

Contribution to the Knowledge of Feeding Habbits of *Lutzomyia* (*Lutzomyia*) *Longipalpis* (Lutz & Neiva, 1912) in Association to Natural Infection by *Leishmania* (*Leishmania*) *Infantum Chagasi* (Cunha & Chagas, 1937)

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Abstract

Background: Brazil faces the expansion and urbanization of American Visceral Leishmaniasis. The presence of the vector in the urban area is one of the major challenges of the Brazilian Program for Control of Visceral Leishmaniasis, which refers the need for a better understanding the behavior of *Lutzomyia longipalpis*, as well as the factors of its adaptation to new habitats. The aim of the study was to combine diagnostic molecular tools capable to identifying natural infection by *Leishmania (L.) infantum chagasi* and the origin of food sources in *L. longipalpis*.

Methods: The specimens were captured in the municipalities of Araguaína/Tocantins, Fortaleza/Ceará, Sobral/Ceará, and Rio de Janeiro/Rio de Janeiro. Molecular diagnoses were performed through Polymerase Chain Reaction using primers that amplify kinetoplast deoxyribonucleic acid in *Leishmania* sp. and the cacophony gene in phlebotomines for the diagnosis of natural infection, primers that amplify the cytochrome b gene, in addition to the sequencing technique, for the study of alimentary habit.

Results: Among the analyzed females, 28.6% were diagnosed in the food evaluation. Among the total of 141 samples that were analyzed, showed a higher positivity for the human food source (62.5%), followed by dogs (27.5%) and birds (10%). In Araguaína, the samples showed positivity for human blood (61.5%) and dogs (38.5%). In Fortaleza and Sobral, specimens showed a positive percentage for human blood (54.5% and 66.7%), followed by dogs (27.3% and 20.0%) and birds (18.2% and 13.3%). In the females of Rio de Janeiro was detected exclusive feeding of human blood. In the natural infection, a general index of positivity was obtained equal to 4.3%. When associated, detection of the power source and natural infection, two infected specimens were found in Araguaína, with dog and human feeding; and in Sobral, 3 specimens infected and positive for human blood.

Conclusions: The results corroborate with studies that demonstrate the eclecticism and food opportunism of *L. longipalpis*, as well as their occurrence in several environments, which are determinant factors of the important process of domiciliary vectorization. The low positivity feeding on human blood may suggest that other mammals, possibly rodents, would be acting as a food source.

Background

In a global setting, leishmaniasis is among the most neglected diseases [1]. Leishmaniasis are emerging endemic in Brazil, being the country with the highest prevalence of those diseases in the Americas [2,3].

In the context of the expansion and the urbanization of American Visceral Leishmaniasis (AVL), with a high case fatality rate, Brazil has not been successful in actions to combat the vector, although it has a well elaborated planning with well defined policies of surveillance and control. It is hypothesized that the presence of the vector in the urban area is one of the major challenges for the Brazilian Visceral Leishmaniasis Control Program, which clearly points to the need to better understand the behavior of *Lutzomyia (Lutzomyia) longipalpis*, in the urban area and the factors of its adaptation to the new habitats, which is unequivocal proof of its ecological valence.

The knowledge of the eating habits of phlebotomines, which are leishmaniasis vectors, can help in the use of more adequate and effective measures for their control, thus prompting the understanding of their adaptation to affected environments and the acceptance of different dietary sources, which are probable determinants of LVA expansion [4-17].

The present study aims to associate molecular diagnostic tools capable of identifying natural infection by *Leishmania (Leishmania) infantum chagasi* and the origin of the food source in *L. (L.) longipalpis* specimens from municipalities with different AVL transmission profiles: Araguaína/Tocantins (intense transmission area, urban profile), Fortaleza/Ceará (intense transmission area, urban profile), Sobral/Ceará (intense transmission area, rural profile), and Rio de Janeiro/Rio de Janeiro sporadic transmission, urban profile). It is worth mentioning that the focus on the locality of Caju, a port area, is the first urban focus of the city of Rio de Janeiro. Finally, we intend to define and better elucidate aspects concerning the epidemiology of AVL in different transmission areas with different epidemiological profiles in Brazil.

Methodology

Municipalities of the origin of the specimens. The municipalities were chosen according to the categorization of AVL transmission and epidemiological profile [3] (Figures 1).

Catch stations. The catch sites were defined considering Probable Infection Sites and sites whose entomological survey had previously identified the presence of *L. (L.) longipalpis*. Phlebotomines were captured using modified CDC (Centers for Disease Control) light traps [18] (HP model, Hoover Pugedo) [19]. The traps were randomly installed from 6:00 p.m. to 6:00 a.m., in the peridomiciliary environment during the years of 2013 and 2016, in periods which were favorable to productive catches. The specimens were identified according to the Galati criteria [20]. Females from all municipalities, containing blood residues and males (used as negative control), were individualized in eppendorf tubes and stored in freezer -200C, with the exception of one sample from the city of Rio de Janeiro, which was analyzed as a pool (10 copies).

Molecular analyses

Obtaining total extract. The samples were analyzed in 100 µl of lysis buffer (10 mM Tris-HCl pH 9.2 containing 10 mM EDTA and 100 µg/ml proteinase K) and stored (-20 ° C) until extraction of the total deoxyribonucleic acid (DNA). DNA Extraction DNA extraction was performed from the lysates using the Wizard SV Genomic DNA Purification System (PROMEGA) commercial kit, according to the manufacturer's specifications. All the steps for DNA extraction were monitored by the inclusion of samples for negative control (male insects captured in the field). Such samples determine the control to possible contaminations, since they do not present DNA of *Leishmania* spp. The laminar fluxes proper for DNA extraction, as well as all the material used during DNA extraction, were properly decontaminated with the use of chlorine and exposure to ultraviolet rays [21,22].

Diagnosis of Natural Infection. For the analysis of natural infection, the Polymerase Chain Reaction (PCR) technique was utilized, where a multiplex assay was used [21] via two pairs of primers that amplified simultaneously. A first 120 bp product for *Leishmania* kDNA (samples of positive females) and another of 220 bp corresponding to phlebotomine DNA (samples of males and females). The first pair amplifies the constant region of the mini-circle of kDNA of the genus *Leishmania*; primer A [5'GGC CCA TAC TAC ACC AAC CCC 3'] and primer B [5'GGG GTA GGG GCG TTC TGC GAA 3'] [23]; the second pair amplifies a specific constituent gene of phlebotomines (cacophony): 5Ll₁cac [5'GTG GCC GAA CAT AAT GTT AG 3'] and 3Ll₁cac [5'CCA CGA ACA AGT TCA ACA TC 3'] [24]. The amplified products were visualized by 2% agarose gel electrophoresis stained with Nancy. Dot-Blot. The amplified product (positive samples) in the PCR was also analyzed by solid phase hybridization with 25 pmol/μL of the subgenus-specific or species-specific probe labeled with 5'-biotin for *Leishmania (L.) infantum chagasi*, primer [5'AAAAATGGGTGCAGAAAT3'] (Table 1). The hybridization reaction was developed with chemiluminescence solution.

Analysis of the Eating Habits. To evaluate eating habits, only engorged females or blood residues were analyzed, where five pairs of primers were used [25,26] for *Homo sapiens* (first A: 5'ATACGCAAATTAACCCCCTAATAA 3' and first B: 5'ATG TTT CAG GCT TCT GAG TAG AGA A 3'), *Canis lupus familiaris* (first A: 5'CTA ACA TCT CTG CTT GAT GGA ACT T3' and primer B: 5'TGC GAA TAA TAG TAC AAT TCC AAT G 3'), *Didelphis albiventris* (first A: 5'TAT GCC TAA TTA TTA TCC AAA TC 3' and primer B: 5'AAA GCC ACC CTA ACC CGA TT 3') and *Gallus gallus* (primer A: 5'AATTAACAACCTCCCTAATCGACCTC 3' and primer B: 5'TGTGAAGGAAGATACAGATGAAGAA 3'). The choice of targets was based on the animals that have some relation with the epidemiology of AVL; birds, because it is known that coops near houses act as an attractive for phlebotomines, as well as functioning as breeding grounds, which facilitates the domiciliation of the vector [27]; dogs, which are considered important domestic reservoirs [17] and the opossums suggested as possible reservoirs [2], and in addition to these, humans. Primers that amplify a region of the cytochrome b (cyt b) gene were also used for all vertebrates: 3'CCC CTC AGA ATG ATA TTT GTC CTC 5' and 3'CCA TCC AAC ATC TCA GCA TGA TGA AA 5' (Table 1).

Sequencing - Given the importance of the first urban focus in the city of Rio de Janeiro (Porto area), a new methodology was applied to part of its samples (total of seven individualized samples and one pooled sample). The products obtained for the cyt b gene, obtained by the above methodology, were purified using the Wizard SV PCR Clean-up System kit (Promega), then sequenced with the same primers used for PCR. Sequencing was performed in an automatic sequencer (ABI PRISM® BigDye™ Terminator Cycle Sequencing) at Oswaldo Cruz Foundation (IOC) (Genomic Platform - DNA sequencing, PDTIS-FIOCRUZ). The sequences obtained were aligned and compared with those already deposited in the NCBI nucleotide database (<http://blast.ncbi.nlm.nih.gov/Blast>).

Results

Identification of eating habits

From the total of *L. (L.) longipalpis* females tested (141), a general positivity index of 29.3% was obtained, with the municipality of Sobral having the highest index, 36.6%. In none of the localities multiple feeds were observed (Table 2).

The different populations of *L. (L.) longipalpis* presented a constant pattern of blood tests in the digestive tract, with a higher positivity for human blood (63.4%), followed by dogs (26.8%) and birds (9.8%); no females fed on opossum were found (Table 3, Fig. 2).

In Araguaína, the samples showed positivity for human blood (61.5%), followed by dogs (38.5%).

The samples from Ceará, Fortaleza and Sobral presented a higher percentage of positivity for human blood (54.5% and 66.7%), followed by dogs (27.3% and 20.0%) and birds (18.2% and 13.3%) (Table 3).

From the positive samples for the city of Rio de Janeiro, only positive results were obtained for human blood (100%). These samples had their results sequenced, one sample analyzed individually (identity index equal to 96%, Query Cover value of 94% and Accession number: KX697544.1), and one sample in the form of a pool equal to 98%, Query Cover value of 97% and Accession number: KX697544.1), although in the pool there were 10 females for evaluations and in order to avoid any false positive result in the pool, only one female was considered positive (Table 3).

Identification of the natural infection by *Leishmania (Leishmania) infantum chagasi*

One of the analyzed specimens was obtained with a general index of positivity of 4.3%. A total of 6 positive specimens were found, with a positive index of 4.9% in Araguaína and 9.8% in Sobral (Table 4, Figs. 3, 4). In the municipalities of Fortaleza and Rio de Janeiro, no positive females were detected for *L. (L.) infantum chagasi*.

Association between identification of eating habits and natural infection

In 141 female *L. (L.) longipalpis*, in 5 (3.6%) specimens, natural infection and the food source were identified, four of which were fed in human blood and one in dog blood (Table 5).

In the municipality of Araguaína, a *L. (L.) longipalpis* female was found infected with *L. (L.) infantum chagasi* fed on dog blood and a female fed on human blood. In the municipality of Sobral, 3 females were naturally infected and fed on human blood, and one female with unidentified food source (Table 5).

Discussion

Several methods are used to identify eating habits in phlebotomines, such as immunological methods, through the precipitin technique [4, 8, 11, 28–30] and immunoenzymatic tests [7, 9, 15, 31–32]; and recently molecular methods using PCR [33–42]. Studies that seek to determine the dietary pattern of phlebotomines are of great ecological and epidemiological relevance, since they may at the same time

increase the knowledge about the habits of this vector, but mainly suggest potential reservoirs of *Leishmania* spp., which can help in the planning of better strategies [11, 33, 37, 40].

The literature has recorded, on the basis of field work, the eclecticism of *L. (L.) longipalpis* in feeding on a wide range of mammals, including dogs, pigs, horses, cattle, and birds [17, 43]. In addition to the favorable environmental conditions, the abundance of food sources is a determinant factor in the population growth of the vector, especially in periurban areas, which brings it closer to humans.

The data from the present study revealed that the specimens of the populations of Araguaína, Fortaleza and Sobral, areas that were classified as intensive transmission for AVL, fed mainly on humans. The anthropophilia of *L. (L.) longipalpis* has already been described in several studies [12, 17, 43], being one of the essential criteria for the species to be incriminated as a vector [44]. In Ceará we have already described the attraction of *L. (L.) longipalpis* by humans, including individuals and populations of the vector originated from Massapê, through the ELISA test proving the ingestion of human blood [15].

Research in Campo Grande (Mato Grosso do Sul) and Marajó Island (Pará) shows the high attraction of the vector by humans [7, 14]. However, investigations in Colombia and Araçatuba (São Paulo) have suggested low anthropophilia [28, 29]. The anthropophilia of *L. (L.) longipalpis* observed in the populations of Araguaína, Fortaleza and Sobral, certainly plays a relevant role in the classification of intense transmission to AVL, which is attributed to these municipalities. On the other hand, the data so far do not suggest that the populations of the different biomes (Cerrado and Caatinga) have different habits regarding the attraction to humans. The fact is that the species is highly anthropophilic, but due to its opportunistic character, characteristic of some species of phlebotomines, one can not expect the same pattern of feeding.

In Araguaína, the feeding of the analyzed specimens showed the attraction to dogs, as observed in the populations of, Fortaleza and Sobral, which are areas that present a high incidence of canine visceral leishmaniasis. In the 1950s, in Sobral [43], dogs had already been suggested as important domestic reservoirs of *L. (L.) infantum chagasi* and as sources of infection for *L. (L.) longipalpis*, in the home environment; in Araçatuba (São Paulo), the highest percentage of canine blood female feeding was observed, correlating its epidemiological role in the AVL transmission chain, with the hypothesis that canine enzootia precedes human transmission [30]. In other countries of South America, such as Colombia, in an area endemic to AVL, the population of *L. (L.) longipalpis* showed a strong attraction to dogs [28]. Studies in Montes Claros (Minas Gerais) shows that even with the adoption of euthanasia for seropositive AVL dogs, the canine population will be continue serving as a source of *Leishmania* sp. infection for sand flies [45]. The data obtained in this study confirm the importance of dogs in the maintenance of the AVL transmission.

However, it is possible to observe a weak attraction of *L. (L.) longipalpis* to birds, different from the analyzes that demonstrate opposite behavior, in Northeast areas [15] and in Raposo (Maranhão) [14]. It is well known that coops near houses act as attractive for phlebotomines [43], which serve as breeding grounds, since immature forms develop in soil that is rich in organic matter. In this context, the contact

between vectors and humans increases, besides allowing the domiciliation of the vector, and consequently the transmission of AVL within the home, a situation that is observed both in the rural area and in the periphery of the cities [12, 17, 27]. In terms of environmental determinants of occurrence of AVL transmission in the home environment, the maintenance of coops near homes increases the risk of human exposure to the phlebotomine vector [40].

The analyses of the present study did not reveal positivity of *L. (L.) longipalpis* to opossum blood, a synanthropic animal, which was suggested as a reservoir of *L. (L.) infantum chagasi*, as already observed in populations of the vector from Sobral (Ceará), Massapê (Ceará) and Jequié (Bahia), Brazil [15] and Colombia [28]. Some studies have reported the natural infection of *Didelphis marsupialis*, by *Leishmania* spp. possibly *L. (L.) infantum chagasi*, thus discussing the role of these mammals as potential reservoirs for AVL [2, 46, 47].

Literature has demonstrated that *L. (L.) longipalpis* is an eclectic, and opportunistic, species in the search for food sources [7, 12, 14, 15, 28–30]. Thus, the non-positivity in the general context of this study, about 71% of the analyzed specimens, may have a direct relation with the chosen targets. In the catch areas, besides the sources tested (humans, dogs, birds and opossums), it was possible to observe other potential sources of food, such as pigs, cats, oxen, horses, rodents, goats, sheep and foxes. Another possibility is the viability of DNA. Studies indicate that molecular assays are able to detect host DNA only within 5 days after the blood supply [33, 34, 40].

In relation to the other females that were fed, where it was not possible to detect the food source, probably other animals may be involved in the maintenance of the local population of *L. (L.) longipalpis*. Rodents must be taken into account, which are frequently observed during catches in areas of work and in the home environment. *Rattus norvegicus* and *Rattus Rattus* were found to be infected with *L. infantum* (= *L. (infantum chagasi)*), with analysis using *Leishmania* Nested PCR and hsp70 PCR-RFLP, suggesting the possibility of participation of this rodent in the zoonotic cycle of the LVA [48, 49].

From the analyses performed with the few (13) copies from Rio de Janeiro (RJ), one specimen was detected with human blood DNA. This fact corroborates with the findings that already indicated the local transmission, demonstrating the first urban focus of the city in the Bairro do Caju; the autochthonous human case, canine cases due to visceral leishmaniasis and vector findings (*L. (L.) longipalpis*) [50–53].

New studies should be done, seeking to better understand the ecoepidemiological dynamics of LVA in the city of Rio de Janeiro. The literature suggests that this recent (and first) urban focus has been installed from passive vector dispersion, due to the transfer of used soil to the flower pots of the Caju Cemetery, from presence of *L. (L.) longipalpis* vector [54]. On the other hand, it can be assumed that in the area there was already the presence of the vector, a population that was in equilibrium and well adapted to the dens of rodents, which are very common in the cemetery. The canine transmission occurred by the introduction of an infected animal, because in the area adjacent to the cemetery there was a kennel, which triggered the local transmission to a considerable number of dogs.

The rate of naturally infected phlebotomines in endemic areas as well as the correct identification of the *Leishmania* species are of great importance for a better understanding of the epidemiology of leishmaniasis. The classic methodology, used until then, consists of the direct search of the parasite by microscopy, through dissection of the digestive tract, followed by microscopic analysis, isolation and culture of parasites, with subsequent identification/diagnosis of the species [55–58]. Although it is considered a "gold standard" technique, in some cases it is very laborious and unfeasible, given the amount of material to be analyzed, time and specific training for this process, sometimes rendering the technique imprecise and not always allowing the isolation and the correct identification of the parasite.

Diagnostic assays using PCR have been developed based on different molecular targets such as ribosome minor subunit genes, mini-exon gene spacer sequences, single-copy nuclear sequences such as the DNA polymerase alpha gene, but the minicircle kDNA are the ones that present the highest sensitivity for detection of *Leishmania* [22, 58–63].

In the present study, the molecular analyses were performed individually, since the real scenario of infection rate and of identification of the food source was searched. When working with a female pool, some information may be lost as it would not be possible to detect the exact amount of positive females (for infection or food source analysis). In addition, we sought to associate the two approaches, food source and natural infection with *Leishmania* sp, in view of this association, individual analysis became mandatory. When parasite load analyses are performed on phlebotomines, one should consider the possibility that parasitic load may be different in each infected female, and the level of individual infection may interfere with the total pool load. Thus, if a sample showed a high parasitic load, this may be due to one or more infected phlebotomines [64]. In Camaçari (Salvador) was performed a compared study in diferents periods of captures from *L. longipalpis*, the results of parasite load was low and did not vary regardless of the season, despite the number of collected sand flies [65].

In this study, it was possible to detect the natural infection of *L. (L.) longipalpis* by *L. (L.) infantum chagasi* in Araguaína and Sobral, which are areas of intense transmission of AVL, with rates of 4.9% and 9, 8% respectively. In Fortaleza, a minimum natural infection rate of 3.7% was detected [61]. These results reinforce the practicality of the use of molecular techniques in the identification of natural infection, since it is known that infection rates are low in nature, even in areas endemic for AVL.

It was also possible to carry out the association between food identification and the detection of natural infection by *L. (L.) infantum chagasi* in 5 specimens; four specimens of *L. (L.) longipalpis* performed blood meal in human, and one in dog, the latter with its role already widely discussed in the transmission of AVL [2, 12, 43]. The non-identification of the source of the food source in a female *L. (L.) longipalpis*, from Sobral, corroborates the idea that, possibly, other mammals (in this untested study) can serve as food sources for well-known eclectic phlebotomines.

Conclusions

The presence of *L. (L.) longipalpis* in the urban area is one of the major challenges for the Brazilian Program for Control of Visceral Leishmaniasis. Studies that contribute to the knowledge of the rate of naturally infected phlebotomines, the correct identification of phlebotomines infected by *L. (L.) infantum chagasi*, and the analysis of the vector food identification, can help to understand the behavior of *L. (L.) longipalpis* in urban areas, as well as their adaptation factors to the new habitats, besides helping to elucidate the expansion of AVL in the Brazilian territory, which is clearly evidenced in the South region. The results generated in this study may contribute to the strategies of more effective controls, besides reinforcing the pepticity of the vector as an important aspect associated with its domiciliation.

Abbreviations

Deoxyribonucleic Acid

DNA

Centers for Disease Control

CDC

Declarations

Ethics approval and consent to participate

Not applicable for that section.

Consent for publication

Not applicable for that section.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Idealization and design of the experiments: MMSA, DPP, EFR. Sampling: MMSA. Interpretation of the experiments: MMSA, DPP. Interpretation of data: MMSA, SAMC, EFR. Writing the manuscript: MMSA, EFR. All authors read and approved the final version of the manuscript.

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Tables

Table 1. Primers used for food source detection and natural infection in *Lutzomyia (Lutzomyia) longipalpis* specimens from Sobral / CE, Fortaleza / CE, Araguaína / TO, Rio de Janeiro / RJ.

Targets	Primers	Temperature
Homo sapiens	ATACGCAAATTAACCCCCTAATAA ATG TTT CAG GCT TCT GAG TAG AGA A	60 °C
Canis familiaris	CTA ACA TCT CTG CTT GAT GGA ACT T TGC GAA TAA TAG TAC AAT TCC AAT G	60 °C
Gallus gallus	AATTAACAACCTCCCTAATCGACCTC TGTGAAGGAAGATACAGATGAAGAA	60 °C
Didelphis sp.	ATT GCA AAA CAC ATC CAC TAG TTT TCT AGT ATT CCT GT	50 °C
Lutzomyia sp. (cacophony)	GTG GCC GAA CAT AAT GTT AG CCA CGA ACA AGT TCA ACA TC	55 °C
Leishmania sp.	(G/C)(G/C)(C/G) CC(A/C) CTA T(A/T)T TAC ACC AAC CCC GGG GTA GGG GCG TTC TGC GAA	55 °C
Leishmania (L.) chagasi	AAAAATGGGTGCAGAAAT	55 °C
cyt b	CCC CTC AGA ATG ATA TTT GTC CTC A CCA TCC AAC ATC TCA GCA TGA TGA AA'	

Table 2. Identification of sources detection in *Lutzomyia (Lutzomyia) longipalpis* females, using the PCR technique.

Municipality	Number of females		
	Females tested	Positive	Negative
N (%)	N (%)		
Araguaína – TO	41	13 (31.7%)	28 (68.3%)
Fortaleza – CE	45	11 (24.4%)	34 (75.6%)
Rio de Janeiro – RJ	14	2 (14.3%)	12 (85.7%)
Sobral – CE	41	15 (36.6%)	26 (63.4%)
Total	141	41	100
%		29.3%	70.9%

Table 3. Distribution of food source types in *Lutzomyia (Lutzomyia) longipalpis* females, by PCR, in the studied areas.

Positives samples	Bird	Dog	Opossum	Human
Araguaína – TO				
Total	NR	5	NR	8
%	-	38.5%	-	61.5%
Fortaleza – CE				
Total	2	3	NR	6
%	18.2%	27.3%	-	54.5%
Rio de Janeiro – RJ				
Total	NR	NR	NR	2
%	-	-	-	100%
Sobral				
Total	2	3	NR	10
%	13.3%	20.0%	-	66.7%
Total of samples	4	11	NR	26
%	9.8%	26.8%	-	63.4%
NR – not reactive				

Table 4. Female of *Lutzomyia* (*Lutzomyia*) *longipalpis* submitted to the search for natural infection by *Leishmania* (*Leishmania*) *infantum chagasi*

Municipality	Positive N (%)	Negative N	Total N
Araguaína – TO	2 (4.9%)	39	41
Fortaleza – CE	0 (0%)	45	45
Rio de Janeiro – RJ	0 (0%)	13	14
Sobral – CE	4 (9.8%)	37	41
Total of samples	6 (4.3%)	134	141

Table 5. Association of identification of natural infection by *Leishmania* (*Leishmania*) *infantum chagasi* and food source detection in *Lutzomyia* (*Lutzomyia*) *longipalpis* females.

Municipality	N	Dog	Human	Unidentified Food Source
Araguaína – TO	2	1	1	-
Fortaleza – CE	-	-	-	-
Rio de Janeiro – RJ	-	-	-	-
Sobral – CE	4	-	3	1
N	6	1	4	1

Figures



Figure 1

Capture sites of *Lutzomyia (Lutzomyia) longipalpis*, Sobral / CE, Fortaleza / CE, Araguaína / TO, Rio de Janeiro / RJ, from 2013 to 2016. Credits: Bruno M Carvalho, Margarete MS Afonso.

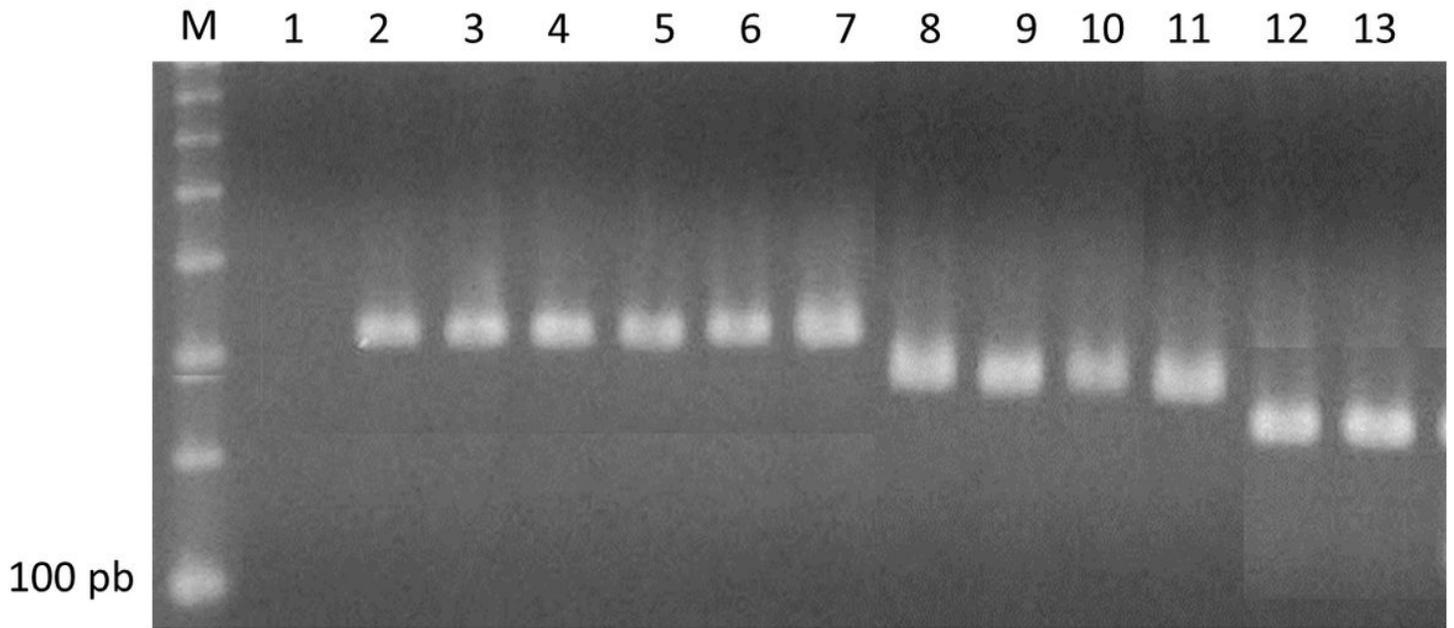


Figure 2

Result of food source detection of *Lutzomyia (Lutzomyia) longipalpis* females, in the worked areas. 1.5% agarose gel electrophoresis. M - Molecular weight marker, 1 - negative reaction control, 2 - 6 positive samples for human, 7 - positive human control, 8 - 10 positive samples for dog, 11 - positive dog control, 12 - positive bird sample, 13 - positive bird control.

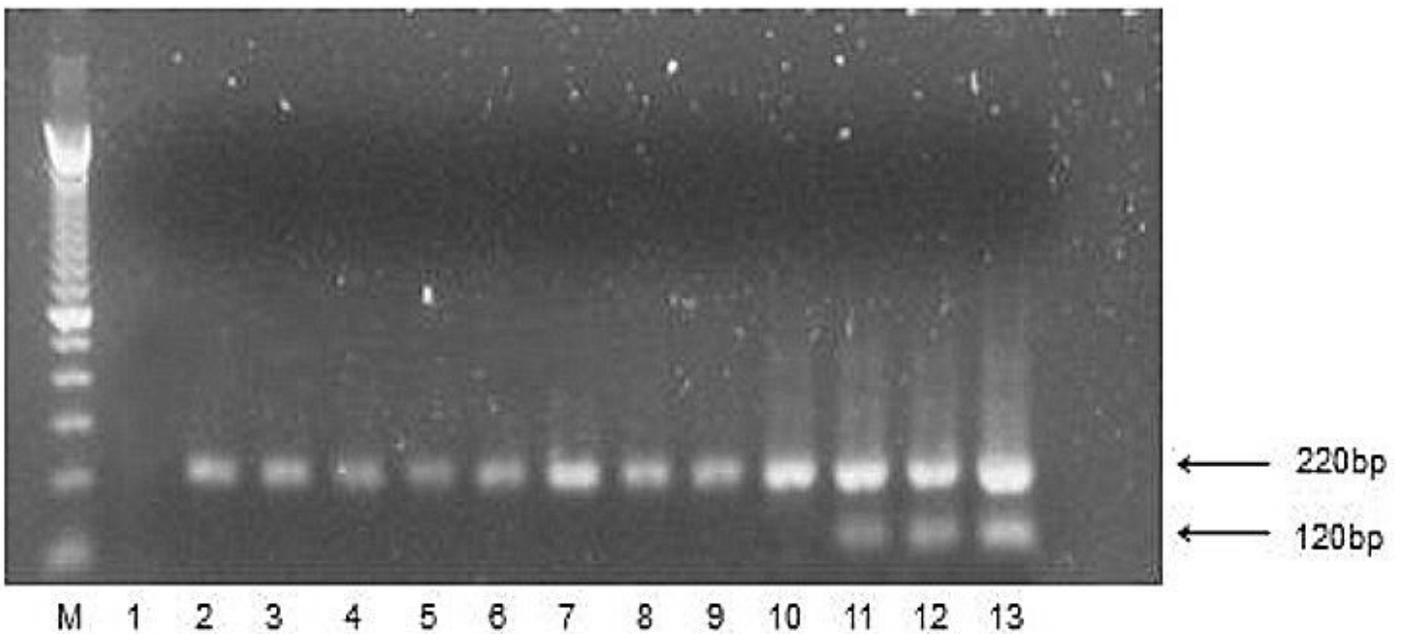


Figure 3

Result of the search for natural infection of *Leishmania (Leishmania) infantum chagasi*, in *Lutzomyia (Lutzomyia) longipalpis*, from Araguaína. 1.5% agarose gel electrophoresis. M - Molecular weight marker, 1 - negative reaction control, 2-3 - *Lutzomyia (L.) longipalpis* males, 4-9 - *Lutzomyia (L.) longipalpis* negative females, 10-12 *Lutzomyia (L.) longipalpis* positive females, 13 - positive control of the reaction.

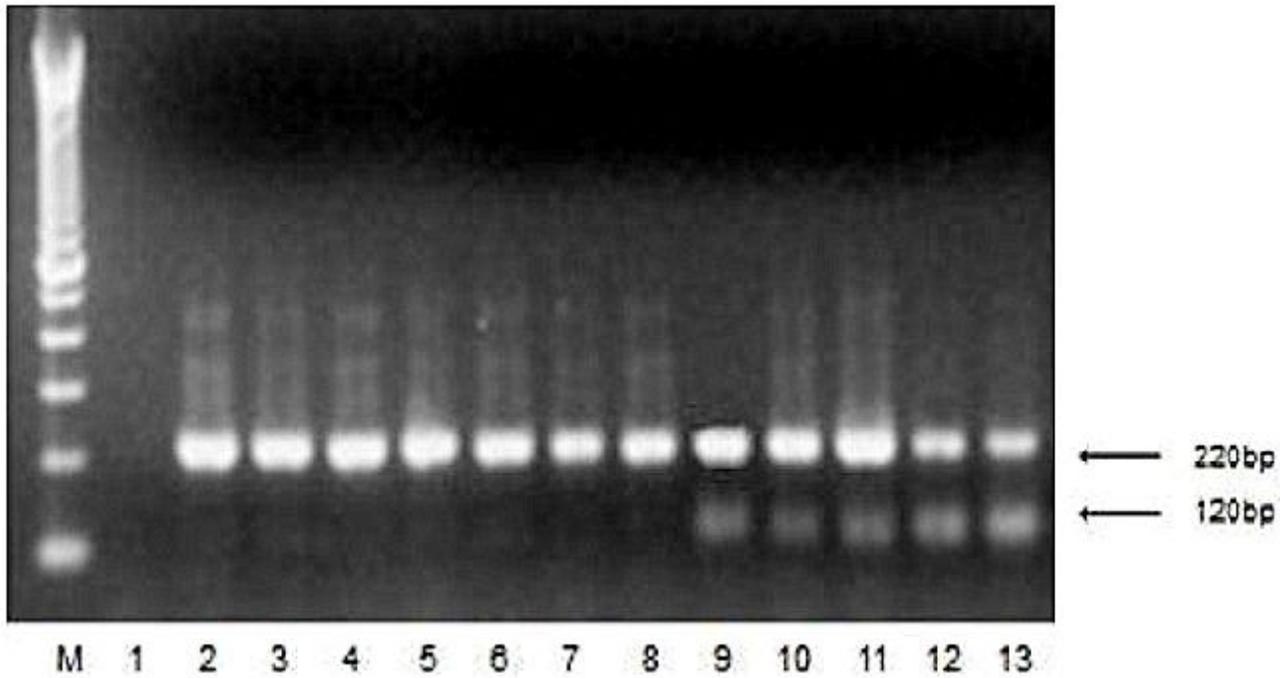


Figure 4

Result of the research of natural infection of *Leishmania (Leishmania) infantum chagasi*, in *Lutzomyia (Lutzomyia) longipalpis*, from Sobral. 1.5% agarose gel electrophoresis. M - Molecular weight marker, 1 - negative reaction control, 2-3 - *Lutzomyia (L.) longipalpis* males, 4-8 - *Lutzomyia (L.) longipalpis* negative females, 9-12 - *Lutzomyia (L.) longipalpis* positive females, 13 - positive control of the reaction.