Production of a kinesin-related recombinant protein (Lbk39) from *Leishmania braziliensis* by *Leishmania tarentolae* promastigotes and its application in the serodiagnosis of leishmaniasis

Lígia Moraes Barizon de Souza, Joyce Carvalho, Michelle D. Bates, Ricardo Rasmussen Petterle, Vanete Thomaz-Soccol, Paul Andrew Bates

**Introduction**

The leishmaniases are multifactorial zoonotic diseases requiring a multidisciplinary One Health approach for diagnosis and control. For leishmaniasis diagnosis, here we describe production of a new recombinant protein based on a kinesin-related gene of *Leishmania braziliensis* (Lbk39), which shows 59% amino acid identity to the *L. infantum* homologue. The Lbk39 gene was synthesized, inserted into the pLEXSY-sat2 vector and transfected into *L. tarentolae* cells by electroporation. Culturing was carried out, and the secreted recombinant protein with a C-terminal histidine tag purified using nickel affinity chromatography on the culture supernatant, yielding a final product at 0.4 mg/mL. An indirect enzyme-linked immunosorbent assay (ELISA) was standardised using sera from 74 Brazilian patients with cutaneous leishmaniasis and 11 with visceral leishmaniasis. Optimal ELISA conditions were established for the Lbk39 antigen in comparison with a crude extract from *L. braziliensis*. The sensitivity, specificity analysis and receiver operating characteristic (ROC) curve were determined with a significance level of 5%. The ROC curve showed a good accuracy with an area under curve (AUC) = 0.967, p < 0.001 (0.941–0.993) for CL patients and an AUC = 100 (100–100) for VL patients. The values of sensitivity and specificity were 88 and 98% for CL and 100 and 100% for VL, respectively. The study showed good production and expression of the target protein and has generated a potential new antigen for the diagnosis of leishmaniasis.

**Keywords:** Diagnosis, Cutaneous leishmaniasis, Visceral leishmaniasis, Recombinant protein, Kinesin, *L. braziliensis*
addition, some Leishmania antigens can drive the differentiation of T-cells that can activate B-lymphocytes to produce immunoglobulins. It is also known that antibodies are produced through neutrophil stimulation at the very beginning of the infection. Although various studies suggest that such antibodies play no role in host protection, they can be useful in diagnosis for determining the presence of the parasite [1,26].

The correct diagnosis of the leishmaniasis is performed through a combination of clinical, epidemiological and laboratory findings [37]. A range of diagnostic tools is available, but none of them are perfect [21]. Some purified recombinant antigens of various Leishmania species have been produced and used in serological assays, such as the rK39 antigen for the serodiagnosis of visceral leishmaniasis (VL) [4,15]. The rK39 antigen is a recombinant protein derived from Leishmania infantum that contains 6.5 tandem copies of a B-cell antigenic epitope composed of 39 amino acids. This antigen is related to a kinesin motor protein, which is well conserved between L. infantum and L. donovani, and the corresponding gene reveals a single open-reading frame that encodes a total of 298 amino acids with a predicted molecular mass of 32.7 kDa [10]. The Leishmania motor protein is involved in various intracellular processes and is present in the amastigote forms of many species.

Production of an antigenic protein of Leishmania, by heterologous expression of its specific epitopes in a prokaryotic system such as Escherichia coli, is a relatively straightforward technique that is both inexpensive for culturing and quick for processing the target recombinant protein. However, such systems lack eukaryotic post-translational activity, which is a significant disadvantage in producing many eukaryotic proteins. Further, high concentrations of the unfolded protein can occur, leading to a decline in effective yield, and culturing at a temperature optimal for E. coli can also reduce yields of recombinant protein and increase protein degradation [20]. The protozoan Leishmania tarentolae, which is not pathogenic to mammals, has been explored as a general eukaryotic host to develop a platform that allows complex eukaryotic protein expression at high levels, and which also has the ability to produce proteins with appropriate post-translational processing [5]. Moreover, the host is easy to manipulate and can be cultivated on a cheap medium with a 6 to 8 h doubling time. The maintenance of a transfected culture of L. tarentolae is performed under specific antibiotic selection and maintains the same level of protein expression after several months of culturing [9,21,24]. Finally, specifically with respect to this study, when the desired recombinant antigen itself is derived from a species of Leishmania use of this system maximises the probability of successful expression.

Based on what has been described above, the aim of this study was to explore the use of L. tarentolae as a host for the expression and secretion of a L. braziliensis kinesin-related recombinant protein, which was identified based on the reference kinesin-related rK39 gene of L. infantum. The diagnostic efficiency of this new antigen was evaluated by developing an indirect ELISA for leishmaniasis detection. Until now, no studies have reported on the levels of antibodies against L. braziliensis kinesin in cutaneous leishmaniasis (CL) patients.

2. Materials and methods

2.1. Serum sample collection

The patients enrolled in the study were divided into four groups, according to clinical classification (Table 1). In Group 0, 50 healthy individuals from a non-endemic area and medically examined to eliminate any previous CL infection, were used to determine the cut off for the ELISA test and the specificity. Patients with L. braziliensis, diagnosed with infection by parasite isolation and clinical examination, were classified in Group 1 (n = 74). Patients with L. infantum diagnosed by serology and PCR were classified in the Group 2 (n = 11). Patients with a positive leishmaniasis diagnosis were treated, by local service staff, in accordance with the guidelines of the Brazilian Ministry of Health, as described in the Manual of surveillance and control of American Integumentary Leishmaniasis [7,8]. Patients with Chagas disease (n = 13), confirmed by serology, were also studied to assess the possibility of cross-reactivity. Patient serum samples were stored frozen (−20 °C) before use.

This study was conducted in accordance with the International Ethical Guidelines for Biomedical Research in Human Beings. In addition, ethical approval was obtained from the Universidade Federal do Paraná Ethical Committee under number 684.244, and in accordance with the law of the Southern Common Market Treaty (Mercosur), Resolution No. 129/96.

A homology search was performed by means of BLAST similarity [2] in the TrinTrypDB database website. Sequences derived from a kinesin-related gene of L. braziliensis, henceforth called Lbk39, and comprised of 828 nucleotides (nt) were used for initial plasmid construction (Suppl. Fig. 1A). These were identified by homology with the kinesin-related gene of L. infantum - Genebank: L07879, described by Burns et al. [10], and containing 39 amino acid repeats. Lbk39 also contains a related 39 amino acid sequence (Suppl. Fig. 1B) and is also predicted to comprise immunologically dominant B-cell epitopes (BepiPred; http://www.cbs.dtu.dk/services/BepiPred/).

For expression of the target recombinant protein, the synthetic gene Lbk39 was assembled from synthetic oligonucleotides by Invitrogen (Germany), and the fragment was inserted into the pLEXSY-sat2 recombinant vector, developed by Jena Bioscience (Germany), and cloned with a 6 × His-tag into the corresponding site of the above-mentioned recombinant vector. The expression vector was designed for integration into the chromosomal 18SrRNA (ssu) locus of the parasite [9], allowing for the true expression of the eukaryotic protein; also, for this specific study, the target protein was selected to be secreted into the culture medium.

Following the construction of the Lbk39 plasmid, the One Shot™TOP10 Chemically Competent Escherichia coli strain (Invitrogen) was chosen for the plasmid cloning and propagation, and the procedure for culturing was followed according to the manufacturer’s instructions, except for the incubation temperature, which was 30 °C for plasmid stability reasons. After that, the plasmid was purified from the E. coli strain using the Geneflow Q-Spin Plasmid DNA Purification Kit and was sent for sequencing. The forward P1442 (5′-CGACGTGAAACAGGCTGTG TAG-3′) and reverse A264 (5′-CATCTATAGAGAAGTACACGTAA 3′) sequencing primers, included in the LEXSY kit, were used to confirm the plasmid identity and sequence.

Table 1

| Group | N   | Description                                      |
|-------|-----|-------------------------------------------------|
| 0     | 50  | Healthy individuals from non-endemic areas - Curitiba |
| 1     | 74  | L. braziliensis CL patients with active lesions and no treatment |
| 2     | 11  | Positive patient for visceral leishmaniasis (VL) |
| 3     | 13  | Patients with Chagas disease (CD)                |

N: number of patients in each group.

with a positive leishmaniasis diagnosis were treated, by local service staff, in accordance with the guidelines of the Brazilian Ministry of Health, as described in the Manual of surveillance and control of American Integumentary Leishmaniasis [7,8]. Patients with Chagas disease (n = 13), confirmed by serology, were also studied to assess the possibility of cross-reactivity. Patient serum samples were stored frozen (−20 °C) before use.

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2.3. Lbk39 plasmid transfection into LEXSY culture and Lbk39 LEXSY culturing

The purified and purified Lbk39 plasmid from the E. coli strain was linearised through digestion with the Swal (Smpl) enzyme, from Streptococcus milleri S - 10 U/μl (Thermo Fischer Scientific), to prepare...
for plasmid transfection into the LEXSY host L. tarentolae, according to the manufacturer’s protocol. To confirm the correct procedure for linearization and to isolate the fragment corresponding to the plasmid, 1% agarose gel-isolation of the expression cassette with an Agarose Gel Extraction Kit (Jena Bioscience) was performed according to the manufacturer’s instruction. The LEXSY strain was previously prepared for transfection according to the LEXSYcon2 Expression Kit manual (for detail see https://www.jenabioscience.com/images/ae3a4f50f1/EGE-1310.pdf). When ready for transfection through electroporation, the cultured cells were handled according to the same manual mentioned above. Other aliquots of LEXSY cells were electroporated without DNA under the same conditions as a negative control. Then the electroporated cells were transferred to tissue culture flasks containing 10-mL Brain Heart Infusion (BHI) medium supplemented with porcine hemin (Jena Bioscience) and penicillin and streptomycin (Pen-Strep, Jena Bioscience) at 26 °C in the dark under aerated conditions. As soon as the cultures became slightly turbid (24 h after electroporation), the specific Streptothricin-class of aminoglycoside antibiotic Nourseothricin (Jena Bioscience) and 1 μL/well of an antibiotic-conjugate solution of antigen diluted in a carbonate–bicarbonate buffer (pH 9.4). On the following day, the plates were washed twice with 200 μL/well of a washing solution (0.9% w/v NaCl, 0.05% v/v Tween 20), and then the wells were blocked with 120 μL of a blocking solution (PBS + 0.1% w/v casein) for 1 h at 37 °C. Afterwards, they were washed twice again with 200 μL/well of the washing solution. Following the washing step, serum samples were diluted in an incubation solution (PBS + 0.25% w/v casein) and were added in their respective wells and incubated at 37 °C for 1 h. Then the plates were washed four times with 200 μL/well of the washing solution, and a polyclonal goat anti-human IgG HRP conjugate (2 mg/mL, SanBio Scientia) was diluted and was added to each well for 1 h at 37 °C. Finally, the reaction was developed by adding 100 μL of a 10.5-mL citrate buffer (4.5% w/v Na2HPO4, 3.25% w/v citric acid, pH 5.0), with 2 mg of o-Phenylenediamine dihydrochloride (2 mg/tablet, Sigma, USA) and 2 μL of 30% (w/w) H2O2 to each well at room temperature for 15 min, avoiding light, and then 20 μL of a solution 1:20 of H2SO4 was added to stop the reaction. Plates were read in a Powerwave HT reader (BioTek) at 492 nm, and values were expressed in absorbance.

To determine whether antibodies present in human serum from uninfected individuals and those infected with CL and other diseases can be detected using Lbk39 epitopes, optimal ELISA conditions were established [14]. A range of serum dilutions (1:100, 1:200, 1:400 and 1:800), antibody-conjugate dilutions (1:5000, 1:10,000 and 1:20,000) and antigen dilutions (0.1 μg, 0.5 μg and 0.85 μg/100 μL/well) were tested in various combinations.

High-binding polystyrene microtiter plates (96 well EIA/RIA 1 × 8 Stripwell Plate, Costar, USA) were coated overnight at 4 °C with 100 μL/well solution of antigen diluted in a carbonate–bicarbonate buffer (pH 9.6). On the following day, the plates were washed twice with 200 μL/well of a washing solution (0.9% w/v NaCl, 0.05% v/v Tween 20), and then the wells were blocked with 120 μL of a blocking solution (PBS + 0.1% w/v casein) for 1 h at 37 °C. Afterwards, they were washed twice again with 200 μL/well of the washing solution. Following the washing step, serum samples were diluted in an incubation solution (PBS + 0.25% w/v casein) and were added in their respective wells and incubated at 37 °C for 1 h. Then the plates were washed four times with 200 μL/well of the washing solution, and a polyclonal goat anti-human IgG HRP conjugate (2 mg/mL, SanBio Scientia) was diluted and was added to each well for 1 h at 37 °C. Finally, the reaction was developed by adding 100 μL of a 10.5-mL citrate buffer (4.5% w/v Na2HPO4, 3.25% w/v citric acid, pH 5.0), with 2 mg of o-Phenylenediamine dihydrochloride (2 mg/tablet, Sigma, USA) and 2 μL of 30% (w/w) H2O2 to each well at room temperature for 15 min, avoiding light, and then 20 μL of a solution 1:20 of H2SO4 was added to stop the reaction. Plates were read in a Powerwave HT reader (BioTek) at 492 nm, and values were expressed in absorbance.

Soluble proteins from the crude extract of L. braziliensis promastigotes (strain MHOM/BR/84/LTB300) were included in the study as a positive control. As additional controls, the pooled positive and negative sera were included in each plate when testing individual sera; each sample was measured in triplicate, and the whole assay described above was performed in duplicate.

2.5. Statistical analysis

The receiver operating characteristic (ROC) curve was derived based on the logistic regression model, considering the classification of the samples (presence or absence of the disease) as a dependent variable and each antigen as an independent variable. Logistic regression model, ROC curve and sensitivity and specificity analyses were performed using R software [35] with an auxiliary pROC system [36]. We used analysis of variance (ANOVA) and Tukey’s test to compare the differences in absorbance between the groups. A significance level of p < 0.05 was adopted.

3. Results

3.1. Lbk39 plasmid construction

Gene synthesis using pLEXSY E.coli/L. tarentolae shuttle vectors was used to construct the Lbk39 plasmid, encoding L. braziliensis sequences homologous to the L. infantum rk39 antigen (Suppl. Fig. 1A). BLAST similarity sequence analysis of the cloned sequence confirmed it comprised a 843-bp product (828 plus 15 bp vector flanking sequences) homologous to the locus LBRI_14_1110 of L. braziliensis (strain MHOM/BR/75/2M924), and which exhibited 84% nucleotide sequence identity and 59% amino acid identity with the equivalent kinesin-related gene of L. infantum (Suppl. Fig. 1C). The predicted protein encoded a protein of 281 amino acids with a predicted molecular mass of 30 kDa, with six copies of 39 AA repeats. The cloned sequence was then inserted into the pLEXSY-sat2 vector for secretion and addition of a C-terminal 6 × His-tag (Suppl. Fig. 1B). The purification of the Lbk39 plasmid yielded 1.5 μg/μL of DNA, and plasmid identity was confirmed by sequencing the purified product, with 100% identity for both forward PI442 and reverse A264 sequencing primers.
3.2. Lbk39 plasmid transfection into Leishmania tarentolae

Linearization of the plasmid through digestion with the Swal (Smil) enzyme generated a 2.9-kbp fragment related to the E. coli part and a larger fragment (approximately 5 kbp) related to the Lbk39 plasmid (Fig. 1A). The linearized plasmid was used to transfet L. tarentolae under antibiotic selection, and after approximately 10 days of Lbk39 LEXSY culturing, the cultures became turbid (10⁷ cells/mL), and there was no noticeable growth of the parasites in the negative control flasks. On the 12th day of culturing, another passage was made, and 2 mL of that dense culture were withdrawn to perform the confirmation of Lbk39 genomic integration through PCR. For this objective, two pairs of primers were used: one of them amplifying from within the expression cassette and the other amplifying to a chromosomal flanking sequence that was not present on the plasmid. The PCR reactions resulted in two DNA fragments of different sizes, one for each pair of primers, as expected and indicated by the manufacturer: a 1.1 kbp fragment size for F3001/A1715 primers and a 2.3 kbp fragment size for F2999/F3002 (Fig. 1B).

3.3. Purification of Lbk39 protein

The Lbk39 protein was expressed as a 6xHis-tagged recombinant protein in the pLEXSY-sat2 vector, inserted into the chromosomal 18S rRNA (ssu) locus of L. tarentolae and designed to be secreted into the culturing media with predicted molecular mass of 31.2 kDa (Suppl. Fig. 1B). The purification procedure and the protein expression were confirmed by SDS-PAGE, with the purified recombinant protein exhibiting a molecular mass of approximately 35 kDa (Fig. 2). The purified Lbk39 recombinant protein was obtained at a final concentration of 0.4 mg/mL.

3.4. Enzyme-linked immunosorbent assay (ELISA)

An indirect ELISA was developed and standardised using the recombinant protein as an antigen for detection of specific anti-Leishmania antibodies in the sera of leishmaniasis patients. The optimum combination of conditions were found to be as follows: antigen concentration of 0.1 μg/100 μL/well; serum samples diluted to 1:200 in incubation solution (PBS + 0.25% w/v casein); and polyclonal goat anti-human IgG HRP conjugate (2 mg/mL) diluted to 1:10,000.

The presence of anti-Leishmania antibodies was determined by comparing antibody levels in patients infected with CL and VL with healthy individuals living in the same endemic area, as well as individuals with Chagas disease, using the Lbk39 recombinant protein as the antigen. The results obtained were compared using the same parameters and the same sample groups using as a positive control antigen a crude extract of L. braziliensis promastigotes. Comparing the two types of Leishmania antigen for patients known to have leishmaniasis, the Lbk39 antigen showed sensitivity of 88% for CL and of 100% for VL patients. The specificity was 98% and 100%, respectively (Table 2 and Fig. 3). Based on the percentage positivity for all serum samples and sensitivity and specificity values, the Lbk39 antigen was able to detect antibodies from both CL and VL patients. The results obtained with the Lbk39 antigen were equivalent to or better than using crude promastigote extract, except for a small reduction in specificity for CL patients (98% versus 100%).

The range of absorbance readings obtained in these assays are shown in Fig. 4. Using the crude antigen, the range of readings obtained was high with the CL patients (Group 1), otherwise the readings were well grouped. However, the crude antigen also gave high readings with patients diagnosed with Chagas disease (Group 3). With the recombinant Lbk39 antigen the cross-reactivity with Chagas patient sera was much reduced, and the readings with the CL patient group were more tightly grouped. Even so the median value for the Chagas patients (Group 3) was still higher than that for CL patients. However, a wider range was found in the VL group using the Lbk39 antigen. ANOVA indicated significant differences between the means of the various groups for both antigens. Pairwise comparisons using Tukey’s test showed these differences were significant (p < 0.05) in all cases except between Groups 1 and 2 (CL and VL patients) with L. braziliensis crude antigen, and between Groups 1 and 3 (CL and Chagas patients) with Lbk39. In all cases the control patients (Group 0) were significantly different to all infection groups.
4. Discussion

Several recombinant proteins have been investigated for their anti-
*Leishmania* antibody responses in patients in attempts to develop the
most suitable antigens for diagnostic purposes, of which the best so far
is the rK39 antigen for VL [4]. However, although recombinant proteins
have been extensively used for specific antibody detection, their use in
diagnostic tests has revealed some problems. For example, they can be
less immunoreactive than the corresponding purified antigen due to the
absence of post-translational modifications, depending on the protein
expression system. Further, their production in high quality and
quantity is almost always laborious and they can be expensive to pro-
duce. Here we explored the use of the pLEXSY/*Leishmania tarentolae*
system for cloning, transfection and recombinant protein production for
leishmaniasis immunodiagnosis, in particular for the diagnosis of
*L. braziliensis* infection.

We designed a sequence based on *L. braziliensis* to generate a pro-
duct that we named Lbk39, which exhibited 59% amino acid identity to
the kinesin-related gene of *L. infantum* (rK39) that is already used in the
serodiagnosis of VL. The Lbk39 sequence was synthesized, inserted into
pLEXSY-sat2 recombinant vector and cloned with a 6×His-tag, then
inserted into the chromosomal 18SrRNA (*ssu*) locus of *L. tarentolae*
and selected to be secreted into the culturing media. Successful expression
of a ~35 kDa protein was achieved, correlating with the predicted
molecular mass of the 289 amino acid 31.2 kDa recombinant protein
(Suppl. Fig. 1B). Therefore, we can now add Lbk39 to the wide range of
proteins that can be expressed in the *L. tarentolae* system [24]. Re-
dcombinant protein production via large-scale fermentation of *L. tar-
etolae* is not expensive and allows yields of 0.1 to 5 mg/L to be
achieved [5,22,23,30]. As used in our study, improved expression is
also obtained if the target gene is followed by the 3' UTR (intergenic
untranslated regions) from a highly expressed gene, because in trypa-
osomatisds the regulation of protein expression generally occurs by a
post-transcriptional process involving the UTRs as in present work
[16,31,39]. Expression and purification of the target antigen in the
current study yielded recombinant Lbk39 antigen at 0.400 mg/mL.

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Table 2
|     | CL |       |     | VL |       |
|-----|----|-------|-----|----|-------|
|     |    | Sensitivity (%) | Specificity (%) | Sensitivity (%) | Specificity (%) |
| *L. braziliensis* | 70 | 100   | 90  | 100 |
| Lbk39 | 88 | 98    | 100 | 100 |

*L. braziliensis*: soluble proteins from crude extract from *Leishmania (Viannia) braziliensis* culture (strain MHOM/BR/84/LTB300.)
Lbk39 was evaluated for the serodiagnosis of CL due to *L. braziliensis* and showed 88% sensitivity and 98% specificity, compared to 98% sensitivity and 100% specificity in VL patients. Absorbance values ranged between 0.46 and 0.016 for CL and between 0.92 and 0.03 for VL patients. The difference between positive and negative sera was similar (30 and 28.4 times for VL and CL, respectively). These findings show that, whilst the target protein was produced from a kinesin-related gene of *L. braziliensis* and performed well with *L. braziliensis* patients, antibodies from patients with VL (*L. infantum*) were also able to recognize Lbk39. The recombinant protein was also recognised by sera from Chagas patients. These are interesting results, however, they do not compromise the use of Lbk39 for serodiagnosis of CL as the other clinical features of infection in VL or Chagas disease are quite different. In fact, given the difficulty in making a positive diagnosis for CL, it may be an advantage and facilitate the usage of Lbk39 as a general immunodiagnostic antigen for both VL and CL, since the main issue is a lack of existing tools for the latter. A greater sampling of patients with cutaneous leishmaniases coming from different regions of Latin America will be needed to confirm if this is a useful approach. The cross-reactivity between the CL and VL patients likely arises from two factors, the first of which is the conservation between Lbk39 and the homologue from *L. infantum* (Suppl. Fig. 1), which, while only 59% at the amino-acid level, is concentrated in several immunogenic repeat motifs. The second factor is the very high antibody response stimulated by VL infection compared to CL, which also explains the relatively high values seen in the ELISA results for VL sera with Lbk39, and the high sensitivity.

The sensitivity and specificity of a leishmaniasis immunodiagnostic test is influenced by various factors such as antigenic and structural properties of the antigen itself, the duration of the infection, number of lesions, and variation in the parasite and host population. Regarding properties of the antigen, although no previous studies have investigated the levels of antibodies against *L. braziliensis* kinesin-related proteins in CL and VL patients until this study, a few have investigated the reaction of antibodies from CL patients against *L. infantum* kinesin-related recombinant proteins. For example, Molinet et al. [28] found that all of 272 serum samples from patients with CL in Brazil were negative when using one commercially available rK39 rapid test. Hartzell et al. [18] observed that both the rK39 rapid test and ELISA using the rK39 antigen demonstrated a positivity of only 10.2 and 28.8%, respectively, in United States soldiers stationed in Afghanistan and Iraq who had contracted CL (mostly due to *L. major*). Likewise, Oliveira et al. [32] evaluated several recombinant antigens that demonstrated the ability to identify *Leishmania infantum*-infected patients and found that CL patients were generally less well identified. Interestingly, only 3 out of 26 CL patients showed a positive result using an antigen that encoded a C-terminal fragment of an *L. infantum* kinesin. One potentially important difference to the current study is that the recombinant antigens described by Oliveira et al. were expressed in *E. coli*, perhaps compromising their sensitivity for CL diagnosis. According to Moreno et al. [29], the high titres of the anti-rK39 antibody in patients with acute VL are explained by expression in the high number of amastigotes present, compared to asymptomatic patients that have lower numbers of amastigotes. However, here we show that Lbk39 is capable of detecting antibodies in CL patients, which also have low numbers of amastigotes. Another factor to consider is the potential diversity of the diagnostic antigen. In that regard, Bhattacharyya et al. [6] showed that there is diversity in rK39 sequences between *L. infantum* and *L. donovani*, which may explain the poorer performance of rK39 in diagnosis of East African VL due to *L. donovani*. Therefore, potential diversity in Lbk39 should also be investigated in further studies [13]. Finally, the reasons why kinesin-related cytoskeletal proteins...
have been found to be good antigens for serodiagnosis is not fully understood but presumably is related to their structure, as they contain repetitive amino acid sequences thus presumably providing multiple stimulation of antibody responses.

Regarding other factors that affect sensitivity and specificity in the current context, duration of the infection is significant. In patients with recent lesions (1 to 6 months of progression), serological negativity is higher, and parasitological tests are more sensitive and specific [12]. Also, in the case of positive serology, the mean titres are significantly higher in patients with multiple lesions, reflecting the higher antigenicity induced by a larger number of parasites. Even within the same species, genetic variability of responses can be high and the antigen used for serological testing can give different results. For example, the rK39 antigen used to detect VL antibodies in several regions of the world, shows variable sensitivity and specificity according to geographical region and ethnic groups [38,27]. Different host immune responses may also be responsible for the variability of serological test results. Goto et al. [17] analysed antibody responses to the rK39 antigen in humans and dogs with VL, and they noticed that humans showed much stronger immune responses to the rK39 antigen than dogs, concluding that the rK39 recombinant antigen is very specific towards detecting VL in humans only. However, Porrozzi et al. [33] revealed that the IgG response to the rK39 antigen was variable in asymptomatic dogs (sensitivity of 66%) and significantly higher in symptomatic dogs (sensitivity of 100%). Likewise, 33% of L. braziliensis-infected dogs were positive for the rK39, and 11% of dogs with leptospirosis were also positive showing cross-reactivity. For intervention programmes of leishmaniasis in humans and dogs, an ideal serodiagnostic test must be able to identify infected and non-infected reservoirs, specifically in dogs [34], thus providing the possibility of guided control and treatment.

In summary, this study showed that the recombinant Lb39 protein produced was able to recognize Leishmania infection in the serum of humans with cutaneous or visceral leishmaniasis in Brazil. This is a particularly important result for the L. braziliensis CL patients, where there is a lack of serological tests. Further work is required to investigate the potential use of this antigen in different population sera, as well as in different geographical regions in order to determine the specific ability to detect anti-Leishmania antibody levels in patients with CL or VL. In addition, analysis of the response to Lb39 by sera of dogs infected with L. infantum should be considered in order to determine how the antigen behaves in different hosts.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.omelet.2019.100111.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

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Appendix A. Supplementary data

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