Visceral Leishmaniasis: vector or vectors? Vectorial capacity and control strategies Trichopygonomyia killickorum - Fossil. Ambar of 20 MYA, República Dominicana.

Foto: José Dilermando Andrade-Filho

Paleoleishmania neotropicum a digenetic Leishmania in Lutzomyia adiketis

American Visceral Leishmaniasis Surveillance and Control Program:

Overall objective: reduce morbidity and lethality in humans

Specific:

- Early detection of VL transmission
- Monitor and reduce canine prevalence
- Detect and treat all human cases early
- Monitor the distribution of *Lutzomyia longipalpis*
- Reduce vector density

Entomological Surveillance Activities

- Entomological survey: detect the presence of *Lu. longipalpis* and its distribution by the municipality
- Entomological research in outbreak: detect the presence of *Lu. longipalpis* in municipalities with transmission where the vector has not been detected yet
- **Research in monthly monitoring unit:** monitor the population fluctuation of *Lu. longipalpis* and relate it to the meteorological conditions and, in some situations, to evaluate the impact of vector control measures



CDC traps









CDC traps



CDC traps

Eletric aspirator



Eletric aspirator

Castro aspirator



Dispersion route of Lutzomyia longipalpis in São Paulo state



Rural area: Cássia dos Coqueiros, E.S. do Pinhal (1994) Socorro, Itupeva, Salto de Pirapora, Pirapora de Bom Jesus



urban area de Araçatuba



E.S. do Pinhal – urban area, 2000











Distribution of Canine VL cases in the São Paulo state according the year of the 1st notification (until 2017)



Distribution of Human VL cases in the São Paulo state according the year of the 1st notification (until 2017)





- (S)-9-methylgermacrene-B
- Cembrene-1
- 3-methil- himachalene
- Cembrene-2

The existence of a species complexes raises the question: Do they have different vectorial capacity?

"Espirito Santo do Pinhal is situated in the Northeast region of the State...... *L. longipalpis* was notified... in rural area in 1994 (Costa et al 1997)... This species was also observed, at low density... in the urban area... from 2000 to 2004. At the present, no human cases of VL have been notified in this municipality or region... All males collected were cembrene 1 chemotype"

- In 2005, was notified the 1st Canine VL case and no human case was detected until now.

- Only 01 Human VL in Votorantim, 2017. Any other case was reported in the municipalities of this region.

"Araçatuba is situated in the West region of the State...The first notification of *L. longipalpis* in the western region of São Paulo State occurred in the urban area of Araçatuba in 1997 (Costa et al. 1997). Since then this species has been found at high density within the urban area of Araçatuba and other municipalities of this region... From 1999 to June 2005, 192 Human VL cases, with 20 deaths, were notified in Araçatuba... All males collected were 9 methylgermacrene-B"

- In Araçatuba, until 2017, 353 human cases were notified with 32 deaths.

Casanova et al 2006

Identification of sex pheromones of Lutzomyia longipalpis (Lutz & Neiva, 1912) populations from the state of São Paulo, Brazil

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In Brazil, four populations of Lutzomyia longipalpis each producing different sex pheromones are recognised. It has been suggested that these chemotype populations represent true sibling species. In this study we present the results of an analysis, by coupled gas cromotography – mass spectrometry, of the pheromones of males L. longipalpis from two different municipalities of the state of São Paulo. Our study showed that L. longipalpis from these two municipalities produced different sex pheromones from each other. This coupled with the remarkable difference between the epidemiological situation in Araçatuba and Espírito Santo do Pinhal, suggests that the (S)-9methylgermacrene-B and cembrene-1 populations may have different vectorial capacities.

Key words: Lutzomyia longipalpis - sex pheromone - (S)-9-methylgermacrene-B - cembrene-1 - Brazil

The sand fly *Lutzomyia longipalpis* (Lutz & Neiva) is the main vector of *Leishmania (L.) chagasi* (Cunha & Chagas), the causative agent of the American Visceral Leishmaniasis (AVL) in the New World. Males of *L. longipalpis* have secretory glands restricted to a pair of dorsolateral pale patches on tergite 4 (one spot form) or with an additional pair of patches on tergite 3 (two spot form) (Lane et al. 1985). These glands release terpenes, which act as a sex pheromone that is highly attractive to intrapopulational conspecific females (Morton & Ward 1989, Ward & Morton 1991, Hamilton et al. 1994, Kelly & Dye 1997). are monocyclic diterpenes partially characterized as cembrene isomers and have been found in males from Sobral, state of Ceará (Hamilton et al. 2005) and males from Jaíbas, state of Minas Gerais (Hamilton et al. 2004). There is no correlation between the numbers of spots on the abdomen and the pheromone extracted from them. This study presents data on the type of sex pheromone found in male *L. longipalpis* from two areas of the state of São Paulo: Espírito Santo do Pinhal and Araçatuba. The epidemiology of AVL in Araçatuba is distinctly different to Espírito Santo do Pinhal.

Espírito Santo do Pinhal is situated in the Northeast

Do the same chemotypes populations occur in the municipalities with the same epidemiological pattern?



RESEARCHARTICLE

Distribution of *Lutzomyia longipalpis* Chemotype Populations in São Paulo State, Brazil

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Abstract

Background

American visceral leishmaniasis (AVL) is an emerging disease in the state of São Paulo, Brazil. Its geographical expansion and the increase in the number of human cases has been linked to dispersion of *Lutzomyia longipalpis* into urban areas. To produce more accurate risk maps we investigated the geographic distribution and routes of expansion of the disease as well as chemotype populations of the vector.



OPENACCESS

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Received: September 30, 2014

Distribution of different Lu. Longipalpis populations in the São Paulo state



Distribution of Human VL cases in the São Paulo state according the year of the 1st notification (until 2017)



Guarujá 2016 - 02 cases Votorantim 2017 - 01 case

Distribution of different *Lu. Longipalpis chemotype* populations in the São Paulo state



Cordeirópolis

- The ecoepidemiological pattern in the western region is defined by the occurrence of human cases, frequent high prevalence of canine cases and a greater number of municipalities where *Lu. longipalpis* is present and occur human and canine VL. Generally, a great number of flies is collected in both manual and CDC light traps. All samples of males analysed were the 9MGB - The expanding population was 9MGB.

- Probably this species has been introduced from Mato Grosso do Sul.

- In contrast, the eastern region, can be characterized by the absence of notified human cases - even where the presence of *Lu. longipalpis* and canine cases have been reported for at least 12 years - low prevalence in dogs and a smaller number of municipalities where the vector is present. The populations of sand flies are generally low in abundance in manual and CDC light trap collections. All samples of males analysed were the cembrene-1 chemotype.

- The native cembrene-1 population had not expanded.

- Spread of the disease is more likely therefore to be due to the expansion of urban areas into rural or wild areas.

Are there differences in ecological parameters that give rise vectorial capacity (e.g. vector abundance, survival, dispersion, host feeding pattern and blood feeding rate)?

RESEARCH





Ecological parameters of the (S)-9methylgermacrene-B population of the *Lutzomyia longipalpis* complex in a visceral leishmaniasis area in São Paulo state, Brazil

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Abstract

Background: Visceral leishmaniasis (VL) is an important public health challenge in Brazil because of the high number of human and canine cases reported annually. *Leishmania infantum* is the etiological agent of VL and *Lutzomyia longipalpis* is its main vector. However, evidence suggests that this taxon constitutes a species complex. In Sao Paulo state, there are two populations of *Lu. longipalpis*, each secreting distinct pheromones, (S)-9-methylgermacrene-B and Cembrene 1; both have been associated with different patterns of VL transmission. The aim of the present study was to investigate the temporal distribution and natural infection of the (S)-9-methylgermacrene-B population of the *Lu. longipalpis* complex in a highly VL endemic area of Sao Paulo state to obtain information that may contribute to the surveillance of this zoonosis and to the planning of preventive and control measures.

Methods: The study was carried out in Panorama municipality, Sao Paulo State. Captures were made during 24 months in seven domiciles. The relation between sand fly abundance and climatic variables, temperature and humidity, was analyzed and natural infection by *Leishmania* spp. in sand fly females was investigated by nested PCR.

Results: A total of 4120 sand flies, with predominance of *Lu. longipalpis* (97.2%) were captured. The highest averages of sand flies/night/trap occurred in the rainy season (November-March) and a positive, significant correlation between sand fly abundance and the temperature and humidity 20 days before the capture days was found. *Leishmania infantum* DNA was detected in three out of 250 pools of females analyzed, giving an estimated minimum infection rate of 1.2%.

Conclusion: The identification of the climatic association between the high abundance of the vector in this highly endemic VL focus constitutes a fundamental point for evaluating future vector and dog control measures and this information increases the data of VL foci in Sao Paulo state that could contribute to the public health authorities in planning prevention and control measures. The identification of natural infection by *Le. infantum* in *Lu. longipalpis* specimens reinforces the importance of entomological surveillance activities in this municipality.



OPEN ACCESS

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A field study of the survival and dispersal pattern of *Lutzomyia longipalpis* in an endemic area of visceral leishmaniasis in Brazil

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Abstract

Zoonotic Visceral leishmaniasis (ZVL) is a neglected tropical disease that in the Americas is caused by the infection of Leishmania infantum and the domestic dog (Canis familiaris) is the main parasite reservoir in urban areas. The parasite is mainly transmitted by populations of the sibling species Lutzomyia longipalpis that has been spreading in countries including Brazil, Argentina, Paraguay and more recently Uruguay. Although bionomic parameters such as population survival and the duration of the gonotrophic cycle are critical in evaluating vector capacity, field studies have rarely been applied to sand fly populations. The present study sought to evaluate basic bionomic parameters related to the vectorial capacity of the (S)-9-methylgermacrene-B population of the Lu. longipalpis complex in a visceral leishmaniasis area of Sao Paulo state. The daily survival rate, the duration of the gonotrophic cycle and the dispersal pattern were evaluated through the mark- release-recapture method. A total of 1,547 males and 401 females were marked and released in five experiments carried out between February 2013 and February 2014. The higher recapture rates occurred within 100 meters of the release point and the estimated daily survival rates varied between 0.69 and 0.89 for females and between 0.69 and 0.79 for males. The minimum duration of the gonotrophic cycle observed was five days. The absolute population size, calculated ranged from 900 to 4,857 females and from 2,882 to 9,543 males. Our results demonstrate a high survival rate of this vector population and low dispersal that could be associated with the presence of all necessary conditions for its establishment and maintenance in the peridomiciles of this area. Our findings contribute to the basic data necessary for the understanding of ZVL dynamics and the evaluation of the implementation of prevention and control measures.

Distribution of Canine VL cases in the São Paulo state according the year of the 1st notification (until 2017)



Cotia e Embu das Artes (since 2003) Guarujá (since 2016) Distribution of Human VL cases in the São Paulo state according the year of the 1st notification (until 2017)



Guarujá (since 2016)



What are the vectors species???

Cotia e Embu das Artes Guarujá ? Sand flies collected in areas with Visceral Leishmaniasis transmission and absence of L. longipalpis, São Paulo state

Embú e Cotia	Guarujá	Santos
Pintomyia fischeri - 1	Nyssomyia intermedia	Nyssomyia intermedia - 3
Migonemyia migonei -	Migonemyia migonei	Nyssomyia neivai
Pintomyia monticola	Psathyromyia pascalei	Migonemyia migonei
Evandromyia edwardsi	Psychodopygus ayrosai	Psathyromyia pascalei
Psychodopygus lloydi	Pintomyia fischeri	Psathyromyia sp
Nyssomyia intermedia		Pintomyia fischeri
		Psathyromyia aragaoi
		Micropygomyia schreiberi
		Sciopemyia sordellii

1 Exl = Experimental infection; NI = Natural Infection 2 = parasite, 3 = PCR; 4 vectorial competence

Do these species have vectorial competence to transmit Leishmania infantum?




Fig. 3.4 Sand fly digestive tract and *Leishmania* development in the vector. Sand fly female ingest amastigotes (AM) together with the blood meal. In the abdominal midgut the blood meal is encased by peritrophic matrix (PM), and amastigotes transform to procyclic promastigotes (PP) which proliferate. After PM disintegrates, PP transform to long nectomonads (NE) which later transform to shorter form called leptomonads (*LE* = short nectomonads). These forms temporarily attach to midgut epithelium and proliferate. In subgenus *Viannia*, haptomonads (HA) attach to cuticular lining of proctodeum. In late-stage infections, leptomonads migrate to thoracic midgut, produce promastigote secretory gel (PSG) and transform either to metacyclics (MC) or haptomonads (HA) attaching to cuticular lining of stomodeal valve (SV) (J. Sadlova and P. Volf)

Permissive vectors: sand flies species that support late-stage development of multiple *Leishmania* species (Volf and Myskova, 2007).

The permissive vectors have epidemiologic importance. They could be responsible for establishing new leishmaniasis foci (Volf and Myskova, 2007)

"Probably the most important exemple of a permissive vector involved in the establishment of a new disease foci is *Lu. longipalpis*, the major New World vector of *L. (L). Infantum...* imported into New World by the dogs that accompanied Spanish and Portuguese immigrants..." (Dvorack et al 2018).

It is crucial to determine a putative vector. It is still necessary to show experimental transmission and Vectorial Capacity!!!



RESEARCH ARTICLE

OPEN ∂ ACCESS

Canine visceral leishmaniasis in the metropolitan area of São Paulo: *Pintomyia fischeri* as potential vector of *Leishmania infantum*

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Abstract – American visceral leishmaniasis is a zoonosis caused by Leishmania infantum and transmitted mainly by Lutzomyia longipalpis. However, canine cases have been reported in the absence of this species in the Greater São Paulo region, where Pintomyia fischeri and Migonemyia migonei are the predominant species. This raises the suspicion that they could be acting as vectors. Therefore, this study sought to investigate specific vector capacity parameters of these species and to compare them with those of Lu. longipalpis s.l. Among these parameters the blood feeding rate, the survival, and the susceptibility to the development of Le. infantum were evaluated for the three species, and the attractiveness of dogs to Pi. fischeri and Mg. migonei was evaluated. The estimated interval between blood meals was shorter for Lu. longipalpis s.l, followed by Pi. fischeri and Mg. migonei. The infection rate with Le. infantum flagellates in Lu. longipalpis was 9.8%, in Pi. fischeri 4.8%, and in Mg. migonei nil. The respective infective life expectancies (days) of Lu. longipalpis, Mg. migonei, and Pi. fischeri were 2.4, 1.94, and 1.68. Both Pi. fischeri and Mg. migonei were captured in the kennel with a predominance (95%) of Pi. fischeri. Considering the great attractiveness of dogs to Pi. fischeri, its susceptibility to infection by Le. infantum, infective life expectancies, and predominance in Greater São Paulo, this study presents evidence of Pi. fischeri as a potential vector of this parasite in the region.

Key words: Vector capacity, Sandfly, Pintomyia fischeri, Migonemyia migonei, Visceral leishmaniasis.

Résumé – Leishmaniose viscérale canine dans la région métropolitaine de São Paulo : Pintomyia fischeri comme vecteur potentiel de Leishmania infantum. La leishmaniose viscérale américaine est une zoonose causée par Leishmania infantum et transmise principalement par Lutzomyia longipalpis. Cependant, des cas canins ont été rapportés dans la région de São Paulo en l'absence de cette espèce, avec Pintomyia fischeri et Migonemyia migonei comme espèces prédominantes, ce qui suggère qu'elles pourraient agir comme vecteurs. Par conséquent, cette étude a cherché à étudier certains paramètres de capacité vectorielle de ces espèces et de les comparer avec ceux de Lu. Longipalpis s.l. Parmi ces paramètres, le taux d'alimentation sanguin, la survie et la susceptibilité au développement de Le. infantum ont été évalués pour les trois espèces, et l'attrait des chiens a été évalué pour Pi. fischeri et Mg. migonei. L'intervalle estimé entre les repas sanguins était plus court pour Lu. longipalpis s.l., suivi par Pi. fischeri et Mg. migonei. Le taux d'infection par des formes flagellées de Le. infantum était de 9.8 % chez Lu. longipalpis, 4.8 % chez Pi. fischeri, et néant chez Mg. migonei. Les espérances de vie infectieuses respectives de Lu. longipalpis, Mg. migonei et Pi. fischeri étaient 2.4, 1.94 et 1.68 jours. Les deux espèces Pi. fischeri et Mg. migonei ont été capturées dans les chenils avec une prédominance (95 %) pour Pi. fischeri.

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The incrimination of species as a vector is based on some criteria:

- Overlapping of the geographic distributions of the vector and human disease
- Repetitive natural infections, in females, of the same *Leishmania* species as occurs in human
- Vectorial Competence: acquire, support and transmit Leishmania experimentally
- Vectorial capacity: ecological factors as: density, dispersion; blood feeding frequence, antropophilia and zoophilia, survival

$VC = ma^2 b S^n / -ln(s)$

- **VC** = vectorial capacity
- **m** = **females density in relation to the host**
- **a** = the host-biting habit in bites per host per day per sand fly
- **b** = the proportions of infected females that become infective
- **S** = female daily survivorship
- n = duration of the extrinsic incubation period in days

Critical population-based parameters of Vectorial Capacity (VC) such as survivorship ("S"), population size ("m"), and gonotrophic cycle duration and host feeding pattern ("a") essentially determine the number of potentially infectious females in the population and the frequency of vector-host contact.

$VC = ma^2 b S^n / -ln(s)$

- **VC** = vectorial capacity
- **m** = **females density in relation to the host**
- **a** = the host-biting habit in bites per host per day per sand fly
- **b** = the proportions of infected females that become infective
- **S** = female daily survivorship
- n = duration of the extrinsic incubation period in days

How can the evaluation of these parameters help to understand the dynamics of transmission and direct and increase the efficiency of control measures?



m = 10.000 $\stackrel{\bigcirc}{+}$ infectadas S = 0.80

Dia 0

1st bloodmeal infective m = 10.000 = 100%



Dia 1 m = 8.000 = 80% Dia 5 2 st bloodmeal infective m = 3.200=32%



 $C = ma^2 S^n / - ln(S)$





Adulticides: small changes in the daily survival rate (S) cause substantial changes in the proportion of females that survive long enough to become infective

 $VC = ma^2 S^n / - ln(S)$

Adulticide

With an additional mortality of 50%, induced by a residual effect insecticide, in pop. of females with S = 0.8, results S = 0.4. At the end of 5 days only $0.4^5 = 0.010 = 1.02\%$, ie 100 females will survive



N= 10.000 fêmeas

Repasto sangue	Dias	S=0,80	N final
1	0		10.000
2	5	(0,80) ⁵ =0, 327 ou 33%	3270
3	10	(0,80) ¹⁰ =0,107 ou 10%	1070
4	15	(0,80) ¹⁵ =0,035 ou 3,5%	351

Adulticida

Repasto sangue	Dias	S=0,40	N final
1	0		10.000
2	5	(0,40) ⁵ =0,010 ou 1%	100
3	10	$(0,40)^{10}=0,0001$ ou $0,01\%$	1
4	15	$(0,40)^{15} = 0,000001$	0

From this analysis it is easy to see that an additional mortality, induced by the application of adulticide residual effect, will further reduce the proportion of potential transmitters. For example, if 50% reduction can be achieved in a pop. of females of *Lu. longipalpis* with 80% of daily survival, we will have a daily survival rate of 40%, that is, only 1% will survive until the 5th day to transmit *Leishmania infantum*. No doubt such a reduction would have a major impact on transmission.

Zooprophylaxis: Would it be possible to use birds (refractory to Leishmania) to reduce the proportion of females that feed on man and / or dog? We are talking about the "a" (a = chicken Blood index / CG)



Additional mortality of 50% (S=0.80 to S= 0.40) + bird feeding on the 5th day

Dia 0	Dia 5	Dia 10
1st bloodmeal infective	S = 40%	S = 40%
m = 10.000 = 100%	2 st bloodmeal in chickens	3 st bloodmeal infective
	m = 100 = 1%	m = 1 = 0,01%
XXXXXX	× ×	×
**********	× × ×	₩
***********	+	

* * * * * * * * * * * * * * * * * * *		VC = ma ² S ⁿ /-In(S)

Zoopr	ophyilaxis with S=0,8		N= 10.000 fêmeas		
	Repasto sangue	Dias	S=0,80	N final	
	1	0		10.000	
	2	5	(0,80) ⁵ =0, 327 ou 33%	3270 Sem transmissão	
	3	10	(0,80) ¹⁰ =0,107 ou 10%	1070	
	4	15	(0,80) ¹⁵ =0,035 ou 3,5%	350	
Adulticide + Zooprophylaxis					
	Repasto sangue	Dias	S=0,40	N final	
	1	0		10.000	
	2	5	(0,40) ⁵ =0,010 ou 1%	100 Sem Transmissão	
	3	10	$(0,40)^{10}=0,0001$ ou $0,01\%$	1	
	4	15	$(0,40)^{15} = 0,000001$	0	

From this analysis it is easy to see that a first or second feed in a bird will cause the transmission to only occur from the third feeding. This will happen on the 10^{th} day. In a population with S = 0.80 only 10% will survive to perform the third blood repast. For a pop. who had the daily survival reduced to 40%, only 0.01%, that is, 1 female in 10,000 will survive long enough (10 days) to transmit to *L. infantum*. No doubt such a reduction may be sufficient to interrupt transmission in an area.

Feromônio: Males of *Lu. longipalpis* produce attractive pheromones for cospecific females (forming "lek"). The application of insecticide in chicken sheds would interrupt the lek and disperse the males to other ecotypes (dog and domicile) (Kelly 1997).

What could happen if we applied an insecticide?





























 $VC = ma^2 S^n / - ln(S)$

Collar impregnated with insecticide: besides acting as a repellent also kills the females that can feed on the dog. It can have a large impact on the decrease of infected females. In "VC" it has effect on "a" for dog and "S".



Adulticide + Zooprophylaxis + Pherome + Collar

```
VC = ma^2 S^n / - ln(S)
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Adulticide + Zooprophylaxis + Pherome + Collar

$VC = ma^2 S^n / - ln(S)$

How to act in "m"

Adulticide + Zooprophylaxis + Pherome + Collar

$VC = ma^2 S^n / - ln(S)$

How to act in "m"

How to decrease the number of vector-host contacts?

Decreasing the density of adults "m" via decreased survival of immature forms would be one more factor to be used in an integrated control proposal.

Environmental management: The purpose of management is to make the environment unsuitable for the creation of immature forms.

$$C = ma^2 S^n / - ln(S)$$

However any control action directed at immature forms (environmental modification or insecticides - biological and chemical) will depend on a better knowledge about breeding sites.

Where are the larvae? What are the preferred breeding sites?

Larval Breeding Sites of *Lutzomyia longipalpis* (Diptera: Psychodidae) in Visceral Leishmaniasis Endemic Urban Areas in Southeastern Brazil

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Abstract

Background: The scarcity of information on the immature stages of sand flies and their preferred breeding sites has resulted in the focus of vectorial control on the adult stage using residual insecticide house-spraying. This strategy, along with the treatment of human cases and the euthanasia of infected dogs, has proven inefficient and visceral leishmaniasis continues to expand in Brazil. Identifying the breeding sites of sand flies is essential to the understanding of the vector's population dynamic and could be used to develop novel control strategies.

Methodology/Principal finding: In the present study, an intensive search for the breeding sites of Lutzomyia longipalpis was conducted in urban and peri-urban areas of two municipalities, Promissão and Dracena, which are endemic for visceral leishmaniasis in São Paulo State, Brazil. During an exploratory period, a total of 962 soil emergence traps were used to investigate possible peridomiciliary breeding site microhabitats such as: leaf litter under tree, chicken sheds, other animal sheds and uncovered debris. A total of 160 sand flies were collected and 148 (92.5%) were *L. longipalpis*. In Promissão the proportion of chicken sheds positive was significantly higher than in leaf litter under trees. Chicken shed microhabitats presented the highest density of *L. longipalpis* in both municipalities: 17.29 and 5.71 individuals per square meter sampled in Promissão and Dracena respectively. A contagious spatial distribution pattern of *L. longipalpis* was identified in the emergence traps located in the chicken sheds.

Conclusion: The results indicate that chicken sheds are the preferential breeding site for *L. longipalpis* in the present study areas. Thus, control measures targeting the immature stages in chicken sheds could have a great effect on reducing the number of adult flies and consequently the transmission rate of *Leishmania* (*Leishmania*) infantum chagasi.






























Conclusion

The estimates of the population parameters of the "VC" allow to evaluate and clarify the relationships between the entomological variables and these with the transmission dynamics of the leishmaniasis. Residual insecticides, insecticide impregnated collars, the use of synthetic pheromones and zooprophylaxis have a clear theoretical background to act in the reduction of vector-host contacts. However, control of immature forms could have a significant effect on reducing the population density of adult forms "m" and, consequently, would improve the efficacy of all other interventions (acting on "a" or " S").

VC = ma² Sⁿ/-In(S)

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