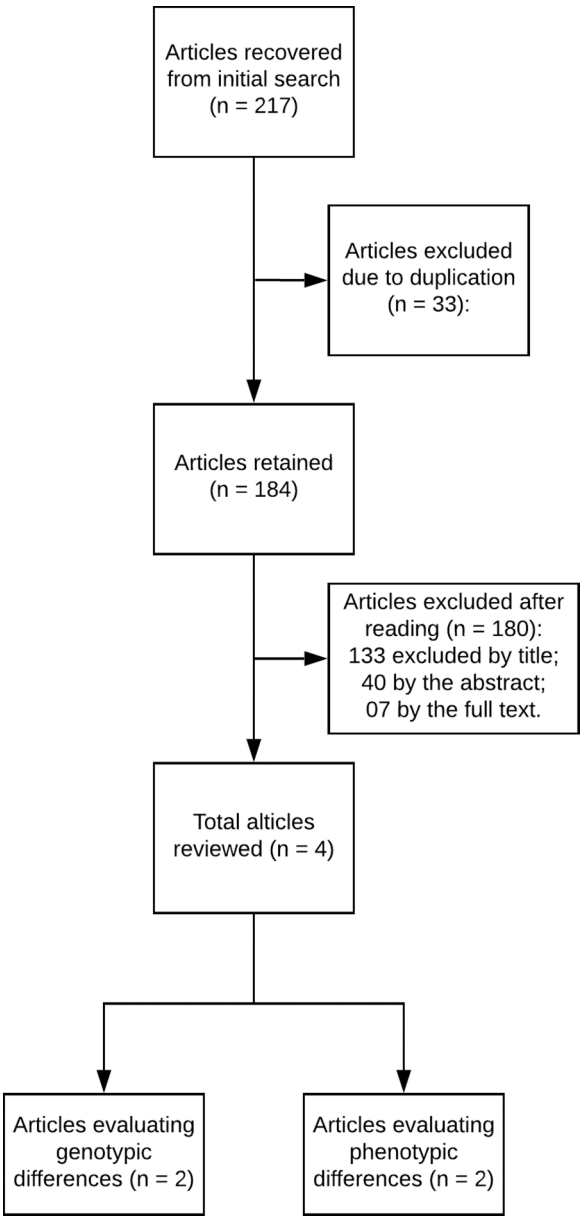

Fig. 1. Methodological flow chart. Demonstration of articles excluded/included in the study and distribution of the groups evaluated.

sub-species or strains, araising of diverse phenotypic aspects, and the formation of novel haplotypes. Such changes can ultimately result in variation of the clinical spectrum of the disease and generation of drug-resistant parasites (Cupolillo et al., 1998; Mirzapour et al., 2019).

Oliveira et al. (2007), analyzed parasite DNA by sequencing the ITS 1, 2, and the 5.8 S subunit of the ribosomal RNA genes, RAPD, and PFGE followed by hybridization with gene-specific probes. The authors showed that the isolated were genetically diverse and that this divergence extended to variations in terms of chromosome size. However, they didn't find any correlation between genetic polymorphism and visceral or cutaneous phenotypes. Similarly, Schönian et al. (2000) didn't find a correlation between genetic variability within the species *Leishmania aethiopica* and the clinical variations of cutaneous leishmaniasis, and Teixeira et al., (2017) suggested that individual sequence variants of *L. donovani* didn't correlate with clinical outcome or adaptation to different hosts. However, several studies have demonstrated a correlation between genetic *Leishmania* diversity and phenotypic manifestations (Boité et al., 2020).

The results found by Oliveira et al., (2007) can be explained by the limitations of the techniques used. Although ITS is a highly sensitive method for *Leishmania* detection (Akhoundi et al., 2017), this technique investigates only a single gene, which may not have been enough to demonstrate the difference among the isolates. In fact, it was previously described the ITS limitations to differentiate the closely related species *L. donovani* and *L. infatum* (Schönian et al., 2003). Also, the primer variations can limit the power of this technic (Hitakarun et al., 2014).

Similarly, RAPD, despite being a simple and inexpensive method to detect DNA polymorphism used as a tool to identify the genetic variability of various classes of microorganisms (Franco-Duarte et al., 2019), has limitations that include variable reproducibility, which can be caused mainly by the size ranges of DNA fragments and the used primers, and appear to be related to the low stringency (Penner et al., 1993). Lastly, as PFGE evaluates the chromosome size classes (Bastien et al., 1998), a common limitation of this technique is the non-discrimination between unrelated isolates (CDC, 2020).

Another theory that could explain these results is that, while there are no differences at the genomic level, there could be differences at the post-transcriptional and post-translational levels that would be directing the different phenotypes. These types of changes have already been shown to be important regulators of virulence and drug resistance (Mandal et al., 2015; Rosenzweig et al., 2008; Sundar and Singh, 2018).

In the second work, Valdivia et al. (2017), characterized isolates of 36 dogs with clinical manifestations of visceral leishmaniasis from an endemic area in Brazil. Among the samples, two were identified as *L. amazonensis* (S3 and S6), which were studied in terms of single nucleotide polymorphisms (SNPs), chromosome number variation, and gene copy number variation by complete genome sequencing.

To confirm the identification of the isolates, the authors compared S3 and S6 in relation to the *L. (L.) infantum* (JPCM5) and *L. (L.) amazonensis* (M2269) reference strains, and they could prove that both isolates were specifically related to *L. amazonensis*. This classification was also confirmed by genome assembly, employing a hybrid assembly approach to generate a draft genome sequence for each isolate and by phylogenetic analysis.

Table 1
Differences between strains of *L. amazonensis* isolated from cutaneous and visceral leishmaniasis in BALB/c mice infection.

Author, Year	Clinical Condition	International Code	Lesion Size	Visceralization (Weeks p.i.)		Histopathology			Spleen Weight	Metastasis	Serology	Death
				Spleen	Lymph.	Lymphocytes	Macrophages	Granuloma				
ALMEIDA et al, 1996 (Almeida et al., 1996)	Cutaneous	MHOM/BR/87/BA125	Larger	3	3	Low	Heavily infected	Absent	Gradual increase	Present	Higher titles	Yes
	Visceral	MHOM/87/BR/BA109	Smaller	3	8	High	Lightly infected	Present	Increase followed by decrease	Absent	Lower titles	No
	Visceral	MHOM/BR/87/BA137	Smaller	15	3	High	Lightly infected	Present	Increase followed by decrease	Absent	Lower titles	No

Table 2
Differences between strains of *L. amazonensis* isolated from cutaneous and visceral leishmaniasis in CBA mice infection.

Author, Year	Clinical Condition	International Code	Lesion Size	Visceralization (Weeks p.i.)			Histopathology Lymphocytes	Macrophages	Neutrophils	Parasitophorous vacuole	IL-4, IL-10, IL-12, and IFN-γ	Nitric oxide	Arginase	Ecto-ATPases
				Spleen	Lymph.	Bone marrow								
SOUZA et al., 2011 (Souza et al., 2011)	Cutaneous	MHOM/BR/87/BA125	Larger	4	11	7	Low	Heavily infected	High	Larger	p>0.05	p>0.05	Higher	Lower
	Visceral	MHOM/87/BR/BA109	Smaller	11	NV	NV	Low	Lightly infected	Low	Smaller			Lower	Higher

In the study by Valdivia et al. (2017), in search of possible variations between the *L. (L.) amazonensis* species, the isolates were compared with the reference strain M2269 by SNP. The results of this study found 15, 550 and 16,178 SNPs in S6 and S3, respectively, in addition to 14,369 SNPs shared between them. When analyzed the S3 and S6 chromosome copy numbers, the authors found that most chromosomes of both isolates had a haploid copy number of one and were disomic, except for Chr30, which presented complete chromosomal amplifications and seemed to be tetrasomic in both isolates. This chromosome is homologous to chromosome 31 in other *Leishmania* species, in which it is consistently polysomic and enriched for amastigote upregulated genes. These genes seem to be involved in the parasite's survival strategies inside the host cells, suggesting that the increase in the number of copies of this chromosome could favor parasite adaptation to the vertebrate infection, survival, and proliferation (Reis-Cunha et al., 2017).

Valdivia et al. (2017) also found gene copy variations in both isolates by single-copy gene normalization, with expansions in gene-related to proteins crucial for the invasion and survival of the parasite in the intracellular environment. The gene copy number variation is the most important form of gene regulation in *Leishmania* and confers phenotypic plasticity among parasite populations (Iantorno et al., 2017). In the S3 and S6 isolates the more frequent gene expansions were found in the RNA helicase, a putative pyroglutamyl peptidase I (PPI), and 15 hypothetical proteins with unknown function. Among the hypothetical proteins, eight were found among the ten largest mean haploid copy numbers for gene, which highlights the importance of the characterization of *Leishmania* genes with unknown function (Valdivia et al., 2017).

Concerning known proteins, PPI is a soluble intracellular enzyme found in various organisms, but little is known about its trypanosomatids function. These proteins prevent peptides from aminopeptidase degradation by hydrolyzing N-terminal L-pyroglutamyl residues from peptides and proteins containing this modification (Schaeffer et al., 2006). Morty et al. (2006), demonstrated that in *Trypanosoma brucei* PPI is responsible for creating the abnormal degradation of products present in the blood, and therefore can be associated with protection against antimicrobial peptides, suggesting that this enzyme play a role as a virulence factor. On the other side, Schaeffer et al. (2006) showed that *Leishmania major* PPI presents an important role in parasite differentiation, but the authors showed that the enzyme itself is not essential for parasite proliferation. Based on these studies, Valdivia et al. (2017) hypothesize the PPI gene in *L. amazonensis* could also act during the transition to infective metacyclic promastigotes.

Also, in the isolates from Valdivia et al. (2017), *in tandem* expansions were identified for the genes of parasite surface antigen 2 (PSA2), elongation factor 1α (EF-1α), apical membrane antigen 1 (AMA1), heat shock proteins (HSPs) and beta-tubulin. The PSA-2 is a family of glycol-proteins expressed in the cell surface both in amastigote and promastigote forms of *Leishmania*. These proteins are highly expressed in metacyclic promastigotes, playing a vital role in determining parasite infection and survival (Devault and Bäuls, 2008). These proteins participate in the evasion of the parasites from complement-mediated lysis (Lincoln et al., 2004), are involved in the macrophage invasion by interaction with CR-3 receptor (Kedzierski et al., 2004), and play an important role in the drug resistance (Bhandari et al., 2013). *Leishmania* EF-1α is a tyrosine phosphatase-1 (SHP-1) binding protein with an important role in the host-parasite relationship and parasite virulence (López-López et al., 2018). These proteins can be secreted in the exosomes acting in the immunosuppression of host cells, priming them for parasite invasion (Nandan et al., 2002; Silverman and Reiner, 2011). They were absent in the *L. mexicana* that cause milder forms of the disease (Valdivia et al., 2017). Until today the function of *Leishmania* AMA1 is not fully known, but a comparative *in-silico* genome analysis suggested that these genes play a function in the parasite interaction with the host membrane cholesterol through leucine residues and helps in parasite internalization (Kumar et al., 2014).

Table 3
Genotypical differences between strains of *L. amazonensis* isolated from cutaneous and visceral leishmaniasis.

Author, Year	Clinical Condition	International Code	SNPs	Chromosome Number	Gene Copy Number	Genome Sequencing
VALDIVIA et al., 2017 (Valdivia et al., 2017)	Cutaneous	MHOM/BR/ 71973/M2269	ND	ND	ND	GenBank
	Visceral	S3	23,921 (llijpcm) 16,178 (M2269)	Duplicate chromosome 30	53 (RNA helicase, putative pyroglutamyl peptidase I (PPI) and several hypothetical proteins) gtpase, PSA2, EF1alfa, ama1, HSP83, beta tubulina, linj 15.0900	Illumina
	Visceral	S6	17,624 (llijpcm) 15,500 (M2269)	Duplicate chromosome 30	62 (RNA helicase, putative pyroglutamyl peptidase I (PPI) and several hypothetical proteins) gtpase, PSA2, EF1alfa, ama1, HSP83, beta tubulina, linj 15.0900	Illumina
OLIVEIRA et al., 2007 (Oliveira et al., 2007)	Cutaneous	MHOM/BR/87/ BA125	ND	ND	ND	ND
	Cutaneous	MHOM/BR/ 1973/M2269	ND	ND	ND	ND
	Cutaneous	MHOM/BR/85/ BA69	ND	ND	ND	ND
	Cutaneous	MHOM/BR/85/ BA73	ND	ND	ND	ND
	Cutaneous	MHOM/BR/85/ BA75	ND	ND	ND	ND
	Cutaneous	MHOM/BR/87/ BA115	ND	ND	ND	ND
	Cutaneous	MHOM/BR/85/ BA56	ND	ND	ND	ND
	Cutaneous	MHOM/BR/00/ BA771	ND	ND	ND	ND
	Visceral	MHOM/BR/ 1985/BA32	ND	ND	ND	ND
	Visceral	MHOM/BR/ 1987/BA109	ND	ND	ND	ND
	Visceral	MHOM/BR/ 1987/BA112	ND	ND	ND	ND
	Visceral	MHOM/BR/87/ BA137	ND	ND	ND	ND

HSPs, located in chromosome 32, have been widely studied in recent decades, especially about their function as diagnostic tools (Siripattanapipong et al., 2017). These proteins are responsible to maintain protein folding under stress conditions, participate in differentiation during the lifecycle of *Leishmania*, playing an important role in the parasite's survival, virulence, and proliferation (Shonhai et al., 2011). Alpha and beta-tubulin proteins are the basic components of microtubules, which are crucial for the mitotic spindle apparatus, transport, and motility (Luis et al., 2013). Polymorphism of the β-tubulin gene family has been demonstrated as sufficient to its variability causes phenotypic differences even at the subgenera level (Luis et al., 1998). Given the fundamental role for parasites, these proteins have also been described as a relevant target for drugs, but also as important in the development of drug resistance (Luis et al., 1998). Knowing the important functions performed by the expanded genes, Valdivia et al. (2017) infer that such genes expansion would be directly related to broad spectrum of diseases phenotypes associated with *L. (L.) amazonensis* infection.

Zhang et al. (2014) carried out a complete genome sequencing of *L (L.) donovani* isolates from Sri Lanka whose patients had two different clinical forms of the disease, VL and CL, and found that one of the polymorphisms found among the isolates was the number of copies of the A2 gene; lesser in isolates that cause skin disease. However, Valdivia et al. (2017) found that in the two *L. (L.) amazonensis* isolates sequenced, the gene that encodes the A2 protein family is collapsed, due to its large repetitive region.

Among the articles selected in the search, two investigated the phenotypic differences triggered by strains of *L. (L.) amazonensis* isolated from patients with different clinical evolution of leishmaniasis. In both studies, it was found that strains isolated from patients with VL developed a milder disease with slower evolution in both BALB/c and CBA mice when compared to strains isolated from patients with CL (Almeida et al., 1996; Souza et al., 2011).

The inflammatory infiltrate in the site of infection observed in these animals was also different, while animals infected with CL strains showed a progressive lesion with the presence of intense inflammation, necrotic area and massive predominance of highly parasitized macrophages, those infected with VL strains showed moderate inflammation with the presence of poorly infected macrophages, lymphocytes and neutrophils (Almeida et al., 1996; Souza et al., 2011). It was also verified that animals infected with VL strains developed granulomas with plasma and giant cells, which may be associated with the animals' resistance to this strain (Almeida et al., 1996). The role of macrophages in the pathogenesis of leishmaniasis has been described and the polarization of macrophages to the type II (M2) phenotype at the beginning of the infection contributes to the establishment of host susceptibility and the phenotypic switch for the type M1 macrophage pattern in later stages of infection favors the worsening of the lesions. Although the authors did not evaluate the macrophage subtypes present at the site of infection, it is possible to infer that CL strains can modulate the change in the macrophage phenotype earlier to favor infection, which might cause these differences in lesion progression (Tomiotto-Pellissier et al., 2018).

In both studies, it was found that strains of VL presented a delay in the time of metastasis to central organs such as the liver and spleen, which might be associated with the generation of the granulomas at the injection site and containment of parasites (Almeida et al., 1996; Souza et al., 2011). In addition, it has also been reported that CBA mice are relatively resistant to infection by strains of *L. (L.) amazonensis* isolated from patients with visceral leishmaniasis, with a delay in the appearance of the lesion (De Souza et al., 2018). Although the authors used strains isolated from patients who developed VL, this picture was not observed in the experimental models, which may indicate that the visceralization of the disease may require other associated factors besides those evaluated in the studies and also be associated with the resistance of the animal model used (Pereira and Alves, 2008).

Almeida, et al. (1996), also evaluated the humoral response of BALB/c mice against the different strains of *L. (L.) amazonensis* and

found that CL have higher levels of total IgG than VL, however when they evaluated the IgG subclasses, it was observed that the levels of IgG1 increased progressively in both strains, being correlated with lesion progression, however, IgG2 levels decrease in CL and increase in VL throughout the infection. The increase in IgG2 may explain the delay in the appearance and size of the lesion seen in strains isolated from visceral patients, since the ulcerative skin lesion is usually due to the destructive pro-inflammatory activity of immune cells, and IgG2 has a low affinity for Fc receptors and does not develop antibody-dependent cell-mediated cytotoxicity. Failure to recognize infected cells does not trigger a cytotoxic response and prevents the death of these cells, attenuating the local inflammatory response

Furthermore, the appearance of both IgG1 and IgG2 in animals infected with VL strains indicates the establishment of a dualistic pattern of immune response Th1 and Th2, and this is probably related to the resistance of animals to this strain (Almeida et al., 1996; Bretscher, 2014). The excess of Th1 response can cause more intense and ulcerative lesions while the predominance of Th2 promotes the appearance of diffuse nodules lesions. Parasite resistance is associated with a balance between these responses, which should promote the destruction of the parasite, but be able to repair tissue damage caused in the process (Silveira et al., 2009). However, Souza et al. (2011) found no difference in the production of IFN-γ, IL-4, and IL-10 in animals infected with strains isolated from patients with CL and VL. Although these cytokines are classic of the Th1 and Th2 response patterns, the patterns are not established exclusively by them, and other Th1/Th2 cytokines, such as TNF-α, IL-1β, and IL-13, may also play a role in the difference of the response between strains. On the other hand, this may also be an indication that the difference in immune response between strains is due to other factors or response patterns, thus, the evaluation of other potential mediators, such as IL-6, IL-17, IL-22, and TGF-β, could better elucidate these differences (Kumar et al., 2017; Maspi et al., 2016; Raphael et al., 2015; Romagnani, 1999).

Souza et al. (2011) also demonstrated that strains from VL present ecto-ATPases activity three times higher than CL strains, respectively. This type of enzyme is responsible for hydrolyzing extracellular nucleotides and plays a role in parasite survival and proliferation (Silva et al., 2008). It is known that infected cells release ATP to the extracellular environment who acts like a damage-associated molecular pattern, activating the inflammatory response. Greater expression of ecto-enzymes that consume these signals can reduce their bioavailability and attenuate the immune response (Giuliani et al., 2019). Besides, it has been reported that ecto-ATPases activity in *L. (L.) amazonensis* is related to drug resistance (Giarola et al., 2014).

Even so, strains of CL showed greater activity of arginase than strains of VL (Souza et al., 2011). In parasites of the *Leishmania* genus, arginase has the role of converting L-arginine into L-ornithine, an important substrate to produce polyamines that are essential in the replication and survival of the parasite. On the other hand, L-arginine is also a substrate for the generation of nitric oxide in macrophages, which could explain the greater infectivity of the CL strains, since the consumption of L-arginine by the parasite would favor its replication and impair the production of microbicidal molecules in infected macrophages (Acuña et al., 2017; Aoki et al., 2019b).

5. Conclusion

The complexity of leishmaniasis clinical manifestations seems to involve the combination of parasite genetic factors and the host's immune background. *L. amazonensis* can cause differents clinical manifestations in humans and dogs and the difference in the disease driven by different *L. (L.) amazonensis* strains may be due to changes in the production of molecules related to the metabolism of the parasite and also to characteristics inherent to the host itself. SNP and chromosomic alterations in the L. *amazonensis* genome could favor parasite adaptation to the vertebrate infection, survival, and proliferation. In addition, strains isolated from CL induce a higher inflammatory condition than strains from VL. However, the exact mechanism that leads to different disease phenotypes caused by the same species of parasite is still uncertain and future studies must be developed to better elucidate this phenomenon. In this sense, the present work highlights the need for further studies involving both the *Leishmania*-associated molecular patterns, the pattern recognition receptors and other molecules of the immune system, as well as the host-pathogen interactions.

Declaration of Competing Interest

The author declares no conflicts of interest.

References

Acuña, S.M., Aoki, J.I., Laranjeira-Silva, M.F., Zampieri, R.A., Fernandes, J.C.R., Muxel, S.M., Floeter-Winter, L.M., 2017. Arginase expression modulates nitric oxide production in Leishmania (Leishmania) *amazonensis*. PLoS One 12. https://doi.org/10.1371/journal.pone.0187186.

Akhoundi, M., Downing, T., Votýpka, J., Kuhls, K., Lukeš, J., Cannet, A., Ravel, C., Marty, P., Delaunay, P., Kasbari, M., Granouillac, B., Gradoni, L., Sereno, D., 2017. Leishmania infections: Molecular targets and diagnosis. Mol. Aspects Med. https://doi.org/10.1016/j.mam.2016.11.012.

Almeida, R.P., Barral-Netto, M., De Jesus, A.M.R., De Freitas, L.A.R., Carvalho, E.M., Barral, A., 1996. Biological behavior of Leishmania *amazonensis* isolated from humans with cutaneous, mucosal, or visceral leishmaniasis in BALB/c mice. Am. J. Trop. Med. Hyg. 54, 178–184. https://doi.org/10.4269/ajtmh.1996.54.178.

Aoki, J.I., Laranjeira-Silva, M.F., Muxel, S.M., Floeter-Winter, L.M., 2019a. The impact of arginase activity on virulence factors of Leishmania *amazonensis*. Curr. Opin. Microbiol. https://doi.org/10.1016/j.mib.2019.06.003.

Aoki, J.I., Laranjeira-Silva, M.F., Muxel, S.M., Floeter-Winter, L.M., 2019b. The impact of arginase activity on virulence factors of Leishmania *amazonensis*. Curr. Opin. Microbiol. https://doi.org/10.1016/j.mib.2019.06.003.

Arora, K., Rai, A.K., 2019. Dependence of Leishmania parasite on host derived ATP: an overview of extracellular nucleotide metabolism in parasite. J. Parasit. Dis. https://doi.org/10.1007/s12639-018-1061-4.

Barral, A., Pedral-Sampaio, D., Grimaldi, G., Momen, H., McMahon-Pratt, D., Ribeiro De Jesus, A., Almeida, R., Badaro, R., Barral-Netto, M., Carvalho, E.M., Johnson, W.D., 1991. Leishmaniasis in Bahia, Brazi: evidence that Leishmania *amazonensis* produces a wide spectrum of clinical disease. Am. J. Trop. Med. Hyg. 44, 536–546. https://doi.org/10.4269/ajtmh.1991.44.536.

Bastien, P., Blaineau, C., Britto, C., Dedet, J.P., Dubessay, P., Pages, M., Ravel, C., Wincker, P., Blackwell, J.M., Leech, V., Levick, M., Norrish, A., Ivens, A., Lewis, S., Bagherzadeh, A., Smith, D., Myler, P., Stuart, K., Cruz, A., Ruiz, J.C., Schneider, H., Sampaio, I., Almeida, R., Papadopoulou, B., Shapira, M., Belli, S., Fasel, N., 1998. The complete chromosomal organization of the reference strain of the Leishmania genome project, L. major "Friedlin. Parasitol. Today 14, 301–303. https://doi.org/10.1016/S0169-4758(98)01275-7.

Bastien, P., Blaineau, C., Pages, M., 1992. Leishmania: Sex, lies and karyotype. Parasitol. Today 8, 174–177. https://doi.org/10.1016/0169-4758(92)90016-U.

Berrêdo-Pinho, M., Peres-Sampaio, C.E., Chrispim, P.P.M., Belmont-Firpo, R., Lemos, A. P., Martiny, A., Vannier-Santos, M.A., Meyer-Fernandes, J.R., 2001. A Mg-dependent ecto-ATPase in Leishmania *amazonensis* and its possible role in adenosine acquisition and virulence. Arch. Biochem. Biophys. 391, 16–24. https://doi.org/10.1006/abbi.2001.2384.

Bhandari, V., Kumar, D., Verma, S., Srividya, G., Negi, N.S., Singh, R., Salotra, P., 2013. Increased parasite surface antigen-2 expression in clinical isolates of Leishmania donovani augments antimony resistance. Biochem. Biophys. Res. Commun. 440, 646–651. https://doi.org/10.1016/j.bbrc.2013.09.113.

Boité, M.C., Späth, G.F., Bussotti, G., Porrozzi, R., Morgado, F.N., Llewellyn, M., Schwabl, P., Cupolillo, E., 2020. Trans-atlantic spill over: deconstructing the ecological adaptation of leishmania infantum in the americas. Genes (Basel). https://doi.org/10.3390/genes11010004.

Bretscher, P.A., 2014. On the Mechanism Determining the Th1/Th2 Phenotype of an Immune Response, and its Pertinence to Strategies for the Prevention, and Treatment, of Certain Infectious Diseases. Scand. J. Immunol. https://doi.org/10.1111/sji.12175.

Burza, S., Croft, S.L., Boelaert, M., 2018. Leishmaniasis. Lancet. https://doi.org/10.1016/S0140-6736(18)31204-2.

Cardoso, F., de, O., Zaverucha-do-Valle, T., Almeida-Souza, F., Abreu-Silva, A.L., Calabrese, K., da, S., 2020. Modulation of cytokines and extracellular matrix proteins expression by leishmania *amazonensis* in susceptible and resistant mice. Front. Microbiol. 11 https://doi.org/10.3389/fmicb.2020.01986.

Carvalho, L.S.A., Junior, I.J.A., Junqueira, L.R., Silva, L.M., Riani, L.R., de Faria Pinto, P., Da Silva Filho, A.A., 2019. ATP-diphosphohydrolases in parasites: localization, functions and recent developments in drug discovery. Curr. Protein Pept. Sci. 20, 873–884. https://doi.org/10.2174/1389203720666190704152827.

CDC, 2020. Pulsed-field Gel Electrophoresis (PFGE) | PulseNet Methods| PulseNet | CDC [WWW Document].

Charest, H., Matlashewski, G., 1994. Developmental gene expression in Leishmania donovani: differential cloning and analysis of an amastigote-stage-specific gene. Mol. Cell. Biol. 14, 2975–2984. https://doi.org/10.1128/mcb.14.5.2975.

Coelho, L.I.C., Paes, M., Guerra, J.A., Barbosa, M.D.G., Coelho, C., Lima, B., Brito, M.E., Brandão Filho, S.P., 2011. Characterization of Leishmania spp. causing cutaneous leishmaniasis in Manaus, Amazonas, Brazil. Parasitol. Res. 108, 671–677. https://doi.org/10.1007/s00436-010-2139-9.

Cupolillo, E., Momen, H., Grimaldi, G., 1998. Genetic diversity in natural populations of new world Leishmania. Mem. Inst. Oswaldo Cruz 93, 663–668. https://doi.org/10.1590/S0074-02761998000500018.

De Souza, C.da S.F., Calabrese, K.da S., Abreu-Silva, A.L., Carvalho, L.O.P., Cardoso, F.de O., Dorval, M.E.M.C., Oshiro, E.T., Quaresma, P.F., Gontijo, C.M.F., Pacheco, R.da S., Rossi, M.I.D., Da Costa, S.C.G., Do Valle, T.Z., 2018. Leishmania *amazonensis* isolated from human visceral leishmaniasis: histopathological analysis and parasitological burden in different inbred mice. Histol. Histopathol. 33, 705–716. https://doi.org/10.14670/HH-11-965.

Devault, A., Bāuls, A.L., 2008. The promastigote surface antigen gene family of the Leishmania parasite: differential evolution by positive selection and recombination. BMC Evol. Biol. 8 https://doi.org/10.1186/1471-2148-8-292.

Dias, E.S., Regina-Silva, S., França-Silva, J.C., Paz, G.F., Michalsky, É.M., Araújo, S.C., Valadão, J.L., de Oliveira Lara-Silva, F., de Oliveira, F.S., Pacheco, R.S., Fortes-Dias, C.L., 2011. Eco-epidemiology of visceral leishmaniasis in the urban area of Paracatu, state of Minas Gerais, Brazil. Vet. Parasitol. 176, 101–111. https://doi.org/10.1016/j.vetpar.2010.11.014.

Downing, T., Imamura, H., Decuypere, S., Clark, T.G., Coombs, G.H., Cotton, J.A., Hilley, J.D., De Doncker, S., Maes, I., Mottram, J.C., Quail, M.A., Rijal, S., Sanders, M., Schönian, G., Stark, O., Sundar, S., Vanaerschot, M., Hertz-Fowler, C., Dujardin, J.C., Berriman, M., 2011. Whole genome sequencing of multiple Leishmania donovani clinical isolates provides insights into population structure and mechanisms of drug resistance. Genome Res. 21, 2143–2156. https://doi.org/10.1101/gr.123430.111.

Fernandes, A.P., Canavaci, A.M.C., McCall, L.I., Matlashewski, G., 2014. A2 and other visceralizing proteins of Leishmania: role in pathogenesis and application for vaccine development. Subcell. Biochem. 74, 77–101. https://doi.org/10.1007/978-94-007-7305-9_3.

Franco-Duarte, R., Černáková, L., Kadam, S., Kaushik, K.S., Salehi, B., Bevilacqua, A., Corbo, M.R., Antolak, H., Dybka-Stępień, K., Leszczewicz, M., Tintino, S.R., de Souza, V.C.A., Sharifi-Rad, J., Coutinho, H.D.M., Martins, N., Rodrigues, C.F., 2019. Advances in chemical and biological methods to identify microorganisms—from past to present. Microorganisms. https://doi.org/10.3390/microorganisms7050130.

Giarola, N.L.L., Silveira, T.S., Inacio, J.D.F., Vieira, L.P., Almeida-Amaral, E.E., Meyer-Fernandes, J.R., 2014. Leishmania *amazonensis*: increase in ecto-ATPase activity and parasite burden of vinblastine-resistant protozoa. Exp. Parasitol. 146, 25–33. https://doi.org/10.1016/j.exppara.2014.08.013.

Giuliani, A.L., Sarti, A.C., Di Virgilio, F., 2019. Extracellular nucleotides and nucleosides as signalling molecules. Immunol. Lett. https://doi.org/10.1016/j.imlet.2018.11.006.

Gonçalves, L.P., Santos, T.V.Dos, Campos, M.B., Lima, L.V.do R., Ishikawa, E.A.Y., Silveira, F.T., Ramos, P.K.S., 2020. Further insights into the eco-epidemiology of American cutaneous leishmaniasis in the Belem metropolitan region, Pará State, Brazil. Rev. Soc. Bras. Med. Trop. 53, e20200255 https://doi.org/10.1590/0037-8682-0255-2020.

Hitakarun, A., Tan-Ariya, P., Siripattanapipong, S., Mungthin, M., Piyaraj, P., Naaglor, T., Siriyasatien, P., Tiwananthagorn, S., Leelayoova, S., 2014. Comparison of PCR methods for detection of Leishmania siamensis infection. Parasites Vect. 7 https://doi.org/10.1186/s13071-014-0458-x.

Hoffmann, A.R., Navarro, I.T., Elemar Camargo Junior, V., Teles Caldart, E., Mitsuka Breganó, R., Mendes Pereira, P., 2012. Leishmania *amazonensis* in dog with clinical diagnosis of visceral leishmaniasis in Paraná State, Brazil – a case reportLeishmania *amazonensis* em cão com quadro clínico de leishmaniose visceral no Estado do Paraná, Brasil – relato de caso. Semin. - Ciencias Agrar. 33, 3265–3270. https://doi.org/10.5433/1679-0359.2012v33n6Supl2p3265.

Iantorno, S.A., Durrant, C., Khan, A., Sanders, M.J., Beverley, S.M., Warren, W.C., Berriman, M., Sacks, D.L., Cotton, J.A., Grigg, M.E., 2017. Gene expression in Leishmania is regulated predominantly by gene dosage. MBio 8. https://doi.org/10.1128/mBio.01393-17.

Kedzierski, L., Montgomery, J., Bullen, D., Curtis, J., Gardiner, E., Jimenez-Ruiz, A., Handman, E., 2004. A Leucine-rich repeat motif of leishmania parasite surface antigen 2 binds to macrophages through the complement receptor 3. J. Immunol. 172, 4902–4906. https://doi.org/10.4049/jimmunol.172.8.4902.

Kumar, R., Bhatia, M., Pai, K., 2017. Role of cytokines in the pathogenesis of visceral leishmaniasis. Clin. Lab. https://doi.org/10.7754/Clin.Lab.2017.170404.

Kumar, S., Gokulasuriyan, R.K., Ghosh, M., 2014. Comparative in-silico genome analysis of Leishmania (Leishmania) donovani: a step towards its species specificity. Meta Gene 2, 782–798. https://doi.org/10.1016/j.mgene.2014.10.003.

Lincoln, L.M., Ozaki, M., Donelson, J.E., Beetham, J.K., 2004. Genetic complementation of Leishmania deficient in PSA (GP46) restores their resistance to lysis by complement. Mol. Biochem. Parasitol. 137, 185–189. https://doi.org/10.1016/j.molbiopara.2004.05.004.

López-López, K., Apodaca-Medina, A.I., León-Sicairos, C.R., Murúa-López, C.C., Ortiz-Navarrete, V.F., Chávez-Ontiveros, J., Beltrán-López, E., López-Moreno, H.S., 2018. Cloning and recombinant expression of elongation factor-1α of Leishmania mexicana. Vector-Borne Zoonotic Dis. 18, 709–712. https://doi.org/10.1089/vbz.2018.2347.

Luis, L., Ramírez, A., Aguilar, C.M., Eresh, S., Barker, D.C., Mendoza-León, A., 1998. The genomic fingerprinting of the coding region of the β-tubulin gene in Leishmania identification. Acta Trop. 69, 193–204. https://doi.org/10.1016/S0001-706X(97)00128-9.

Luis, L., Serrano, M.L., Hidalgo, M., Mendoza-León, A., 2013. Comparative analyses of the ί µί»½-tubulin gene and molecular modeling reveal molecular insight into the colchicine resistance in kinetoplastids organisms. Biomed. Res. Int. 2013 https://doi.org/10.1155/2013/843748.

Mandal, G., Mandal, S., Sharma, M., Charret, K.S., Papadopoulou, B., Bhattacharjee, H., Mukhopadhyay, R., 2015. Species-specific antimonial sensitivity in leishmania is driven by post-transcriptional regulation of AQP1. PLoS Negl. Trop. Dis. 9 https://doi.org/10.1371/journal.pntd.0003500.

Mannaert, A., Downing, T., Imamura, H., Dujardin, J.C., 2012. Adaptive mechanisms in pathogens: universal aneuploidy in Leishmania. Trends Parasitol. https://doi.org/10.1016/j.pt.2012.06.003.

Maspi, N., Abdoli, A., Ghaffarifar, F., 2016. Pro- and anti-inflammatory cytokines in cutaneous leishmaniasis: a review. Pathog. Glob. Health. https://doi.org/10.1080/20477724.2016.1232042.

Mirzapour, A., Spotin, A., Behniafar, H., Azizi, H., Maleki, B., Shakeraminia, H., Javad, S., Tabaei, S., 2019. Intra-species diversity of Leishmania major and L. tropica from clinical isolates of cutaneous Leishmaniasis in Southwest Iran inferred by ITS1-rDNA - PubMed. Iran J. Public Heal. 48, 892–901.

Morty, R.E., Bulau, P., Pellé, R., Wilki, S., Abe, K., 2006. Pyroglutamyl peptidase type I from Trypanosoma brucei: a new virulence factor from African trypanosomes that de-blocks regulatory peptides in the plasma of infected hosts. Biochem. J. 394, 635–645. https://doi.org/10.1042/BJ20051593.

Nandan, D., Yi, T., Lopez, M., Lai, C., Reiner, N.E., 2002. Leishmania EF-1α activates the Src homology 2 domain containing tyrosine phosphatase SHP-1 leading to macrophage deactivation. J. Biol. Chem. 277, 50190–50197. https://doi.org/10.1074/jbc.M209210200.

Oliveira, J.P., Fernandes, F., Cruz, A.K., Trombela, V., Monteiro, E., Camargo, A.A., Barral, A., de Oliveira, C.I., 2007. Genetic diversity of Leishmania *amazonensis* strains isolated in northeastern Brazil as revealed by DNA sequencing, PCR-based analyses and molecular karyotyping. Kinetoplastid Biol. Dis. 6 https://doi.org/10.1186/1475-9292-6-5.

Paletta-Silva, R., Vieira, D.P., Vieira-Bernardo, R., Majerowicz, D., Gondim, K.C., Vannier-Santos, M.A., Lopes, A.H., Meyer-Fernandes, J.R., 2011. Leishmania *amazonensis*: Characterization of an ecto-3'-nucleotidase activity and its possible role in virulence. Exp. Parasitol. 129, 277–283. https://doi.org/10.1016/j.exppara.2011.07.014.

Peacock, C.S., Seeger, K., Harris, D., Murphy, L., Ruiz, J.C., Quail, M.A., Peters, N., Adlem, E., Tivey, A., Aslett, M., Kerhornou, A., Ivens, A., Fraser, A., Rajandream, M. A., Carver, T., Norbertczak, H., Chillingworth, T., Hance, Z., Jagels, K., Moule, S., Ormond, D., Rutter, S., Squares, R., Whitehead, S., Rabbinowitsch, E., Arrowsmith, C., White, B., Thurston, S., Bringaud, F., Baldauf, S.L., Faulconbridge, A., Jeffares, D., Depledge, D.P., Oyola, S.O., Hilley, J.D., Brito, L.O., Tosi, L.R.O., Barrell, B., Cruz, A.K., Mottram, J.C., Smith, D.F., Berriman, M., 2007. Comparative genomic analysis of three Leishmania species that cause diverse human disease. Nat. Genet. 39, 839–847. https://doi.org/10.1038/ng2053.

Penner, G.A., Bush, A., Wise, R., Kim, W., Domier, L., Kasha, K., Laroche, A., Scoles, G., Molnar, S.J., Fedak, G., 1993. Reproducibility of random amplified polymorphic DNA (RAPD) analysis among laboratories. Genome Res. 2, 341–345. https://doi.org/10.1101/gr.2.4.341.

Pereira, B.A.S., Alves, C.R., 2008. Immunological characteristics of experimental murine infection with Leishmania (Leishmania) *amazonensis*. Vet. Parasitol. https://doi.org/10.1016/j.vetpar.2008.09.015.

Pessenda, G., Silva, J.S.da, 2020. Arginase and its mechanisms in Leishmania persistence. Parasite Immunol. 42, e12722. https://doi.org/10.1111/pim.12722.

Pinheiro, C.M., Martins-Duarte, E.S., Ferraro, R.B., Fonseca de Souza, A.L., Gomes, M.T., Lopes, A.H.C.S., Vannier-Santos, M.A., Santos, A.L.S., Meyer-Fernandes, J.R., 2006. Leishmania *amazonensis*: Biological and biochemical characterization of ecto-nucleoside triphosphate diphosphohydrolase activities. Exp. Parasitol. 114, 16–25. https://doi.org/10.1016/j.exppara.2006.02.007.

Raphael, I., Nalawade, S., Eagar, T.N., Forsthuber, T.G., 2015. T cell subsets and their signature cytokines in autoimmune and inflammatory diseases. Cytokine. https://doi.org/10.1016/j.cyto.2014.09.011.

Raymond, F., Boisvert, S., Roy, G., Ritt, J.F., Légaré, D., Isnard, A., Stanke, M., Olivier, M., Tremblay, M.J., Papadopoulou, B., Ouellette, M., Corbeil, J., 2012. Genome sequencing of the lizard parasite Leishmania tarentolae reveals loss of genes associated to the intracellular stage of human pathogenic species. Nucleic Acids Res. 40, 1131–1147. https://doi.org/10.1093/nar/gkr834.

Reis-Cunha, J.L., Valdivia, H.O., Bartholomeu, D.C., 2017. Gene and chromosomal copy number variations as an adaptive mechanism towards a parasitic lifestyle in trypanosomatids. Curr. Genom. 19 https://doi.org/10.2174/1389202918666170911161311.

Romagnani, S., 1999. Th1/Th2 cells. Inflamm. Bowel Dis. 5, 285–294. https://doi.org/10.1097/00054725-199911000-00009.

Rosenzweig, D., Smith, D., Myler, P.J., Olafson, R.W., Zilberstein, D., 2008. Post-translational modification of cellular proteins during Leishmania donovani differentiation. Proteomics 8, 1843–1850. https://doi.org/10.1002/pmic.200701043.

Santos, A.L.S., 2014. Proteins and Proteomics of Leishmania and Trypanosoma. Subcell. Biochem. https://doi.org/10.1007/978-94-007-7305-9.

Schaeffer, M., de Miranda, A., Mottram, J.C., Coombs, G.H., 2006. Differentiation of Leishmania major is impaired by over-expression of pyroglutamyl peptidase I. Mol. Biochem. Parasitol. 150, 318–329. https://doi.org/10.1016/j.molbiopara.2006.09.004.

Schönian, G., Akuffo, H., Lewin, S., Maasho, K., Nylén, S., Pratlong, F., Eisenberger, C.L., Schnur, L.F., Presber, W., 2000. Genetic variability within the species Leishmania aethiopica does not correlate with clinical variations of cutaneous leishmaniasis.

Mol. Biochem. Parasitol. 106, 239–248. https://doi.org/10.1016/S0166-6851(99)00216-9.
Schönian, G., Nasereddin, A., Dinse, N., Schweynoch, C., Schallig, H.D.F.H., Presber, W., Jaffe, C.L., 2003. PCR diagnosis and characterization of Leishmania in local and imported clinical samples. Diagn. Microbiol. Infect. Dis. 47, 349–358. https://doi.org/10.1016/S0732-8893(03)00093-2.
Shonhai, A.G., Maier, A.M., Przyborski, J.L., Blatch, G., 2011. Intracellular protozoan parasites of humans: the role of molecular chaperones in development and pathogenesis. Protein Pept. Lett. 18, 143–157. https://doi.org/10.2174/092986611794475002.
Silva, E.de A.M.da, de Oliveira, J.C., Figueiredo, A.B., de Souza Lima Júnior, D., Carneiro, C.M., Rangel Fietto, J.L., Crocco Afonso, L.C., 2008. Extracellular nucleotide metabolism in Leishmania: influence of adenosine in the establishment of infection. Microbes. Infect. 10, 850–857. https://doi.org/10.1016/j.micinf.2008.04.016.
Silveira, F.T., Lainson, R., De Castro Gomes, C.M., Laurenti, M.D., Corbett, C.E.P., 2009. Immunopathogenic competences of Leishmania (V.) braziliensis and L. (L.) *amazonensis* in American cutaneous leishmaniasis. Parasite Immunol. https://doi.org/10.1111/j.1365-3024.2009.01116.x.
Silverman, J.M.axwel., Reiner, N.E., 2011. Leishmania exosomes deliver preemptive strikes to create an environment permissive for early infection. Front. Cell. Infect. Microbiol. https://doi.org/10.3389/fcimb.2011.00026.
Siripattanapipong, S., Kato, H., Tan-ariya, P., Mungthin, M., Leelayoova, S., 2017. Comparison of recombinant proteins of Kinesin 39, Heat Shock Protein 70, Heat Shock Protein 83, and Glycoprotein 63 for antibody detection of Leishmania martiniquensis infection. J. Eukaryot. Microbiol. 64, 820–828. https://doi.org/10.1111/jeu.12415.
Smith, D.F., Peacock, C.S., Cruz, A.K., 2007. Comparative genomics: from genotype to disease phenotype in the Leishmaniases. Int. J. Parasitol. https://doi.org/10.1016/j.ijpara.2007.05.015.
Soong, L., Henard, C.A., Melby, P.C., 2012. Immunopathogenesis of non-healing American cutaneous Leishmaniasis and progressive visceral Leishmaniasis. Semin. Immunopathol. https://doi.org/10.1007/s00281-012-0350-8.
Souza, V.L., Veras, P.S.T., Welby-Borges, M., Silva, T.M.C., Leite, B.R., Ferraro, R.B., Meyer-Fernandes, J.R., Barral, A., Costa, J.M.L., de Freitas, L.A.R., 2011. Immune and inflammatory responses to leishmania *amazonensis* isolated from different clinical forms of human leishmaniasis in CBA mice. Mem. Inst. Oswaldo Cruz 106, 23–31. https://doi.org/10.1590/s0074-02762011000100004.
Sterkers, Y., Lachaud, L., Bourgeois, N., Crobu, L., Bastien, P., Pagès, M., 2012. Novel insights into genome plasticity in Eukaryotes: mosaic aneuploidy in *Leishmania*. Mol. Microbiol. 86, 15–23. https://doi.org/10.1111/j.1365-2958.2012.08185.x.
Sundar, S., Singh, B., 2018. Understanding Leishmania parasites through proteomics and implications for the clinic. Expert Rev. Proteomics. https://doi.org/10.1080/14789450.2018.1468754.
Teixeira, D.G., Monteiro, G.R.G., Martins, D.R.A., Fernandes, M.Z., Macedo-Silva, V., Ansaldi, M., Nascimento, P.R.P., Kurtz, M.A., Streit, J.A., Ximenes, M.F.F.M., Pearson, R.D., Miles, A., Blackwell, J.M., Wilson, M.E., Kitchen, A., Donelson, J.E., Lima, J.P.M.S., Jeronimo, S.M.B., 2017. Comparative analyses of whole genome sequences of Leishmania infantum isolates from humans and dogs in northeastern Brazil. Int. J. Parasitol. 47, 655–665. https://doi.org/10.1016/j.ijpara.2017.04.004.
Tolezano, J.E., Uliana, S.R.B., Taniguchi, H.H., Araújo, M.F.L., Barbosa, J.A.R., Barbosa, J.E.R., Floeter-Winter, L.M., Shaw, J.J., 2007. The first records of Leishmania (Leishmania) *amazonensis* in dogs (Canis familiaris) diagnosed clinically as having canine visceral leishmaniasis from Araçatuba County, São Paulo State, Brazil. Vet. Parasitol. 149, 280–284. https://doi.org/10.1016/j.vetpar.2007.07.008.
Tomiotto-Pellissier, F., Bortoleti, B.T., da, S., Assolini, J.P., Gonçalves, M.D., Carloto, A.C.M., Miranda-Sapla, M.M., Conchon-Costa, I., Bordignon, J., Pavanelli, W.R., 2018. Macrophage polarization in Leishmaniasis: broadening horizons. Front. Immunol. https://doi.org/10.3389/fimmu.2018.02529.
Valdivia, H.O., Almeida, L.V., Roatt, B.M., Reis-Cunha, J.L., Pereira, A.A.S., Gontijo, C., Fujiwara, R.T., Reis, A.B., Sanders, M.J., Cotton, J.A., Bartholomeu, D.C., 2017. Comparative genomics of canine-isolated Leishmania (Leishmania) *amazonensis* from an endemic focus of visceral leishmaniasis in Governador Valadares, southeastern Brazil. Sci. Rep. 7 https://doi.org/10.1038/srep40804.
Wanderley, J.L.M., Deolindo, P., Carlsen, E., Portugal, A.B., DaMatta, R.A., Barcinski, M.A., Soong, L., 2019. CD4+ T cell-dependent macrophage activation modulates sustained ps exposure on intracellular amastigotes of leishmania *amazonensis*. Front. Cell. Infect. Microbiol. 9 https://doi.org/10.3389/fcimb.2019.00105.
WHO, 2021. Leishmaniasis [WWW Document]. URL https://www.who.int/news-room/fact-sheets/detail/leishmaniasis (accessed 6.13.21).
Zhang, W.-W., Matlashewski, G., 2010. Screening Leishmania donovani-specific genes required for visceral infection. Mol. Microbiol. 77, 505–517. https://doi.org/10.1111/j.1365-2958.2010.07230.x.
Zhang, W.-W., Peacock, C.S., Matlashewski, G., 2008. A Genomic-based approach combining in vivo selection in mice to identify a novel virulence gene in Leishmania. PLoS Negl. Trop. Dis. 2, e248. https://doi.org/10.1371/journal.pntd.0000248.
Zhang, W.W., Ramasamy, G., McCall, L.I., Haydock, A., Ranasinghe, S., Abeygunasekara, P., Sirimanna, G., Wickremasinghe, R., Myler, P., Matlashewski, G., 2014. Genetic analysis of Leishmania donovani Tropism using a naturally attenuated cutaneous strain. PLoS Pathog. 10 https://doi.org/10.1371/journal.ppat.1004244.