

Haematophagic behavior in laboratory of *Lutzomyia cruzi* (Mangabeira) (Diptera: Psychodidae) in relation to three mammalian blood sources in Manaus, Brazil

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ABSTRACT

The sand fly *Lutzomyia cruzi* is considered as one of vectors of visceral leishmaniasis in Brazil. This work examined optimum feeding age, feeding time, host preference, fecundity rates, and female blood meal volume taken by single females from a closed colony of *L. cruzi*. Mean feeding time was longer on hamsters, 6.6 minutes, than on humans, 5.7 minutes. 49.1% of the 48h-old flies fed on humans and 43.3% of 72h-old flies fed on hamsters. Of a total of 120 females, 61% fed on humans and 25% fed on hamsters. Total fecundity was significantly higher in females fed on hamster than on human or opossum. Laboratory-reared *L. cruzi* females fed earlier, more promptly, and preferably on humans than on hamsters when offered these blood-meal sources simultaneously. The blood-meal volume is higher in females fed on hamsters than other hosts (human and opossum).

KEY-WORDS

Lutzomyia cruzi, vector of visceral leishmaniasis, fecundity, blood meal volume.

Comportamento hematofágico de Lutzomyia cruzi (Diptera: Psychodidae) em relação a três fontes sanguíneas de mamíferos em laboratório, Manaus, Brasil

RESUMO

O flebotomíneo *Lutzomyia cruzi* é incriminado como um dos vetores de leishmaniose visceral no Brasil. Foram estudados: a idade ótima, a preferência alimentar, os índices de fecundidade e o volume de alimentação sanguínea realizado com fêmeas de *L. cruzi* colonizadas em laboratório. O tempo médio de alimentação foi maior em hamster, seguido de humano. Verificou-se que 49,1% das fêmeas alimentaram-se em humano com 48h enquanto em hamster, 43,3% alimentaram-se com 72h. Das 120 fêmeas observadas, 61% realizaram a alimentação em humanos e 25% em hamster. A fecundidade total foi maior nas fêmeas alimentadas em hamster, humano e mucura, respectivamente. Observou-se que fêmeas de *L. cruzi* colonizadas alimentam-se mais facilmente e preferencialmente em humanos do que em hamster quando ambas as fontes de alimentação são oferecidas simultaneamente. O volume da alimentação sanguínea foi maior nas fêmeas alimentadas em hamster.

PALAVRAS-CHAVE

Lutzomyia cruzi, vetor de leishmaniose visceral, fecundidade, volume sanguíneo.

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INTRODUCTION

Leishmaniasis is an important public health problem in Brazil. Phlebotomine sand flies (Diptera: Psychodidae) are the vectors of leishmaniasis, as well as other vertebrate pathogens. The phlebotomine sand fly *Lutzomyia longipalpis* (Lutz & Neiva) (Diptera: Psychodidae) is the primary vector of visceral leishmaniasis, currently considered the mainly Neotropical vector of this disease. Santos *et al.* (1998) demonstrated that *Lutzomyia cruzi* (Mangabeira) is a vector of visceral leishmaniasis in Corumbá and Ladário municipalities, in the Mato Grosso do Sul state, Brazil.

Despite the medical importance of *L. cruzi*, little is known about its biology or ecology. Justiniano *et al.* (2001) examined the biology of this vector under laboratory conditions, while Oliveira *et al.* (2003) and Santos *et al.* (2003) examined some ecological aspects of the species.

After the *L. cruzi* incrimination as a vector of visceral leishmaniasis, studies in laboratory colonies were necessary to know the biology of the species to corroborate that incrimination. This led to discoveries about colonization characteristics and biological cycle of *L. cruzi* (Justiniano *et al.*, 2001; Brazil *et al.*, 2002; Chagas *et al.*, 2002). Watts *et al.* (2005) studied sex pheromones and the phylogeographic structure of some *Lutzomyia longipalpis* species complex, including reared *L. cruzi*; Almeida *et al.* (2004) described the patterns of exochorion ornaments on eggs; Spiegel *et al.* described the sensilla on the terminalia of *L. cruzi*; Hodgkinson *et al.* (2002) studied the mitochondrial cytochrome B haplotypes polymorphism in *L. cruzi* and *L. longipalpis* populations.

New World sand fly colonies were observed under various laboratory conditions, varying factors, such as temperature, humidity, light level, and larval food sources (Sherlock & Sherlock, 1959; Killick-Kendrick *et al.*, 1977; Rangel *et al.*, 1987; Luitgards-Moura *et al.* (2000). There are few laboratory studies addressing haematophagic behavior by sand flies using different blood meal sources, optimal feeding age, fecundity, fertility, and female blood meal volume (Ready, 1979; Benito-de-Martín *et al.*, 1994; Queiróz, 1995; Ximenes *et al.*, 2001).

According to the review of Lehane (1991), physiological factors are mainly important in determining host choice, in the sense that once the insect has become associated with a narrow range of hosts; then specialization in its physiology will occur which may limit the range of other hosts it can exploit. This is because natural selection will ensure that all of the systems of the insect will become tuned to the exploitation of the resources of its major hosts that may restrict the insect's ability to deal with unusual situations. Several experimental studies have shown that the fecundity of blood-sucking insects depends on the host on which the insect is fed, reduced food intake or rate of digestion (Rothschild, 1975). Krinsky *et al.*, (1952) showed that in some cases choice of the wrong host can, for physiological reasons,

lead to the death of the insect by, for example, the crystals of oxyhaemoglobin that may form in the blood of a guinea-pig (*Cavia* sp.) can cause the rupture of the intestine of several blood-sucking insects.

Our objective is to examine how food source affects the haematophagic behavior of colonized *L. cruzi*. We examined timing of first blood meal, optimal feeding age, food source preference for hamster (*Mesocricetus auratus* Waterhouse) and man, and fertility, fecundity, and female blood meal volume for hamster, man and opossum (*Didelphis marsupialis* Linnaeus) and human.

MATERIAL AND METHODS

L. cruzi matrices were originated from the Corumbá Municipality (18°59'44"S; 57°37'16"W), in the Mato Grosso do Sul state, Brazil, and laboratory colonies were established using techniques described by Killick-Kendrick *et al.* (1977), Killick-Kendrick & Killick-Kendrick (1991) and Justiniano *et al.* (2004).

OPTIMAL FEEDING AGE, TIMING, AND FOOD SOURCE PREFERENCE

Optimal feeding age is defined as the age at which the largest numbers of females of similar age females take their first blood meal. One, two, and three days after their emergence, 20 females and 20 male of sand flies were offered blood meals from two sources: hamster under Ketalar® anesthesia, and human (forearm of the author). Food sources were offered for one hour in the laboratory (27 °C and 92% humidity).

FECUNDITY, FERTILITY, AND BLOOD MEAL VOLUME

We defined fecundity as the number of eggs successfully deposited plus the eggs retained in the ovaries. Fertility was defined as the number of eggs successfully deposited (Queiróz, 1995). Hamster, human, and common opossum (under Ketalar® anesthesia) were offered as haematophagic sources for *L. cruzi* females. We recorded and compared fecundity and fertility rates after the females laid eggs and died.

Blood meal volume was measured per individual female using the hemoglobinometry method (Brigel *et al.*, 1979), where hemoglobin is converted into an erythrocyte suspension lysate by a stable complex, hemiglobincyanide (HiCN). Immediately after feeding, the gut of each female was dissected and the Malpighian tubes removed to avoid uric acid interfering with the trials.

We placed each blood-filled gut into microtubes with 600µl of Drabkin reagent (Legowski & Boroviczeny, 1962), composed of: 1.0g sodium bicarbonate (NaHCO₃), 0.1 g potassium carbonate (K₂CO₃), 0.05 g potassium cyanide (KCN), 0.2 g potassium ferricyanide [K₃Fe(CN)₆], and distilled water. We incubated samples at 15-23 °C for 20 minutes and then used a

540 nm spectrophotometer to determine optical density (OD).

We constructed calibration curves for each blood-meal source (human, hamster, opossum), which determined the ODs for different known blood volumes for each host (0.1; 0.2; 0.4; 0.6; 0.8; 1.0; 2.0; 3.0; 4.0; 5.0 µl). To some samples, we were unable to accurately measure the amount of blood ingested by the female; we used the observed OD and these linear equations and then obtained a linear regression to determine ingested blood volume in these cases.

We performed four replicates of the fecundity and fertility experiments (100 total females) for each blood meal source, and analyzed the results using parametric analysis of variance (ANOVA) or non-parametric Kruskal-Wallis one-way ANOVA, depending upon whether or not the data was distributed normally (Zar, 1996).

RESULTS

OPTIMAL FEEDING AGE, TIMING, AND FOOD SOURCE PREFERENCE

Of 120 *L. cruzi* females offered human and hamster food sources, 61 (50.8 %) fed on human, 30 (25.0 %) on hamster, and 29 (24.1 %) did not take a blood meal (Table 1). The optimal feeding age for human food sources was the second day after emergence, with 49.1 % (30) of the females taking a human blood meal when reaching this age. Optimal age for females feeding on hamsters was three days post-emergence, with 43.3 % (13) individuals taking a blood meal. Blood meals were longer when feeding on hamsters (mean time = 6.6 min, range = 2.2-8.9), than humans (mean time = 5.7 min, range = 2.0-13.0).

FECUNDITY, FERTILITY, AND BLOOD MEAL VOLUME

Females that fed on hamster blood had significantly higher fecundity than females that fed on either humans or opossums ($H = 23.6$ $df = 2$ $P < 0.001$ Kruskal-Wallis). Fertility was also higher, but not significantly, in females that fed on hamster blood than those that fed on human or opossum blood ($F = 1.96$ $df = 2$ $P = 0.14$ ANOVA). Females that fed on hamster blood also retained significantly more eggs in their ovaries than those that fed on other sources ($H = 18.6$ $df = 2$ $P < 0.001$) (Table 2).

Table 1 - Optimal feeding age and length (min) of blood meal in laboratory for *Lutzomyia cruzi* on human and hamster food sources.

	Blood sources					
	Man			Hamster		
	24h	48h	72h	24h	48h	72h
Mean±SD	5,6±3.1	6,1±2.5	5,1±2.4	7,5±4.4	5,2±1.7	7,0±5.2
Minimum	2,0	2,1	2,6	2,5	2,2	2,5
Maximum	10,2	12,7	12,2	16,0	7,4	19,0
Total ♀♀	10,0	30,0	21,0	8,0	9,0	13,0

N = 120 (Number of females), SD = Standard deviation

Female blood meal volume was greater when feeding on hamsters (mean volume = 1.5 µl) than on humans (mean volume = 0.7 µl) or opossums (mean volume = 1.1 µl). Linear regression equations for blood meal volume and optical density were as follows: hamster, $y = 8.47x - 0.99$, $r = 0.99$, $n = 23$; opossum, $y = 7.51x - 0.93$, $r = 1$; human, $y = 5x - 0.26$, $r = 0.98$, $n = 23$ (Figure 1).

DISCUSSION

The optimal age for *L. cruzi* to feed on humans was two days post-emergence. This is quite different from the optimal age for another human-biting leishmaniasis vector, *L. whitmani* (Antunes & Coutinho), with an optimal age of six or seven days post-emergence (Queiróz, 1995). We found the optimal age for *L. cruzi* to feed on hamsters was three days post-emergence. Still, our results are slightly earlier than optimal ages for *L. migonei* (França) and *L. evandroi* (Costa Lima & Antunes), four days post-emergence and *L. lenti* (Mangabeira), five days post-emergence (Queiróz, 1995). Our results are similar to other studies in that they confirmed that phlebotomine food source preference may vary with age. Since our experiments offered equal chances to feed on either food source and as choice varied with age, perhaps food source choice is regulated by a specific mechanism, rather than being simply a question of host availability (Queiróz, 1995; Nieves & Pimenta, 2002).

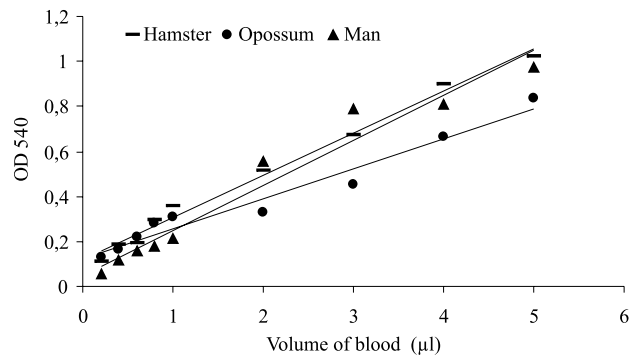


Figure 1 – Linear regression of HiCN (OD_{540}) and blood sample from hamster ($Y = 8.47X - 0.99$, $r = 0.99$, $n = 23$), opossum ($Y = 7.51X - 0.93$, $r = 1$, $n = 23$), and human ($Y = 5.00X - 0.26$, $r = 0.98$, $n = 23$).

Table 2 - Number of eggs laid and retained in *Lutzomyia cruzi* females that fed on human, hamster, or opossum blood in laboratorial conditions.

	Man		Hamster		Opossum	
	Mean ± SD	Total	Mean ± SD	Total	Mean ± SD	Total
Laid eggs	23,9 ± 18,4 (0-73)	2309	27,9 ± 24,8 (0-89)	2797	27,4 ± 23,7 (0-99)	2740
Retained eggs	13,8 ± 14,8 (0-56)	1389*	23,5 ± 23,1 (0-83)	2359*	10,4 ± 14,3 (0-52)	1047*
Total		3698*		5156*		3787*

* The difference is significant $P < 0,001$ (Kruskal-Wallis on Way ANOVA).
N = 100 (Number of females), SD = Standard deviation

In this study we found that 16.4% of *L. cruzi* females took a blood meal on the first day after emergence. The majority of phlebotomine females do not feed during the first 24 hours after emergence. Other studies show slightly higher levels of first-day feeding. Approximately 20% one-day old *L. migonei* individuals took a blood meal (Queiróz, 1995), while 30% of *Phlebotomus papatasi* (Scopoli) individuals were found to feed on their first day post-emergence (Adler & Theodor, 1927).

Mean feeding time was longer in hamster sources (6.6 min) than in humans (5.7 min) for *L. cruzi*. The species *L. longipalpis* was found to feed between 2-5 minutes, while *L. sanguinaria* (Fairchild & Hertig), *L. gomezi* (Nitzulescu), and *L. cruciata* (Coquillett) spent about five minutes to complete a blood meal on a variety of hosts: humans, dogs (*Canis familiaris* Linnaeus), and fox (*Cerdocyon thous* (Linnaeus)) (Sherlock & Sherlock, 1959). Sand flies have a pool feeding mouth morphology, which may require some spending time for feeding. Females secrete anti-haemostatic salivary substances to facilitate the feeding process, in which they generate a haematoma on the skin of the host. This could explain why the majority of species do not take a blood meal on the first days after emergence, since females usually require 48h to develop the ability to produce sufficient amounts of saliva (Cupp *et al.*, 1993).

We found that *L. cruzi* preferred human blood sources (50.8 %) over hamster (25.0 %). *Phlebotomus perniciosus* Newstead was found to prefer rabbit sources over hamster (Benito-de-Martín *et al.*, 1994), while *L. lenti* and *L. evandroi* preferred hamsters over human blood sources (Queiróz, 1995). Although *L. migonei* was found to bite hamsters more frequently than other offered sources, this species bit humans at the same rate as *L. whitmani*, which showed a strong preference for humans (Queiróz, 1995). The ability of *Leishmania* Ross to survive and develop during phlebotomine digestion of blood is fundamental for the successful establishment of the parasite population in the vector (Nieves & Pimenta, 2002). When the infection rates of seven different vertebrate species were compared after experimental exposure to *Leishmania braziliensis* Viana and *L. migonei*, four species, including humans, had higher infection rates than the other three species (Nieves & Pimenta, 2002). While this study showed that phlebotomine food-source preference may be important in determining infection rates, these results demonstrate that vertebrate hosts may have varying susceptibility to infection, which can also play an important role in determining regional infection rates.

We found the highest fecundity in females that fed on hamster blood, versus human or opossum blood. *L. longipalpis* females that fed on hamster blood, versus other mammal sources (including humans), had the highest numbers of mature oocytes (Ready, 1979). *L. intermedia* (Lutz & Neiva) female egg deposition rates did not differ depending on food source, in a study that offered either hamster or mouse (*Mus musculus*

Linnaeus) blood (Miranda *et al.*, 2001). Wild *L. umbratilis* (Ward & Fraiha) specimens deposited the most eggs when fed on human blood, but retained more eggs when fed on opossum blood (Chagas *et al.*, 2002).

Despite the large variability in the mean number of eggs deposited by each phlebotomine species, as well as among individuals within species, on average, individuals deposit between 20 and 30 eggs (Forattini, 1973). *L. shannoni* (Dyar) was found to deposit an average of 22.7 eggs and retain 24.3 (Ferro *et al.*, 1998). In this study, *L. cruzi* deposited an average of 27.9 eggs and retained 23.5. These numbers are similar to those of *L. longipalpis* (Luitgards-Moura *et al.*, 2000), are not surprising considering they are in the same sub-genus and share colonization characteristics.

Female blood meal volume was greater when sand flies fed on hamsters (1.5 µl) than other food sources. Normal blood meal volume usually approximates an individual's total body weight (0.5-0.6 mg) (Theodor, 1936; Chaniotis, 1967). Blood meal volume is important for fecundity because it determines how many oocytes develop to maturity (Ready, 1979). For *L. longipalpis*, mature oocyte numbers are linearly related to mammalian food sources, which relates to ingested blood volume, since blood of some vertebrate species are more nutritious than others (Ready, 1979). For *Culex pipiens* Linnaeus, the numbers of mature oocytes depends not on blood-meal volume, but on the protein content of the blood (Bellamy & Bracken, 1971), as some amino acids are essential for oogenesis. Colonies of sand flies are invaluable for the knowledge of insect biology, physiology, behavior, and vectorial efficiency. The knowledge of blood volume ingested by phlebotomine is important, because with this knowledge it is possible to quantify the exact quantity, for example, of *Leishmania* ingested in experiments of laboratorial infection (Queiróz, 1995).

ACKNOWLEDGMENTS

To the late Dr. Raul de Queiróz, adviser of the first and last two authors. His PhD thesis, an important source cited here, is still unpublished.

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Rcebido em 06/10/2006
Aceito em 02/02/2007