PLOS ONE

RESEARCH ARTICLE

Potential antigenic targets used in immunological tests for diagnosis of tegumentary leishmaniasis: A systematic review

Mariana Lourenço Freire*, Felipe Dutra Rêgo, Gláucia Cota, Marcelo Antônio Pascoal-Xavier, Edward Oliveira

Instituto René Rachou, Fundação Oswaldo Cruz, Belo Horizonte, Minas Gerais, Brazil

* marianalfreire@hotmail.com

OPEN ACCESS

Citation: Freire ML, Rêgo FD, Cota G, Pascoal-Xavier MA, Oliveira E (2021) Potential antigenic targets used in immunological tests for diagnosis of tegumentary leishmaniasis: A systematic review. PLoS ONE 16(5): e0251956. https://doi.org/10.1371/journal.pone.0251956

Editor: Albert Schriefer, Universidade Federal da Bahia, BRAZIL

Received: January 6, 2021

Accepted: May 6, 2021

Published: May 27, 2021

Data Availability Statement: All relevant data are within the manuscript and its Supporting information files.

Funding: Conselho Nacional de Desenvolvimento Científico e Tecnológico, grant number 301159/2016-5 to Edward Oliveira Conselho. Nacional de Desenvolvimento Científico e Tecnológico, grant number 301384/2019-3 to Gláucia Cota. Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, grant number Finance Code 001 to Mariana Lourenço Freire. Conselho Nacional de

Abstract

Immunological tests may represent valuable tools for the diagnosis of human tegumentary leishmaniasis (TL) due to their simple execution, less invasive nature and potential use as a point-of-care test. Indeed, several antigenic targets have been used with the aim of improving the restricted scenario for TL-diagnosis. We performed a worldwide systematic review to identify antigenic targets that have been evaluated for the main clinical forms of TL, such as cutaneous (CL) and mucosal (ML) leishmaniasis. Included were original studies evaluating the sensitivity and specificity of immunological tests for human-TL, CL and/or ML diagnosis using purified or recombinant proteins, synthetic peptides or polyclonal or monoclonal antibodies to detect *Leishmania*-specific antibodies or antigens. The review methodology followed PRISMA guidelines and all selected studies were evaluated in accordance with QUADAS-2. Thirty-eight original studies from four databases fulfilled the selection criteria. A total of 79 antigens were evaluated for the detection of antibodies as a diagnostic for TL, CL and/or ML by ELISA. Furthermore, three antibodies were evaluated for the detection of antigen by immunochromatographic test (ICT) and immunohistochemistry (IHC) for CL-diagnosis. Several antigenic targets showed 100% of sensitivity and specificity, suggesting potential use for TL-diagnosis in its different clinical manifestations. However, a high number of proof-of-concept studies reinforce the need for further analysis aimed at verifying true diagnostic accuracy in clinical practice.

Introduction

Tegumentary Leishmaniasis (TL) is a neglected tropical disease caused by different species of the genus *Leishmania* (Kinetoplastea: Trypanosomatidae), transmitted to vertebrate hosts by sand flies (Diptera: Psychodidae) [1]. TL is considered an emergent and re-emergent disease, since a worrisome increase in its incidence has been reported [1]. On a the global scale, the number of new autochthonous TL cases reported annually to the World Health Organization

Desenvolvimento Científico e Tecnológico, grant number 155839/2018-7 to Felipe Dutra Rêgo.

Competing interests: The authors have declared that no competing interests exist.

(WHO) increased from 71,486 to 251,553 during 1998 to 2018 [2]. Several factors are involved with the spread of TL, such as human migration from rural to urban areas, conflicts and wars, disturbances in microenvironments due to climate change and human intervention and deterioration of socioeconomic conditions in endemic countries [3].

TL comprises a broad spectrum of clinical manifestations ranging from single or multiple ulcerative skin lesions (cutaneous leishmaniasis—CL), to diffuse (diffuse leishmaniasis-DL) and mucosal (mucosal leishmaniasis—ML) lesions, with the last two being typical in the Americas. TL is associated with physical deformities and psychological alterations, affecting the health and wellness of the patient [4, 5].

The range of clinical manifestations can hinder rapid and accurate diagnoses, a key step to initiate treatment promptly and control the disease. Although several advances, TL-diagnosis remains based on the triad of epidemiological background, clinical signs and laboratory diagnosis, including direct and histopathological examination of skin biopsy and molecular detection of *Leishmania* DNA. Despite high specificity, low sensitivities have been described for direct and histopathological examination, especially in New World countries, where chronic cases and ML are frequent [6–9]. Molecular techniques are complex, expensive, still without a standardized protocol for routine use and are restricted to reference and research centers. Therefore, these limitations make the TL-diagnosis scenario restricted, particularly in resource limited settings [10–12].

In this sense, immunological tests may present remarkable advantages for TL-diagnosis, due to the use of less invasive sampling compared to skin biopsy and their potential to be automated, quantitative and used as point-of-care tests. The anti-*Leishmania* delayed-type hypersensitivity reaction, known as the Montenegro skin test (MST), has been the most used immunological test for CL-diagnosis in Brazil, even though it presents significant limitations such as positive results associated with previous leishmaniasis or asymptomatic infections [13, 14]. Nonetheless, the production of the MST antigen was discontinued in Brazil, hampering even more CL-diagnosis in the country [15]. Other immunological tests, mainly Enzyme-Linked Immunosorbent Assay (ELISA), have presenting promising results in the Americas and beyond [7].

Several studies using soluble *Leishmania* antigen (SLA) in ELISA for TL-diagnosis, have presented variable sensitivity especially due to antigen preparation and antigenic differences among *Leishmania* isolates and species. Moreover, reduced specificity due to the cross-reactivity with other infectious diseases has been frequently reported [16–18]. Since CL-patients commonly produce low levels of anti-*Leishmania* antibodies, there is growing interest in high sensitivity antigens for immunological tests. Different methodologies have been employed, such as bioinformatics tools [19–23], cDNA expression library [24], phage display [25, 26], immunoproteomic approach [18, 27–32] and isolation and purification of glycoconjugates [33, 34] to identify potential antigens. Furthermore, immunological tools have already been used to detect *Leishmania* antigens using monoclonal and polyclonal antibodies by immunochromatographic test (ICT) or immunohistochemistry (IHC), such as the CL Detect Rapid Test (InBios International Inc., Seattle, WA, USA), which detects peroxidoxin from *Leishmania* and has been used especially in Old World countries, with limited sensitivity [35, 36].

In this sense, we consider immunnodiagosis as potential tools to increase the access and improve TL-diagnosis. Although systematic reviews have been conducted on some aspects of this form of diagnosis, it is essential to identify potential antigenic targets that have been evaluated as TL-immunodiagnostic, point out knowledge gaps that still remain and encourage other studies to allow its application in clinical practice [37, 38]. In this way, we performed a worldwide systematic review to identify potential antigenic targets, with reported sensitivity and specificity, used as TL-immunodiagnostic.

Material and methods

Protocol and registration

The review protocol was registered in the International Prospective Record of Systematic Reviews (PROSPERO: CRD42020213311) and was developed based on the Cochrane Handbook for Systematic Reviews of Diagnostic Test Accuracy [39]. This review followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) (S1 Table) [40].

Information sources and study selection

Structured searches were conducted in the following databases: MEDLINE, Virtual Health Library, Embase and Cochrane. A comprehensive list of key terms including tegumentary leishmaniasis and its different clinical forms AND immunological diagnosis or targets (antigens and antibodies) AND techniques or outcomes (sensitivity and specificity), was constructed in MEDLINE (S1 Fig). Similar searches were adapted to each database. Complementary searches were performed by analysis of reference lists of selected articles. Searches were performed on 23rd March 2020, without restriction of publication date.

Inclusion and exclusion criteria

Original research articles reporting on the performance (sensitivity and specificity) of immunological tests based on the detection of antibodies or antigens using purified or recombinant proteins, synthetic peptides or polyclonal or monoclonal antibodies for diagnosis of human-TL, CL or ML were included. Exclusion criteria were: evaluation of serological tests based on SLA; only non-human samples were tested (e.g. canine samples); both sensitivity and specificity of the immunological tests were not presented or were impossible to be calculated; less than five samples were tested; the absence of information about the reference test and a non-specific *Leishmania* antigen was used.

Selection process

For each database, all publications were retrieved and duplicate citations were excluded by EndNote software [41]. Based on the inclusion and exclusion criteria, two independent reviewers analyzed each publication by title and abstract using Rayyan software [42]. Articles with no reason for rejection were included for full text reading. All discrepancies were solved by consensus after discussion. Selected studies were read in full to confirm their eligibility, to extract data or to exclude if exclusion criteria were identified during this step.

Data extraction

Data were independently extracted by two researchers (MLF and FDR) directly from full-length articles and were checked by a third researcher (EO). In case of disagreements, the final decision was reached by consensus. In this study, data were extracted and a 2x2 contingency table set up for immunological tests, containing the true positives, false positives, true negatives and false negatives. Furthermore, the following items were extracted: origin of the participants; the immunological test used; antigen or antibody types; *Leishmania* species and reference standard test used for disease confirmation. The phase of development of each study was classified according to Leeflang & Allerberger (2019) [43].

Study quality assessment

The quality of the studies was assessed using the second version of Quality Assessment of Studies of Diagnostic Accuracy Approach (QUADAS-2) [44]. This tool allows a more transparent rating of risk of bias for studies included in systematic reviews on diagnostic accuracy.

Data synthesis

The performance of antigenic targets was presented in four groups according immunological tests and clinical form: 1) ELISA for TL; 2) ELISA for CL; 3) Other immunological tests for CL and 4) ELISA for ML. The performance outcomes for each antigen or antibody were sensitivity (probability of a positive test among cases or disease confirmed individuals) and specificity (probability of a negative test among controls or individuals without disease). Forest plots showing sensitivity and specificity values of all antigens, including 95% confidence intervals (CI) and Summary Receiver Operating Characteristic (SROC) curves were created using RevMan 5.3.

Several studies considered a set of results for the same antigen (e.g. different cut-off points were available or different non-case groups were used in the analysis, such as healthy patients and those with other diseases). If possible, these results were grouped and only one sensitivity rate and one specificity rate including all evaluated patients. When impossible, we chose to present data that reflect the best field conditions (e.g. non-case group of patients with other diseases) or the better performance (e.g. cut-off point with best performance).

Results

Literature search

A total of 1642 articles from four databases were initially identified. Of this total, 261 were excluded due to duplicity (the same study was found in different databases). The title and abstract of each of the 1381 articles were checked and 139 were selected for full text reading. Finally, 98 articles presented exclusion criteria and so 38 were included (Fig 1).

Descriptive analysis of included studies

The characteristics of all included studies are presented in Table 1. In several studies, test performance was analyzed according to the clinical form (CL and ML) or globally (TL). In 19 studies, the antigenic targets were evaluated for TL-diagnosis, in 21 for CL and in 9 for ML. Sample size ranged from 26 to 500 patients. A total of three different immunological tests using purified or recombinant proteins, synthetic peptides or polyclonal or monoclonal antibodies were reported: ELISA, ICT and IHC. Different reference standard tests were used to confirm leishmaniasis cases. Thirty-one studies (81.6%) considered at least one parasitological method as a reference standard test, such as microscopy examination or *in vitro* culture for isolation of the parasite. On the other hand, seven studies (18.4%) considered some immunological or molecular tests as a reference standard. A total of 89.5% (34 out of 38) of the studies was classified as phase I (proof-of-concept), and the remaining 10.5% (4 out of 38) was classified as phase III.

ELISA for TL diagnosis

Nineteen studies used ELISA to evaluate the performance of a total of 56 antigens for TL-diagnosis, without specification of the clinical form (CL or ML). These studies evaluated 38 recombinant proteins, 14 synthetic peptides and 4 purified proteins. Forty-seven antigens were evaluated in studies that considered at least one parasitological method, such as microscopy

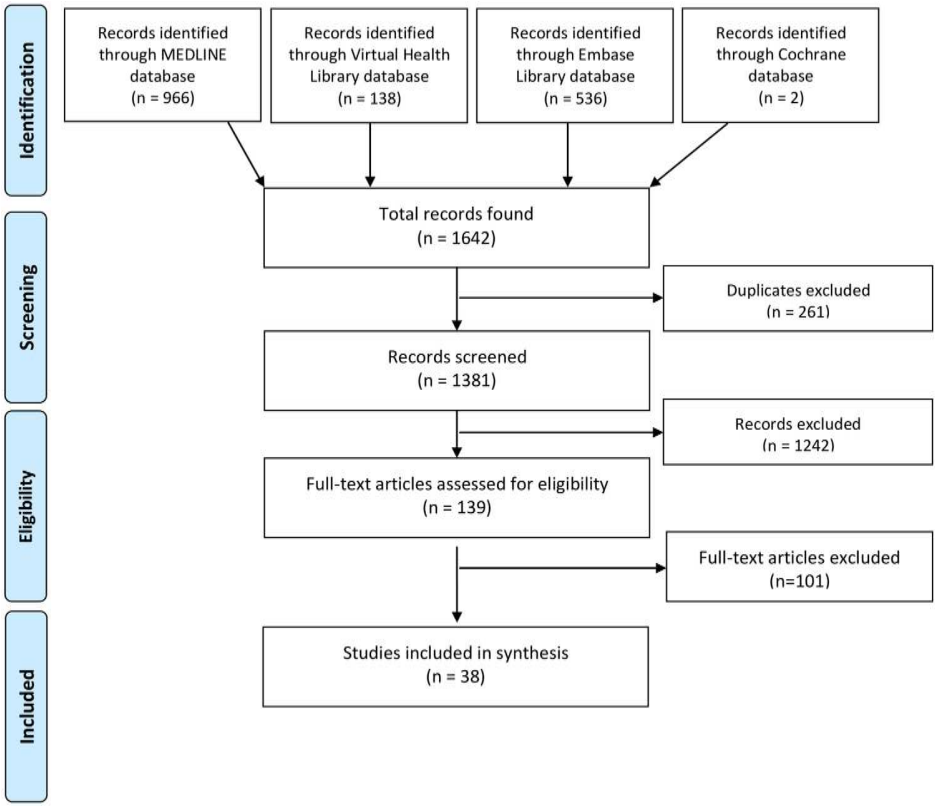

Fig 1. Flow diagram illustrating the study selection process according to PRISMA.

https://doi.org/10.1371/journal.pone.0251956.g001

examination or *in vitro* culture isolation of the parasite, as a reference standard test. The number of TL-patients ranged from 20 to 219 and the number of non-TL patients ranged from 8 to 281. The highest performance (100% of sensitivity and specificity) was reported for four recombinant proteins (cytochrome c oxidase; hypothetical protein XP_003886492.1; putative IgE histamine releasing factor; tryparedoxin peroxidase) and four synthetic peptides (A10, B7, C12 and H7) selected by the phage display technique [18, 25, 27, 30]. Nine other antigens were evaluated in studies that considered at least one immunological method as a reference standard test. For these antigens the sensitivity ranged from 39.8% to 76.9% and the specificity from 53.4% to 97%. The forest plots for sensitivity and specificity of ELISA considering parasitological methods and other tests as reference standard tests for TL-diagnosis are presented in Fig 2; more details about each evaluated antigen are available in S2 Table.

ELISA for CL diagnosis

Seventeen studies used ELISA to evaluate the performance of 44 antigens for CL-diagnosis, which comprised 20 recombinant proteins, 13 synthetic peptides and 11 purified proteins. The performance of 35 antigens was evaluated considering at least one parasitological method as a reference standard test. Among these, the sample size for studies of CL-patients ranged from 12 to 74 and for non-CL-patients from 10 to 177. Peroxidoxin was the only antigen presenting 100% sensitivity and specificity [19]. Nine antigens were evaluated considering at least one immunological test as a reference standard. Overall, HSP83 presented the highest performance (100% sensitivity and specificity) [46] (Fig 3, S3 Table).

Table 1. Characteristics of the studies included in the systematic review.

Reference	Country	Case (n)	No-case (n)	Reference standard test	Test platform	Protein targets	Type	Clinical form evaluated	Phase
Bennis et al., 2018 [35]	Morocco	136	83	Microscopy and/or PCR	ICT	Peroxidoxin	pAb	CL	III
Carmelo et al., 2002 [45]	Peru	24	44	Microscopy and culture	ELISA	H1 and 7 peptides	RP / SP	CL	I
Carvalho et al., 2017 [29]	Brazil	57	55	Microscopy and PCR	ELISA	HP (XP_001469551.1)	RP	TL	I
Celeste et al., 2004 [46]	Brazil	26	20	MST and/or hitopathology and IFAT	ELISA	HSP83	RP	CL and ML	I
Celeste et al., 2014 [47]	Brazil	26	109	Microscopy and Immunological	ELISA	HSP83	RP	CL and ML	I
Coelho et al., 2016 [30]	Brazil	24	28	Microscopy and PCR	ELISA	Cytochrome c oxidase and Putative IgE histamine releasing factor	RP	TL	I
Costa et al., 2016 [25]	Brazil	50	10	Microscopy and PCR	ELISA	A10, C11, C12 B10, B7 and H7	SP	TL	I
de Silva et al., 2017 [48]	Sri Lanka	59	22	PCR	ICT	Peroxidoxin	pAb	CL	III
Duarte et al., 2015 [27]	Brazil	43	40	Microscopy, PCR and MST	ELISA	Enolase; eukaryotic initiation factor 5a; HP (LbrM.30.3350); tryparedoxin peroxidase and β-tubulin	RP	TL	I
Gomes-Silva et al., 2008 [33]	Brazil	58	171	Microscopy and immunological	ELISA	Con-A and Jaca bound fraction	PP	TL	I
González et al., 2002 [49]	Peru	20	19	Microscopy and culture	ELISA	23085, 23089 and 23083	SP	TL	I
Jensen et al. 1996 [50]	Sudan	33	88	Microscopy and histopathology	ELISA	GPB and Gp63	SP / PP	CL	I
Kenner et al., 1999 [51]	Central America	41	20	Culture	IHC	G2D10	mAb	CL	I
Lage et al., 2019 [18]	Brazil	50	75	Microscopy and PCR	ELISA	A2 and HP (XP_003886492.1)	RP	TL	I
Lima et al., 2017 [52]	Brazil	45	50	Microscopy, PCR and MST	ELISA	HP (XP_001566959.1)	RP	TL, CL and ML	I
Lima et al., 2018 [31]	Brazil	40	143	Microscopy and PCR	ELISA	Enolase; eukaryotic initiation factor 5a; HP (XP_001566959.1) and β-tubulin	RP	TL	I
Link et al., 2017 [26]	Brazil	57	30	ELISA	ELISA	P1 and MIX (P1 + P2 + P3)	SP	CL	I
Longoni et al., 2014 [53]	Colombia	51	10	Microscopy	ELISA	Fe-SOD	PP	CL	I
Marin et al., 2009 [54]	Spain	113	32	Microscopy	ELISA	Fe-SOD	PP	CL and ML	I
Menezes-Souza et al., 2014a [19]	Brazil	65	70	Microscopy and PCR	ELISA	Peroxidoxin	RP	TL, CL and ML	I
Menezes-Souza et al., 2014b [20]	Brazil	65	70	Microscopy and PCR	ELISA	HSP83 and 3 peptides	RP/ SP	TL, CL and ML	I
Menezes-Souza et al., 2015a [21]	Brazil	65	70	Microscopy and PCR	ELISA	Cathepsin L-like and peptide	RP / SP	TL	I
Menezes-Souza et al., 2015b [22]	Brazil	65	70	Microscopy and PCR	ELISA	MAPK3 and MAPK4	RP	TL	I
Montoya et al., 1997 [55]	Colombia/ Peru	78	39	Serologic	ELISA	T26-U2 and T26-U4	RP	TL	I
Padilla et al., 2003 [56]	Peru	18	8	ELISA	ELISA	Acidic ribosomal P2β proteins	RP	TL	I

(*Continued*)

Table 1. (Continued)

Reference	Country	Case (n)	No-case (n)	Reference standard test	Test platform	Protein targets	Type	Clinical form evaluated	Phase
Salles et al., 2019 [32]	Brazil	40	100	Microscopy and PCR	ELISA	Small myristoylated protein-3 and peptide	RP/ SP	TL	I
Sato et al., 2017 [57]	Brazil	219	281	Microscopy, PCR and/or histopathology	ELISA	LB6H e Lb8E	RP	TL	I
Schallig et al., 2019 [58]	Suriname	79	14	Microscopy or PCR	ICT	Peroxidoxin	pAb	CL	III
Shirian et al., 2014 [59]	Iran	100	30	Cytology or histology and PCR	IHC	IS2-2B4 (A11) and XLVI-5B8- B3 (T1)	mAb	CL	I
Skraba et al., 2014 [60]	Brazil	60	177	Microscopy	ELISA	Mix (36 and 48–56 kDa)	PP	CL	I
Soto et al., 1996 [61]	Spain	21	30	Microscopy and IFAT	ELISA	Acidic ribosomal protein family (LiP2a-Q and LiP2b-Q)	RP	ML	I
Souza et al., 2013 [62]	Brazil	102	180	MST, immunologic, histopathology and/or therapeutic test	ELISA	H2A; H2B; H3; H4; HSP70; KMP11	RP	TL, CL and ML	I
de Souza et al., 2018 [34]	Brazil	30	119	Microscopy and culture	ELISA	NGP 0204; NGP2333; NGP 2334; NGP 2203	PP	CL	I
de Souza et al., 2019 [63]	Brazil	74	63	Microscopy	ELISA	Lbk39	RP	CL	I
Vidigal et al., 2008 [64]	Brazil	48	114	Microscopy, MST and IFAT	ELISA	Fraction 8—peak 2	PP	CL	I
Vink et al., 2018 [36]	Afghanistan	257	17	Microscopy and/or PCR	ICT	Peroxidoxin	pAb	CL	III
Yeganeg et al., 2009 [65]	Iran	30	41	Microscopy	ELISA	Fe SOD-B1	RP	CL	I
Zurita et al., 2003 [66]	Peru	50	36	Culture	ELISA	HSP70 and 5 peptides	RP	TL, CL and ML	I

CL—cutaneous leishmaniasis; ML—mucosal leishmaniasis; TL—tegumentary leishmaniasis; HP—hypothetical protein; RP—recombinant protein; PP—purified protein; SP—synthetic peptide; mAb—monoclonal antibody; pAb—polyclonal antibody.

https://doi.org/10.1371/journal.pone.0251956.t001

Other immunological tests for CL diagnosis

The performance of ICT and IHC using different monoclonal and/or polyclonal antibodies is presented in Fig 4 and detailed in S4 Table. Four studies evaluated the CL Detect Rapid Test (InBios International Inc., Seattle, WA, USA) in different countries. The sensitivity ranged from 35.6 to 67.6 and the specificity was higher than 80%. For IHC, two monoclonal antibodies were employed to detect antigens in fixed skin fragments. The highest performance was reported for IS22B4/XLVI5B8 mAbs, with 96% and 100% sensitivity and specificity, respectively [59].

ELISA for ML results

Nine studies used ELISA to evaluate the performance of 23 antigens for ML-diagnosis, which comprised 19 recombinant proteins, three synthetic peptides and one purified protein. The sample size from ML-patients in these studies ranged from 14 to 53 and from non-ML-patients from 20 to 92. At least one parasitological method was used as a reference standard test for the evaluation of sixteen antigens. The highest performance was obtained for Hypothetical protein XP_001467126.1, with 100% sensitivity and 98% specificity [27]. Seven antigens were evaluated in studies considering at least one immunological test as a reference standard. As noted for

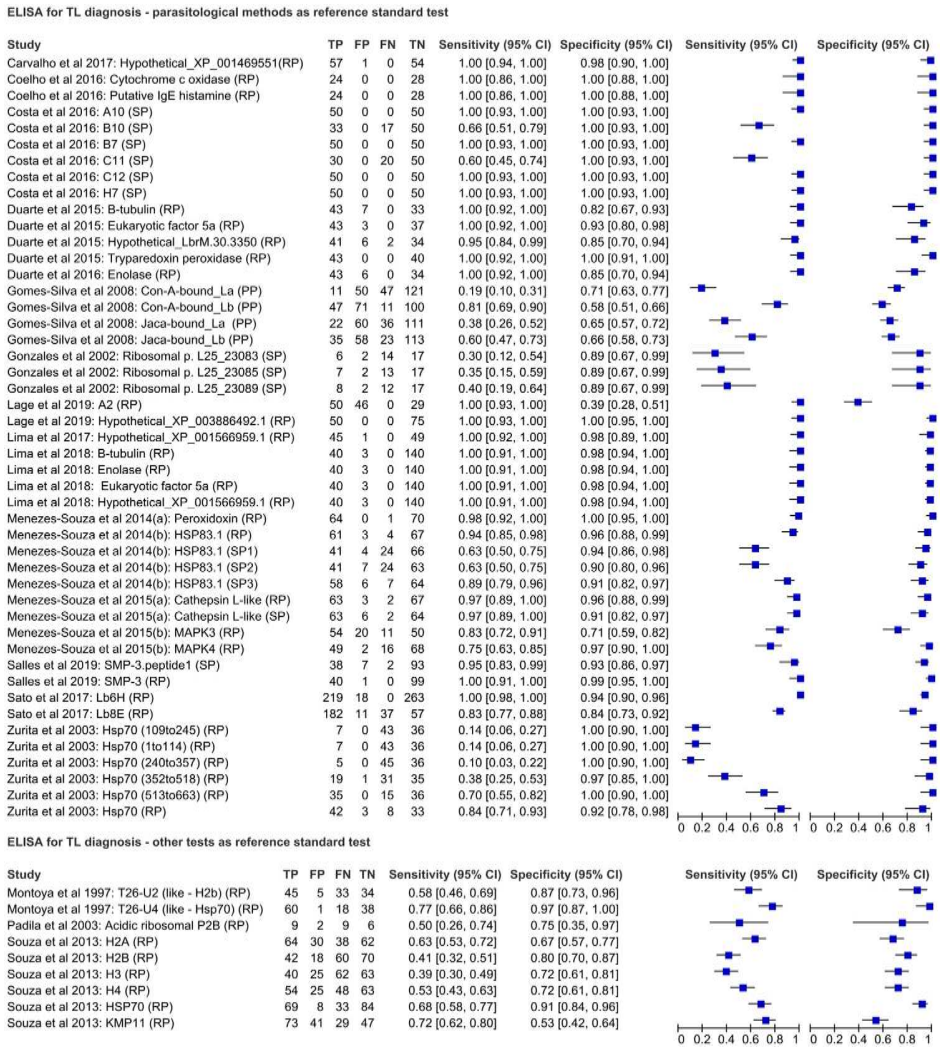

Fig 2. Forest plot representing sensitivity and specificity indices of ELISA using different antigenic targets for TL diagnosis.

https://doi.org/10.1371/journal.pone.0251956.g002

CL-diagnosis, 100% sensitivity and specificity were reported for HSP83 [46]. The performance of these antigens is presented in Fig 5 and more details are available in S5 Table.

The SROC curves with the antigen performances for the diagnostic of different clinical forms, using parasitological or other tests (such as ELISA and MST) as a reference standard, are presented in Fig 6. The antigens tended to have greater accuracy in studies that have used the parasitological methods as reference standard tests, regardless of TL-clinical manifestation.

Quadas-2 based quality assessment

Quality assessment of the study according to risk of bias and concern with applicability (low, high and unclear) is shown in Fig 7. Of the 38 studies assessed, 21 had high risk of bias in patient selection. The risk was unclear for the index test in 34 studies and for flow and timing in 30 studies. Nineteen studies had high concerns regarding applicability of patient selection criteria.

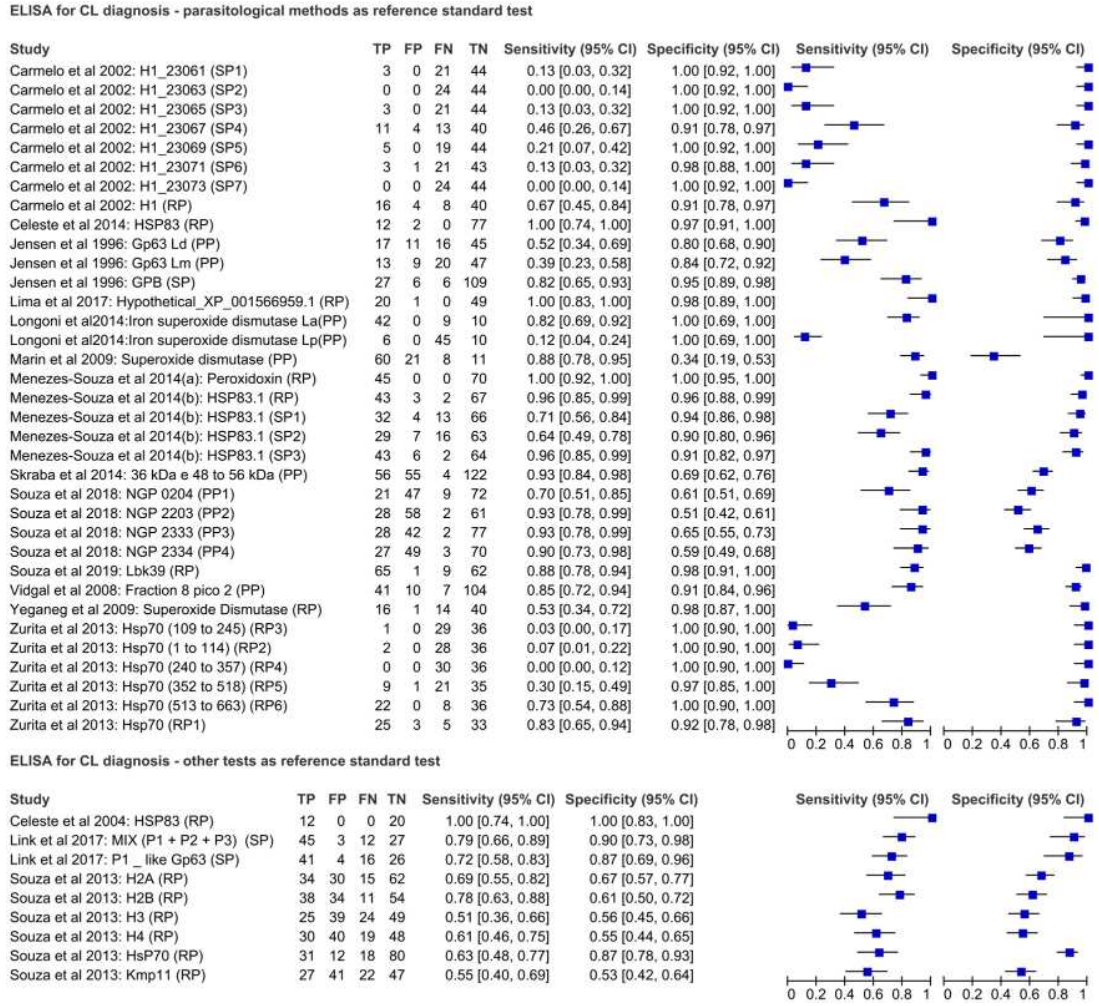

Fig 3. Forest plot representing sensitivity and specificity indices of ELISA using different antigenic targets for CL-diagnosis.

https://doi.org/10.1371/journal.pone.0251956.g003

Discussion

TL is considered a multifactorial disease, responsible for psychological and social impacts due to scars and mutilating lesions generating stigma and self-deprecation in affected patients [67]. Improvements in healthcare access and laboratory diagnosis are needed to overcome the impacts of this disease and should be encouraged [68]. According to WHO's Special Programme for Research and Training in Tropical Diseases (TDR), the ideal test must be

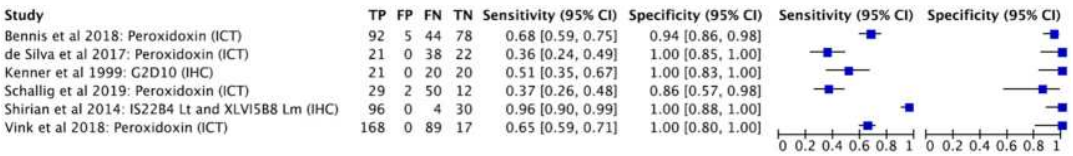

Fig 4. Forest plot representing sensitivity and specificity indices of other immunological tests using different antigenic targets for CL-diagnosis.

https://doi.org/10.1371/journal.pone.0251956.g004

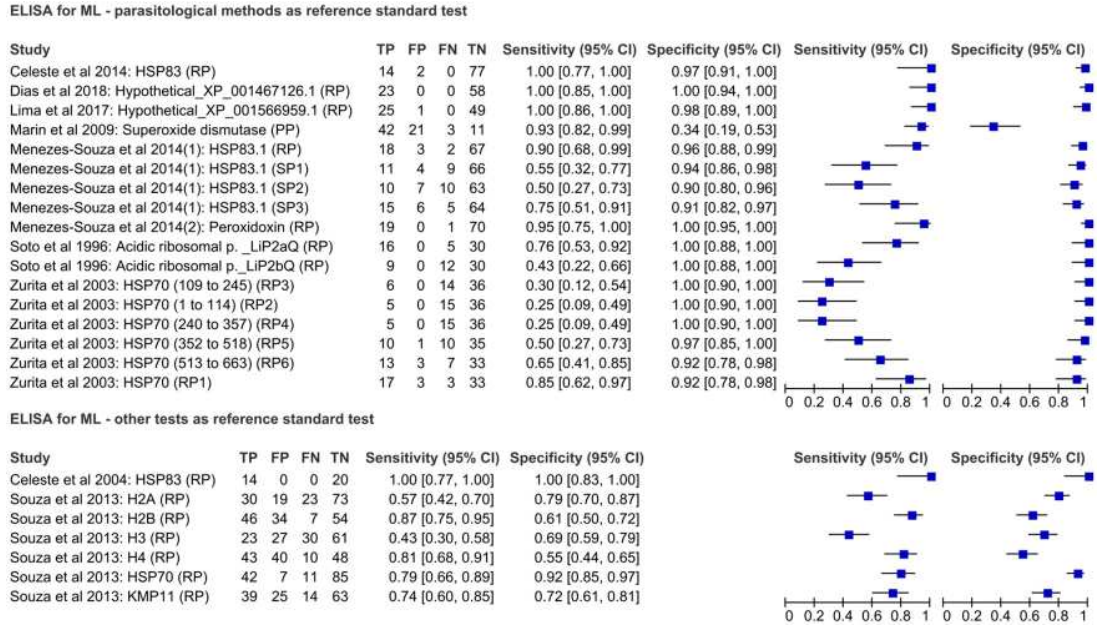

Fig 5. Forest plot representing sensitivity and specificity indexes of other immunological tests using different antigenic targets for ML-diagnosis.

https://doi.org/10.1371/journal.pone.0251956.g005

affordable, sensitive, specific, user-friendly, rapid, equipment-free and delivered to end-users (ASSURED) [69]. Immunological tests may fill these criteria since they are usually easy to perform, accessible and require minimally invasive sample collection. Therefore, the identification of sensitive and specific antigenic targets seems to be a promising step toward the improvement of TL-diagnosis.

The studies analyzed here were conducted from 1996 to 2019, however, almost 50% of them were conducted in the last five years, mostly in Brazil or another country in the Americas. The increase in the number of studies is coincident with the interruption of the production of MST antigen in Brazil in 2015, which extinguished the simple and rapid immunodiagnostic for TL [15]. This fact may have boosted research aimed at finding new diagnostic tools.

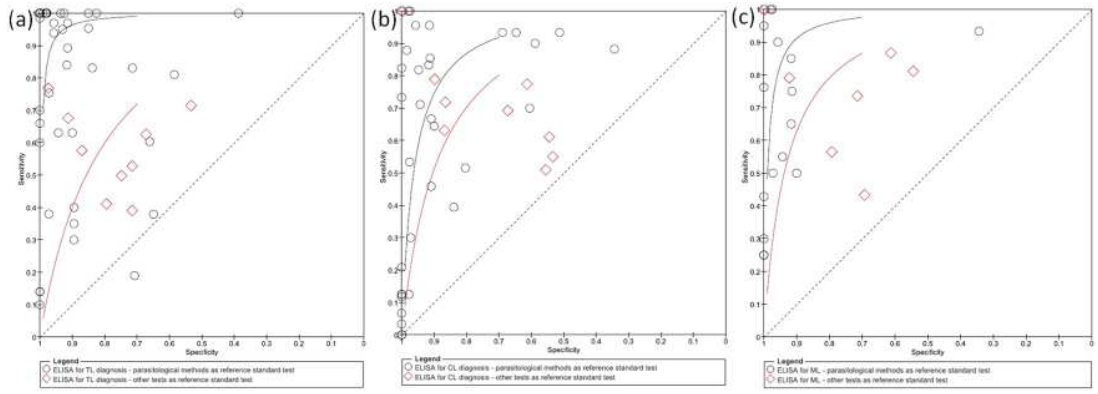

Fig 6. SROC curve for diagnosis of TL (a), CL (b) and ML (c) according to reference standard test.

https://doi.org/10.1371/journal.pone.0251956.g006

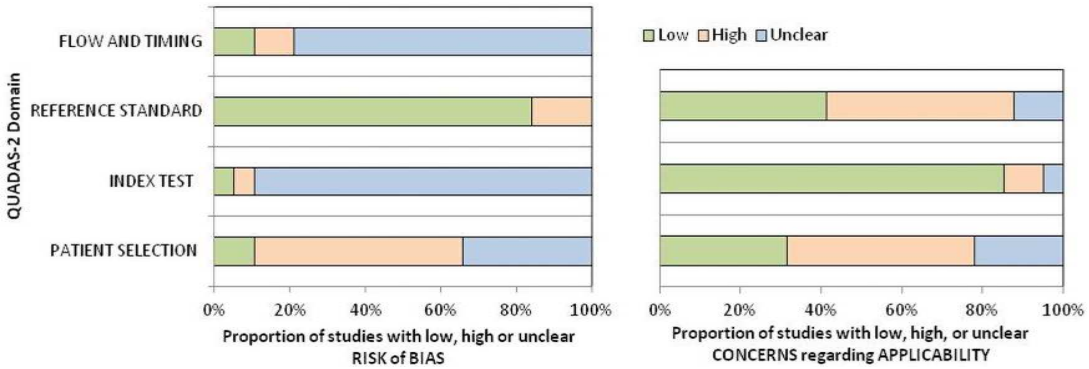

Fig 7. Risk of bias assessed by the QUADAS-2 tool according to different study characteristics (patient selection, index test, reference standard and flow and timing).

https://doi.org/10.1371/journal.pone.0251956.g007

Parasitological diagnosis was considered a reference standard test in 89.5% of the studies. Despite this technique being highly specific for TL-diagnosis, its sensitivity is limited and inversely correlated with disease duration [9, 70]. However, no test seems to present sufficiently high sensitivity and specificity to be used as a gold standard test. We observed a tendency for index tests to be more accurate if parasitological tests were used as a reference standard than other reference test such as MST and histopathology (Fig 6). Polymerase chain reaction (PCR) was used as a reference standard test in 18 studies, generally in association with parasitological diagnosis. Overall, PCR appears to be a more suitable reference test, however, a standard protocol is urgently needed and encouraged, since distinct extraction methods, protocols and molecular targets have been used overtime [71].

The ability to accurately identify TL-patients is essential for a diagnostic test, in view of the range of clinical forms, disease severity and treatment toxicity. Several studies have included patients with Chagas disease as non-TL cases, however, despite phylogenetic proximity, the inclusion of patients with clinical signs that do not resemble TL is at least questionable. For tests with diagnostic purposes, a better sample panel needs to be encouraged, including diseases such as sporotrichosis, paracoccidioidomycosis, hanseniasis, vasculitis, syphilis and other dermal or mucosal diseases, that represent confounding factors in clinical practice.

Despite the distinct profiles in immune response usually reported for each clinical form of TL, some antigens presented high values of sensitivity, even for CL-patients. In general, higher levels of antibodies have been reported for ML-patients compared to CL-patients, the latter being characterized by a moderate Th1 immune response [72, 73]. In this way, it seems that problems related to antibody detection in CL-patients may be reduced by using sensitive targets and well-standardized procedures [16]. Some antigenic targets were evaluated for TL-diagnosis without distinction of clinical form and, consequently, immune response profile. We believe that the accuracy of these antigenic targets may be improperly estimated in these specific cases.

This systematic literature review found 79 different antigens, comprising 40 recombinant proteins, 24 synthetic peptides and 15 purified proteins. The identification and more refined selection of protein targets using recombinant proteins or synthetic peptides allows the development of more standardized techniques due to the possibility of generating the purest inputs. Some protein-families have been widely evaluated as antigenic targets for TL-immunodiagnosis, such as heat shock proteins (HSPs), histones and peroxiredoxins, with promising results.

HSPs represent a highly conserved family of intracellular proteins of varying molecular weights in prokaryotic and eukaryotic cells, including cytosolic, mitochondrial, nuclear and endoplasmic reticulum resident proteins. They act as a chaperon in peptide folding and in the translocation of proteins to organelles, the prevention of protein aggregation, and the stabilization and degradation of proteins [74, 75]. HSPs have usually been identified by amino acid sequence homology and molecular weight, with HSP70 and HSP83 being the most abundant [76, 77]. These proteins are constitutively expressed throughout the life cycle of *Leishmania*, increasing expression in the vertebrate host due to variation in temperature and pH [78]. The recombinant proteins HSP70 and HSP83, and the synthetic peptides extracted from those proteins, have been widely evaluated for TL-diagnosis [20, 24, 46, 47, 62, 66]. The performance of these targets seems to be promising, with HSP83 presenting sensitivity of over 90% and high specificity with few cross reactions [20, 46, 47].

Histones are conserved proteins bound to DNA establishing chromatin structure in eukaryotes. Several biological functions have been described for histones during *Leishmania* infection in susceptible hosts. Core nucleosomal *Leishmania* histones have been proposed as prominent intracellular pathoantigens, since immunological responses against histones seem to be involved in the pathological mechanisms of visceral leishmaniasis (VL) [79, 80]. In this way, this protein family has been extensively employed in ELISA for both human and canine VL [81–84]. The presence of antibodies against rH2B of *L. peruviana* [55], rH1 of *L. braziliensis* [45] and rH2A, rH2B, rH3 and rH4 of *L. infantum* have been detected in sera from CL or ML patients. CARMELO et al. (2002) demonstrated that the antibody against histone H1 was specific for the parasite without cross reaction with human histones. However, moderate cross reactivity has been observed in a sample panel composed of Systemic Lupus Erythematosus (SLE) and Chagas disease [45, 55, 62].

Peroxidoxin, also known as thiol-specific antioxidant protein, as well as tryparedoxin peroxidase, are peroxiredoxins, an antioxidant enzyme family [85–87]. This protein family has been described in a wide variety of organisms and several biological functions have been reported for *Leishmania* parasites, such as virulence factor and protection against reactive oxygen and nitrogen species [88]. In this manner, they are directly associated with cell proliferation, senescence, apoptosis, and circadian rhythms [89]. These proteins have been described in the secretome of *L. braziliensis* and the antigenicity of tryparedoxin peroxidase has also been evaluated for both human and canine VL-diagnosis [90–92]. Peroxidoxin is the protein target identified by the CL Detect Rapid Test (InBios International Inc.) for CL-diagnosis. Variable performance has been reported for this ICT, according to endemic region and, consequently, the *Leishmania* species involved, with better results for infections caused by *L. tropica*, with sensitivity ranging 65.4–73% and specificity 92–100% [35, 36]. This test, however, has not been evaluated in Brazil.

Other recombinant proteins, such as cytochrome c oxidase, putative IgE histamine releasing factor, prohibitin, eukaryotic initiation factor 5a, cathepsin L-like peptide and small myristoylated protein-3, as well as hypothetical proteins, were evaluated in preliminary studies demonstrating potential as candidates for TL-immunodiagnosis, and so more studies are desirable [27, 29, 30, 93].

Some promising synthetic peptides have been identified and employed in ELISA. The use of small fragments containing potent antigenic determinants is able to minimize non-specific reactions. LINK et al. (2017) identified three peptides by phage display, probably from GP63 glycoprotein, and presented 79% sensitivity in ELISA [26]. COSTA et al. (2016) found high performance for three clones (A10, C12 and H7) in discriminating TL-patients from patients with other diseases and healthy individuals (100% sensitivity and specificity) [25]. However, these short linear peptides may have some drawbacks, such as limited passive adsorption on

polystyrene titration plates (ELISA-standard procedure), inability to identify serum antibodies that recognize conformational epitopes and problems considering reproducibility due to variation in inter-assay reactivity producing different batches [94].

Despite the advantages, the absence of post-translational modifications of bacterially-expressed and chemically synthesized proteins comprises an important limitation for the employment of this biotechnology for immunodiagnosis. In this way, purified proteins can represent significant advantages, especially regarding immunoreactivity. This review found iron-superoxide dismutase to be a purified protein with interesting results, with more than 80% sensitivity for CL or ML diagnosis. However, being purified proteins, sensitivity and specificity may vary according to the type, source, and purity of the antigen used [53, 54].

Three polyclonal and monoclonal antibodies were evaluated for detecting *Leishmania* antigen by ICT and IHC [35, 36, 48, 51, 58, 59]. The phase III studies included were ICT tests, that is, prospective studies in which the index and reference test were performed simultaneously in patients with clinical suspicion [35, 36, 48, 58]. This is a commercial test that, despite its low sensitivity, has been useful in some localities due to the simple realization and high specificity, reducing the number of CL patients referred for diagnosis confirmation. High performance was observed in phase I studies for species-specific monoclonal antibody (IS2-2B4—A11/XLVI-5B8-B3) employed in IHC, with 96% sensitivity and 100% specificity [59]. More robust studies using monoclonal or polyclonal antibodies for TL-diagnosis need to be encouraged evaluating the performance in clinical practice.

The strength of the present literature review is that it employed a comprehensive search strategy with four databases. One of the meaningful limitations may be the limited number of studies evaluating the same protein target, and so results need to be interpreted with caution. For this reason, a meta-analysis was not performed here. Additionally, it is important to consider that the risk of bias for many of the included studies was unclear and/or was high for some of the evaluated parameters: "Patient selection", "Flow and Timing" and "Index test". Here, we identified a large number of antigenic targets that could help clinical diagnosis. However, the high number of proof-of-concept and phase I studies highlights the need to move forward with more refined and mainly prospective studies including patients with clinical suspicion of TL from different endemic regions and the most sensitive reference standard tests, to evaluate the diagnostic accuracy of antigenic targets reported in clinical practice.

Supporting information

S1 Fig. Terms used in MEDLINE search.
(TIF)

S1 Table. PRISMA checklist.
(DOCX)

S2 Table. Antigenic targets used in ELISA for diagnosis of tegumentary leishmaniasis.
(DOCX)

S3 Table. Antigenic targets used in ELISA for diagnosis of cutaneous leishmaniasis.
(DOCX)

S4 Table. Antigenic targets for diagnosis of cutaneous leishmaniasis by other tests.
(DOCX)

S5 Table. Antigenic targets used in ELISA for diagnosis of mucosal leishmaniasis.
(DOCX)

Acknowledgments

We are grateful for support from the Programa de Pós Graduação em Ciências da Saúde of the Instituto René Rachou and Coordination for the Improvement of Higher Education Personnel (CAPES).

Author Contributions

Conceptualization: Mariana Lourenço Freire, Marcelo Antônio Pascoal-Xavier, Edward Oliveira.

Data curation: Mariana Lourenço Freire, Felipe Dutra Rêgo, Edward Oliveira.

Formal analysis: Mariana Lourenço Freire, Felipe Dutra Rêgo, Gláucia Cota, Edward Oliveira.

Funding acquisition: Edward Oliveira.

Methodology: Mariana Lourenço Freire, Felipe Dutra Rêgo, Gláucia Cota, Edward Oliveira.

Project administration: Marcelo Antônio Pascoal-Xavier, Edward Oliveira.

Supervision: Edward Oliveira.

Writing – original draft: Mariana Lourenço Freire, Felipe Dutra Rêgo, Gláucia Cota, Marcelo Antônio Pascoal-Xavier, Edward Oliveira.

Writing – review & editing: Mariana Lourenço Freire, Felipe Dutra Rêgo, Gláucia Cota, Marcelo Antônio Pascoal-Xavier, Edward Oliveira.

References

1. PAHO/WHO. Leishmaniases. Epidemiological Report of the Americas. 2019.: https://iris.paho.org/handle/10665.2/51734
2. WHO. Global leishmaniasis surveillance, 2017–2018, and first report on 5 additional indicators. Wkly Epidemiol Rec. 2020; 95: 265–280. https://www.who.int/publications/i/item/who-wer9525
3. Reithinger R, Dujardin JC, Louzir H, Pirmez C, Alexander B, Brooker S. Cutaneous leishmaniasis. Lancet Infect Dis. 2007; 7: 581–596. https://doi.org/10.1016/S1473-3099(07)70209-8 PMID: 17714672
4. d. C Toledo AC, da Silva RE, Carmo RF, Amaral TA, Luz ZMP, Rabello A. Assessment of the quality of life of patients with cutaneous leishmaniasis in Belo Horizonte, Brazil, 2009–2010. A pilot study. Trans R Soc Trop Med Hyg. 2013; 107: 335–336. https://doi.org/10.1093/trstmh/trt021 PMID: 23474473
5. Chahed MK, Bellali H, Ben Jemaa S, Bellaj T. Psychological and Psychosocial Consequences of zoonotic cutaneous leishmaniasis among women in Tunisia: Preliminary findings from an exploratory study. PLoS Negl Trop Dis. 2016; 10: e0005090. https://doi.org/10.1371/journal.pntd.0005090 PMID: 27788184
6. Al-Hucheimi SN, Sultan BA, Al-Dhalimi MA. A comparative study of the diagnosis of Old World cutaneous leishmaniasis in Iraq by polymerase chain reaction and microbiologic and histopathologic methods. Int J Dermatol. 2009; 48: 404–408. https://doi.org/10.1111/j.1365-4632.2009.03903.x PMID: 19335428
7. Goto H, Lindoso JAL. Current diagnosis and treatment of cutaneous and mucocutaneous leishmaniasis. Expert Rev Anti Infect Ther. 2010; 8: 419–433. https://doi.org/10.1586/eri.10.19 PMID: 20377337
8. Sotto MN, Yamashiro-Kanashiro EH, da Matta VLR, de Brito T. Cutaneous leishmaniasis of the New World: diagnostic immunopathology and antigen pathways in skin and mucosa. Acta Trop. 1989; 46: 121–130. https://doi.org/10.1016/0001-706x(89)90006-5 PMID: 2565073
9. Weigle KA, de Davalos M, Heredia P, Molineros R, Saravia NG, D'Alessandro A. Diagnosis of cutaneous and mucocutaneous leishmaniasis in Colombia: a comparison of seven methods. Am J Trop Med Hyg. 1987; 36: 489–496. https://doi.org/10.4269/ajtmh.1987.36.489 PMID: 2437815
10. Kar K. Serodiagnosis of leishmaniasis. Crit Rev Microbiol. 1995; 21: 123–152. https://doi.org/10.3109/10408419509113537 PMID: 7639932
11. Reithinger R, Coleman PG. Treating cutaneous leishmaniasis patients in Kabul, Afghanistan: cost-effectiveness of an operational program in a complex emergency setting. BMC Infect Dis. 2007; 7: 3. https://doi.org/10.1186/1471-2334-7-3 PMID: 17263879

12. de Paiva-Cavalcanti M, de Morais RCS, Pessoa-e-Silva R, Trajano-Silva LAM, Gonçalves-de-Albuquerque S da C, Tavares D de HC, et al. Leishmaniases diagnosis: An update on the use of immunological and molecular tools. Cell and Bioscience. BioMed Central Ltd.; 2015. pp. 1–10. https://doi.org/10.1186/s13578-015-0021-2 PMID: 26097678

13. Guarín N, Palma G, Pirmez C, Valderrama L, Tovar R, Saravia N. Comparative immunohistological analysis of the Montenegro skin test reaction in asymptomatic infection and in acute and chronic cutaneous leishmaniasis. Biomédica. 2006. http://www.scielo.org.co/scielo.php?script=sci_arttext&pid=S0120-41572006000500006. Accessed 29 Mar 2021. PMID: 17361840

14. Sassi A, Louzir H, Ben-Salah A, Mokni M, Ben-Osman A, Dellagi K. Leishmanin skin test lymphoproliferative responses and cytokine production after symptomatic or asymptomatic *Leishmania major* infection in Tunisia. Clin Exp Immunol. 1999; 116: 127–132. https://doi.org/10.1046/j.1365-2249.1999.00844.x PMID: 10209516

15. Braz LMA. Tegumentary leishmaniasis diagnosis: What happened with MST (Montenegro skin test) in Brazil? Rev Inst Med Trop Sao Paulo. 2019; 61: 1–3. https://doi.org/10.1590/S1678-9946201961017 PMID: 30864622

16. Romero GAS, Orge M de la GO, de F Guerra MV, Paes MG, de O Macêdo V, de Carvalho EM. Antibody response in patients with cutaneous leishmaniasis infected by *Leishmania* (*Viannia*) *braziliensis* or *Leishmania* (*Viannia*) *guyanensis* in Brazil. Acta Trop. 2005; 93: 49–56. https://doi.org/10.1016/j.actatropica.2004.09.005 PMID: 15589797

17. Caballero ZC, Sousa OE, Marques WP, Saez-Alquezar A, Umezawa ES. Evaluation of serological tests to identify *Trypanosoma cruzi* infection in humans and determine cross-reactivity with *Trypanosoma rangeli* and *Leishmania* spp. Clin Vaccine Immunol. 2007; 14: 1045–1049. https://doi.org/10.1128/CVI.00127-07 PMID: 17522327

18. Lage DP, Machado AS, Ramos FF, Silveira PC, Dias DS, Ribeiro PAF, et al. A biomarker for tegumentary and visceral leishmaniasis based on a recombinant *Leishmania* hypothetical protein. Immunobiology. 2019; 224: 477–484. https://doi.org/10.1016/j.imbio.2019.05.008 PMID: 31164242

19. Menezes-Souza D, De Oliveira Mendes TA, Pinto Nagem RA, De Oliveira Santos TT, Teixeira Silva AL, Santoro MM, et al. Mapping B-cell epitopes for the peroxidoxin of *Leishmania* (*Viannia*) *braziliensis* and its potential for the clinical diagnosis of tegumentary and visceral leishmaniasis. PLoS One. 2014; 9. https://doi.org/10.1371/journal.pone.0099216 PMID: 24921246

20. Menezes-Souza D, De Mendes TAO, De Gomes MS, Reis-Cunha JL, Nagem RAP, Carneiro CM, et al. Epitope mapping of the HSP83.1 protein of *Leishmania braziliensis* discloses novel targets for immunodiagnosis of tegumentary and visceral clinical forms of leishmaniasis. Clin Vaccine Immunol. 2014; 21: 949–959. https://doi.org/10.1128/CVI.00151-14 PMID: 24807053

21. Menezes-Souza D, de O Mendes TA, de S Gomes M, Bartholomeu DC, Fujiwara RT. Improving serodiagnosis of human and canine leishmaniasis with recombinant Leishmania braziliensis Cathepsin L-like protein and a synthetic peptide containing its linear B-cell epitope. PLoS Negl Trop Dis. 2015; 9: e3426. https://doi.org/10.1371/journal.pntd.0003426 PMID: 25569432

22. Menezes-Souza D, de Oliveira Mendes TA, de Araújo Leão AC, de Souza Gomes M, Fujiwara RT, Bartholomeu DC. Linear B-cell epitope mapping of MAPK3 and MAPK4 from *Leishmania braziliensis*: implications for the serodiagnosis of human and canine leishmaniasis. Appl Microbiol Biotechnol. 2015; 99: 1323–1336. https://doi.org/10.1007/s00253-014-6168-7 PMID: 25359475

23. Florez MM, de Oliveira CI, Puerta C, Guzman F, Ayala M, Montoya G, et al. Synthetic peptides derived from ribosomal proteins of *Leishmania* spp. in mucocutaneous leishmaniasis: Diagnostic usefulness. Protein Pept Lett. 2017; 24: 982–988. https://doi.org/10.2174/0929866524666170728143924 PMID: 28758598

24. Amorim AG, Carrington M, Miles MA, Barker DC, Almeida LC, de Almeida ML. Identification of the C-terminal region of 70 kDa heat shock protein from *Leishmania* (*Viannia*) *braziliensis* as a target for the humoral immune response. Cell Stress Chaperones. 1996; 1: 177–187. PMID: 9222603

25. Costa LE, Salles BCS, Alves PT, Dias ACS, Vaz ER, Ramos FF, et al. New serological tools for improved diagnosis of human tegumentary leishmaniasis. J Immunol Methods. 2016; 434: 39–45. https://doi.org/10.1016/j.jim.2016.04.005 PMID: 27090730

26. Link JS, Alban SM, Soccol CR, Pereira GVM, Thomaz Soccol V. Synthetic peptides as potential antigens for cutaneous leishmaniosis diagnosis. J Immunol Res. 2017; 2017: 5871043. https://doi.org/10.1155/2017/5871043 PMID: 28367456

27. Duarte MC, Pimenta DC, Menezes-Souza D, Magalhães RDM, Diniz JLCP, Costa LE, et al. Proteins selected in *Leishmania* (*Viannia*) *braziliensis* by an immunoproteomic approach with potential serodiagnosis applications for tegumentary leishmaniasis. Clin Vaccine Immunol. 2015; 22: 1187–1196. https://doi.org/10.1128/CVI.00465-15 PMID: 26376929

28. Lima BSS, Pires SFF, Fialho LCCJ, Oliveira EJJ, Machado-de-Avila RAA, Chavez-Olortegui C, et al. A proteomic road to acquire an accurate serological diagnosis for human tegumentary leishmaniasis. J Proteomics. 2017; 151: 174–181. https://doi.org/10.1016/j.jprot.2016.05.017 PMID: 27262223

29. Carvalho AMRSS, Costa LE, Salles BCSS, Santos TTO, Ramos FF, Lima MP, et al. An ELISA immunoassay employing a conserved *Leishmania* hypothetical protein for the serodiagnosis of visceral and tegumentary leishmaniasis in dogs and humans. Cell Immunol. 2017; 318: 42–48. https://doi.org/10.1016/j.cellimm.2017.06.001 PMID: 28602279

30. Coelho EAF, Costa LE, Lage DP, Martins VT, Garde E, de Jesus Pereira NC, et al. Evaluation of two recombinant *Leishmania* proteins identified by an immunoproteomic approach as tools for the serodiagnosis of canine visceral and human tegumentary leishmaniasis. Vet Parasitol. 2016; 215: 63–71. https://doi.org/10.1016/j.vetpar.2015.11.006 PMID: 26790739

31. Lima MP, Costa LE, Lage DP, Dias DS, Ribeiro PAF, Machado AS, et al. Diagnostic application of recombinant *Leishmania* proteins and evaluation of their *in vitro* immunogenicity after stimulation of immune cells collected from tegumentary leishmaniasis patients and healthy individuals. Cell Immunol. 2018; 334: 61–69. https://doi.org/10.1016/j.cellimm.2018.09.006 PMID: 30287082

32. Salles BCS, Dias DS, Steiner BT, Lage DP, Ramos FF, Ribeiro PAF, et al. Potential application of small myristoylated protein-3 evaluated as recombinant antigen and a synthetic peptide containing its linear B-cell epitope for the serodiagnosis of canine visceral and human tegumentary leishmaniasis. Immunobiology. 2019; 224: 163–171. https://doi.org/10.1016/j.imbio.2018.09.003 PMID: 30266201

33. Gomes-Silva A, Souza MA, Afonso-Cardoso SR, Andrade LR, Dietze R, Lemos E, et al. Serological reactivity of different antigenic preparations of *Leishmania* (*Leishmania*) *amazonensis* and the *Leishmania braziliensis* complex. Rev Soc Bras Med Trop. 2008; 41: 135–141. https://doi.org/10.1590/s0037-86822008000200001 PMID: 18545832

34. De Souza LMB, Thomaz Soccol V, Petterle RR, Bates MD, Bates PA, et al. Analysis of *Leishmania* mimetic neoglycoproteins for the cutaneous leishmaniasis diagnosis. Parasitology. 2018; 145: 1938–1948. https://doi.org/10.1017/S0031182018000720 PMID: 29806570

35. Bennis I, Verdonck K, el Khalfaoui N, Riyad M, Fellah H, Dujardin JC, et al. Accuracy of a rapid diagnostic test based on antigen detection for the diagnosis of cutaneous leishmaniasis in patients with suggestive skin lesions in Morocco. Am J Trop Med Hyg. 2018; 99: 716–722. https://doi.org/10.4269/ajtmh.18-0066 PMID: 29988004

36. Vink MMT, Nahzat SM, Rahimi H, Buhler C, Ahmadi BA, Nader M, et al. Evaluation of point-of-care tests for cutaneous leishmaniasis diagnosis in Kabul, Afghanistan. EBioMedicine. 2018; 37: 453–460. https://doi.org/10.1016/j.ebiom.2018.10.063 PMID: 30396855

37. Zanetti ADS, Sato CM, Longhi FG, Ferreira SMB, Espinosa OA. Diagnostic accuracy of enzyme-linked immunosorbent assays to detect anti-leishmania antibodies in patients with American tegumentary leishmaniasis: A systematic review. Rev Inst Med Trop Sao Paulo. 2019; 61: e42. https://doi.org/10.1590/S1678-9946201961042 PMID: 31432991

38. Pena HP, Belo VS, Xavier-Junior JCC, Teixeira-Neto RG, Melo SN, Pereira DA, et al. Accuracy of diagnostic tests for American tegumentary leishmaniasis: a systematic literature review with meta-analyses. Trop Med Int Heal. 2020; 25: 1168–1181. https://doi.org/10.1111/tmi.13465 PMID: 32677284

39. Higgins J, Thomas J. Cochrane Handbook for Systematic Reviews of Interventions | Cochrane Training. 2020. https://training.cochrane.org/handbook/current

40. McInnes MDF, Moher D, Thombs BD, McGrath TA, Bossuyt PM, Clifford T, et al. Preferred reporting items for a systematic review and meta-analysis of diagnostic test accuracy studies the PRISMA-DTA Statement. JAMA—J Am Med Assoc. 2018; 319: 388–396. https://doi.org/10.1001/jama.2017.19163 PMID: 29362800

41. Lorenzetti DL, Ghali WA. Reference management software for systematic reviews and meta-analyses: An exploration of usage and usability. BMC Med Res Methodol. 2013; 13: 141. https://doi.org/10.1186/1471-2288-13-141 PMID: 24237877

42. Ouzzani M, Hammady H, Fedorowicz Z, Elmagarmid A. Rayyan-a web and mobile app for systematic reviews. Syst Rev. 2016; 5: 1–10.

43. Leeflang MMG, Allerberger F. How to: evaluate a diagnostic test. Clin Microbiol Infect. 2019; 25: 54–59. https://doi.org/10.1016/j.cmi.2018.06.011 PMID: 29906592

44. Whiting PF, Rutjes AWS, Westwood ME, Mallett S, Deeks JJ, Reitsma JB, et al. Quadas-2: A revised tool for the quality assessment of diagnostic accuracy studies. Ann Intern Med. 2011; 155: 529–536. https://doi.org/10.7326/0003-4819-155-8-201110180-00009 PMID: 22007046

45. Carmelo E, Martinez E, Gonzalez AC, Pinero JE, Patarroyo ME, Del Castillo A, et al. Antigenicity of *Leishmania braziliensis* histone H1 during cutaneous leishmaniasis: localization of antigenic determinants. Clin Diagn Lab Immunol. 2002; 9: 808–811. https://doi.org/10.1128/cdli.9.4.808-811.2002 PMID: 12093677

46. Celeste BJ, Angel SO, Castro LGM, Gidlund M, Goto H. *Leishmania infantum* heat shock protein 83 for the serodiagnosis of tegumentary leishmaniasis. Brazilian J Med Biol Res. 2004; 37: 1591–1593. https://doi.org/10.1590/s0100-879x2004001100001 PMID: 15517072

47. Celeste BJ, Sanchez MCA, Ramos-Sanchez EM, Castro LGM, Costa FAL, Goto H. Recombinant *Leishmania infantum* heat shock protein 83 for the serodiagnosis of cutaneous, mucosal, and visceral leishmaniases. Am J Trop Med Hyg. 2014; 90: 860–865. https://doi.org/10.4269/ajtmh.13-0623 PMID: 24615136

48. De Silva G, Somaratne V, Senaratne S, Vipuladasa M, Wickremasinghe R, et al. Efficacy of a new rapid diagnostic test kit to diagnose Sri Lankan cutaneous leishmaniasis caused by *Leishmania donovani*. Gannavaram S, editor. PLoS One. 2017; 12: 1–14. https://doi.org/10.1371/journal.pone.0187024 PMID: 29135995

49. González AC, Martínez E, Carmelo E, Piñero JE, Alonso V, Del Castillo A, et al. Analysis of NLS and rRNA binding motifs in the L25 ribosomal protein from *Leishmania* (*Viannia*) *braziliensis*: Investigation of its diagnostic capabilities. Parasitology. 2002; 125: 51–57. https://doi.org/10.1017/s0031182002001804 PMID: 12166520

50. Jensen AT, Gaafar A, Ismail A, Christensen CB, Kemp M, Hassan AM, et al. Serodiagnosis of cutaneous leishmaniasis: Assessment of an enzyme-linked immunosorbent assay using a peptide sequence from gene B protein. Am J Trop Med Hyg. 1996; 55: 490–495. https://doi.org/10.4269/ajtmh.1996.55.490 PMID: 8940979

51. Kenner JR, Aronson NE, Bratthauer GL, Turnicky RP, Jackson JE, Tang DB, et al. Immunohistochemistry to identify *Leishmania* parasites in fixed tissues. Journal of Cutaneous Pathology 1999 pp. 130–136. https://doi.org/10.1111/j.1600-0560.1999.tb01817.x PMID: 10235378

52. Lima MP, Costa LE, Duarte MC, Menezes-Souza D, Salles BCS, de Oliveira Santos TT, et al. Evaluation of a hypothetical protein for serodiagnosis and as a potential marker for post-treatment serological evaluation of tegumentary leishmaniasis patients. Parasitol Res. 2017; 116: 1197–1206. https://doi.org/10.1007/s00436-017-5397-y PMID: 28150041

53. Longoni SS, Marín C, Sánchez-Moreno M, Longoni SS, Marin C, Sanchez-Moreno M, et al. Excreted *Leishmania peruviana* and *Leishmania amazonensis* iron-superoxide dismutase purification: Specific antibody detection in Colombian patients with cutaneous leishmaniasis. Free Radic Biol Med. 2014; 69: 26–34. https://doi.org/10.1016/j.freeradbiomed.2014.01.012 PMID: 24440468

54. Marin C, Longoni SS, Urbano JJ, Minaya G, Mateo H, De Diego JA, et al. Enzyme-linked immunosorbent assay for superoxide dismutase-excreted antigen in diagnosis of sylvatic and andean cutaneous leishmaniasis of Peru. Am J Trop Med Hyg. 2009; 80: 55–60. https://doi.org/10.4269/ajtmh.2009.80.55 PMID: 19141840

55. Montoya Y, Leon C, Talledo M, Nolasco O, Padilla C, Muñoz-Najar U, et al. Recombinant antigens for specific and sensitive serodiagnosis of Latin American tegumentary leishmaniasis. Trans R Soc Trop Med Hyg. 1997; 91: 674–676. https://doi.org/10.1016/s0035-9203(97)90520-4 PMID: 9580116

56. Padilla R. C, Montoya P. Y. Characterization and immunoreactivity of *Leishmania braziliensis* P2ß acidic ribosomal protein. Rev Peru Med Exp Salud Publica. 2003; 20: 92–96.

57. Sato CM, Sanchez MCA, Celeste BJ, Duthie MS, Guderian J, Reed SG, et al. Use of recombinant antigens for sensitive serodiagnosis of American tegumentary leishmaniasis caused by different *Leishmania* species. J Clin Microbiol. 2017; 55: 495–503. https://doi.org/10.1128/JCM.01904-16 PMID: 27927927

58. Schallig H, Hu RVPF, Kent AD, van Loenen M, Menting S, Picado A, et al. Evaluation of point of care tests for the diagnosis of cutaneous leishmaniasis in Suriname. BMC Infect Dis. 2019; 19: 25. https://doi.org/10.1186/s12879-018-3634-3 PMID: 30616544

59. Shirian S, Oryan A, Hatam G-RR, Panahi S, Daneshbod Y, et al. Comparison of conventional, molecular, and immunohistochemical methods in diagnosis of typical and atypical cutaneous leishmaniasis. Arch Pathol Lab Med. 2014; 138: 235–240. https://doi.org/10.5858/arpa.2013-0098-OA PMID: 24476521

60. Skraba CM, Pedroso RB, Fiorini A, Rosado FR, Aristides SMA, Lonardoni MVC, et al. Diagnosis of American cutaneous leishmaniasis by enzyme immunoassay using membrane antigens of *Leishmania* (*Viannia*) *braziliensis*. Diagn Microbiol Infect Dis. 2014; 78: 411–417. https://doi.org/10.1016/j.diagmicrobio.2013.08.020 PMID: 24485589

61. Soto M, Requena JM, Quijada L, Alonso C. Specific serodiagnosis of human leishmaniasis with recombinant *Leishmania* P2 acidic ribosomal proteins. Clin Diagn Lab Immunol. 1996; 3: 387–391. PMID: 8807201

62. Souza AP, Soto M, Costa JMLL, Boaventura VS, de Oliveira CI, Cristal JR, et al. Towards a more precise serological diagnosis of human tegumentary leishmaniasis using *Leishmania* recombinant proteins. PLoS One. 2013; 8: e66110. https://doi.org/10.1371/journal.pone.0066110 PMID: 23776617

63. de Souza LMB, Carvalho J, Bates MD, Petterle RR, Thomaz-Soccol V, Bates PA, et al. Production of a kinesin-related recombinant protein (Lbk39) from *Leishmania braziliensis* by *Leishmania tarentolae* promastigotes and its application in the serodiagnosis of leishmaniasis. One Heal. 2019; 8: 100111. https://doi.org/10.1016/j.onehlt.2019.100111 PMID: 31788531

64. Vidigal CP, Marcussi VM, Marcussi LM, Mikcha JMG, Arraes SMAA, Lonardoni MVC, et al. Enzyme immunoassay using *Leishmania* (*Viannia*) *braziliensis* antigens for laboratorial diagnosis of American cutaneous leishmaniasis. Acta Trop. 2008; 107: 208–212. https://doi.org/10.1016/j.actatropica.2008.04.026 PMID: 18561892

65. Yeganeh F, Barkhordari F, Omidi M, Samiei A, Adeli A, Mahboudi F, et al. Cloning and expression of *Leishmania major* superoxide dismutase b1: A potential target antigen for serodiagnosis of leishmaniasis. Iran J Immunol. 2009; 6: 130–140. PMID: 19801786

66. Zurita AI, Rodriguez J, Pinero JE, Pacheco R, Carmelo E, del Castllo A, et al. Cloning and characterization of the *Leishmania* (*Viannia*) *braziliensis* HSP70 gene. Diagnostic use of the C-terminal fragment rlb70(513–663). J Parasitol. 2003; 89: 372–378. https://doi.org/10.1645/0022-3395(2003)089[0372:CACOTL]2.0.CO;2 PMID: 12760657

67. Bennis I, Belaid L, De Brouwere V, Filali H, Sahibi H, Boelaert M. The mosquitoes that destroy your face. Social impact of cutaneous leishmaniasis in south-eastern Morocco, A qualitative study. Fortin A, editor. PLoS One. 2017; 12: e0189906. https://doi.org/10.1371/journal.pone.0189906 PMID: 29261762

68. WHO. Accelerating work to overcome the global impact of neglected tropical diseases. 2012. https://www.who.int/neglected_diseases/NTD_RoadMap_2012_Fullversion.pdf

69. Land KJ, Boeras DI, Chen XS, Ramsay AR, Peeling RW. REASSURED diagnostics to inform disease control strategies, strengthen health systems and improve patient outcomes. Nat Microbiol. 2019; 4: 46–54. https://doi.org/10.1038/s41564-018-0295-3 PMID: 30546093

70. Jara M, Adaui V, Valencia BM, Martinez D, Alba M, Castrillon C, et al. Real-time PCR assay for detection and quantification of *Leishmania* (*Viannia*) organisms in skin and mucosal lesions: Exploratory study of parasite load and clinical parameters. J Clin Microbiol. 2013; 51: 1826–1833. https://doi.org/10.1128/JCM.00208-13 PMID: 23554201

71. De Oliveira DM, Valdrinez M, Lonardoni C, Teodoro U, Gomes T, Silveira V, et al. Comparison of different primes for PCR-based diagnosis of cutaneous leishmaniasis. Brazilian J Infect Dis. 2011; 15: 204–210. https://doi.org/10.1016/s1413-8670(11)70176-3

72. Cuba CC, Llanos-Cuentas EA, Barreto AC, Magalhães A V., Lago EL, Reed SG, et al. Human mucocutaneous leishmaniasis in Três Braços, Bahia—Brazil: an area of *Leishmania braziliensis braziliensis* transmission. I. Laboratory diagnosis. Rev Soc Bras Med Trop. 1984; 17: 161–167. https://doi.org/10.1590/s0037-86821984000400002

73. Pedrosa Valli LC, Azeredo Passos VM, Dietze R, Lee Callahan H, Berman JD, Grogl M. Humoral immune responses among mucosal and cutaneous leishmaniasis patients caused by *Leishmania braziliensis*. J Parasitol. 1999; 85: 1076–1083. https://doi.org/10.2307/3285671 PMID: 10647040

74. Stewart GR, Young DB. Heat-shock proteins and the host-pathogen interaction during bacterial infection. Curr Opin Immunol. 2004; 16: 506–510. https://doi.org/10.1016/j.coi.2004.05.007 PMID: 15245747

75. Priya S, Sharma SK, Goloubinoff P. Molecular chaperones as enzymes that catalytically unfold misfolded polypeptides. FEBS Lett. 2013; 587: 1981–1987. https://doi.org/10.1016/j.febslet.2013.05.014 PMID: 23684649

76. Clare DK, Saibil HR. ATP-driven molecular chaperone machines. Wittinghofer A, editor. Biopolymers. 2013; 99: 846–859. https://doi.org/10.1002/bip.22361 PMID: 23877967

77. Wiesgigl M, Clos J. Heat shock protein 90 homeostasis controls stage differentiation in *Leishmania donovani*. Mol Biol Cell. 2001; 12: 3307–3316. https://doi.org/10.1091/mbc.12.11.3307 PMID: 11694568

78. Zilka A, Garlapati S, Dahan E, Yaolsky V, Shapira M. Developmental regulation of heat shock protein 83 in *Leishmania*: 3′ processing and mRNA stability control transcript abundance, and translation is directed by a determinant in the 3′-untranslated region. J Biol Chem. 2001; 276: 47922–47929. https://doi.org/10.1074/jbc.M108271200 PMID: 11598129

79. Chang KP, McGwire BS. Molecular determinants and regulation of *Leishmania* virulence. Kinetoplastid Biol Dis. 2002; 1: 1. https://doi.org/10.1186/1475-9292-1-1 PMID: 12234388

80. Chang KP, Reed SG, McGwire BS, Soong L. *Leishmania* model for microbial virulence: The relevance of parasite multiplication and pathoantigenicity. Acta Tropica. Acta Trop; 2003. pp. 375–390. https://doi.org/10.1016/s0001-706x(02)00238-3 PMID: 12659975

81. Maalej IA, Chenik M, Louzir H, Ben Salah A, Bahloul C, Amri F, et al. Comparative evaluation of ELISAs based on ten recombinant or purified *Leishmania* antigens for the serodiagnosis of Mediterranean visceral leishmaniasis. Am J Trop Med Hyg. 2003; 68: 312–320. PMID: 12685637

82. Soto M, Requena JM, Quijada L, García M, Guzman F, Patarroyo ME, et al. Mapping of the linear antigenic determinants from the *Leishmania infantum* histone H2A recognized by sera from dogs with leishmaniasis. Immunol Lett. 1995; 48: 209–214. https://doi.org/10.1016/0165-2478(95)02473-5 PMID: 8867853

83. Soto M, Requena JM, Quijada L, Gomez LC, Guzman F, Patarroyo ME, et al. Characterization of the antigenic determinants of the *Leishmania infantum* histone H3 recognized by antibodies elicited during canine visceral leishmaniasis. Clin Exp Immunol. 1996; 106: 454–461. https://doi.org/10.1046/j.1365-2249.1996.d01-865.x PMID: 8973612

84. Soto M, Requena JM, Quijada L, Perez MJ, Nieto CG, Guzman F, et al. Antigenicity of the *Leishmania infantum* histones H2B and H4 during canine viscerocutaneous leishmaniasis. Clin Exp Immunol. 1999; 115: 342–349. https://doi.org/10.1046/j.1365-2249.1999.00796.x PMID: 9933463

85. Jirata D, Kuru T, Genetu A, Barr S, Hailu A, Aseffa A, et al. Identification, sequencing and expression of peroxidoxin genes from *Leishmania aethiopica*. Acta Trop. 2006; 99: 88–96. https://doi.org/10.1016/j.actatropica.2006.08.001 PMID: 16962062

86. Levick MP, Tetaud E, Fairlamb AH, Blackwell JM. Identification and characterisation of a functional peroxidoxin from *Leishmania major*. Mol Biochem Parasitol. 1998; 96: 125–137. https://doi.org/10.1016/s0166-6851(98)00122-4 PMID: 9851612

87. Webb JR, Campos-Neto A, Ovendale PJ, Martin TI, Stromberg EJ, Badaro R, et al. Human and murine immune responses to a novel *Leishmania major* recombinant protein encoded by members of a multicopy gene family. Infect Immun. 1998; 66: 3279–89. http://www.ncbi.nlm.nih.gov/pubmed/9632596 https://doi.org/10.1128/IAI.66.7.3279-3289.1998

88. Barr SD, Gedamu L. Role of peroxidoxins in *Leishmania chagasi* survival. Evidence of an enzymatic defense against nitrosative stress. J Biol Chem. 2003; 278: 10816–10823. https://doi.org/10.1074/jbc.M212990200 PMID: 12529367

89. Castro H, Rocha MI, Silva R, Oliveira F, Gomes-Alves AG, Cruz T, et al. Functional insight into the glycosomal peroxiredoxin of *Leishmania*. Acta Trop. 2020; 201: 105217. https://doi.org/10.1016/j.actatropica.2019.105217 PMID: 31605692

90. Cuervo P, De Jesus JB, Saboia-Vahia L, Mendonça-Lima L, Domont GB, Cupolillo E. Proteomic characterization of the released/secreted proteins of *Leishmania (Viannia) braziliensis* promastigotes. J Proteomics. 2009; 73: 79–92. https://doi.org/10.1016/j.jprot.2009.08.006 PMID: 19703603

91. Santarém N, Silvestre R, Cardoso L, Schallig H, Reed SG, Cordeiro-da-Silva A. Application of an improved enzyme-linked immunosorbent assay method for serological diagnosis of canine leishmaniasis. J Clin Microbiol. 2010; 48: 1866–74. https://doi.org/10.1128/JCM.02402-09 PMID: 20164286

92. Todolí F, Pérez-Filgueira M, Galindo I, Gómez-Sebastián S, Escribano JM, Rodríguez-Cortés A, et al. Seroreactivity against raw insect-derived recombinant KMPII, TRYP, and LACK *Leishmania infantum* proteins in infected dogs. Vet Parasitol. 2009; 164: 154–161. https://doi.org/10.1016/j.vetpar.2009.05.032 PMID: 19570612

93. Rodrigues MR, Santos LMO, Miyazaki CK, Martins VT, Ludolf FR, Kursancew AC, et al. Immunodiagnosis of human and canine visceral leishmaniasis using recombinant *Leishmania infantum* Prohibitin protein and a synthetic peptide containing its conformational B-cell epitope. J Immunol Methods. 2019; 474: 112641. https://doi.org/10.1016/j.jim.2019.112641 PMID: 31400411

94. Gomara M, Haro I. Synthetic peptides for the immunodiagnosis of human diseases. Curr Med Chem. 2007; 14: 531–546. https://doi.org/10.2174/092986707780059698 PMID: 17346145