Effect of a Second Bloodmeal on the Oesophagus Colonization by *Leishmania mexicana* Complex in *Lutzomyia evansi* (Diptera: Psychodidae)

Alejandra Vivenes, Milagros Oviedo, Julio César Márquez, James Montoya-Lerma*/+

Centro de Investigaciones J. W. Torrealba, Trujillo, Venezuela *CIDEIM, Apartado Aéreo 5390, Cali, Colombia and Departamento de Biología, Universidad del Valle, Apartado Aéreo 25360, Cali, Colombia

Migration and colonization of the oesophagus by Leishmania mexicana *parasites were enhanced after digestion of a second bloodmeal intake in* Lutzomyia evansi. *This event has epidemiological significance since it affects the infection susceptibility of this sand fly species, which is a proven vector of* L. chagasi *in Colombian and Venezuelan visceral leishmaniasis foci. Also, it may explain the host seeking behaviour displayed by some partially bloodfed flies found inside houses.*

Key words: leishmaniasis - parasite development - refeeding - sand flies - *Lutzomyia evansi*

The vectorial capacity of any leishmaniasis vector depends not only on its ability to develop and harbour parasites in its intestine but also the colonization of the foregut by infective promastigotes after a complete digestion of the infective bloodmeal. Also, an infected fly should be able to locate, bite and transmit the parasites to a new reservoir or host. Hence, both biochemical and physiological mechanisms might be expected to control host-seeking behaviour and parasite development in a vector, respectively. In the study of vector capacity two assumptions are widely accepted: firstly, at least for haematophagous Diptera, host seeking is stopped by a full bloodmeal (Klowden 1990) and, secondly, that a full infecting bloodmeal is sufficient for an appropriate sand fly vector to become infective. However, recent observations prompted us to revisit these assumptions in sand flies. Firstly, in two geographically separated areas in Colombia and Venezuela, hungry gravid and semi-gravid *Lutzomyia evansi* females were caught indoors hence, displaying an active host-seeking behaviour (Montoya-Lerma 1996, M Oviedo, pers. obs.). Secondly, our studies on the biology of *Lu. evansi* suggest that this sand fly has a very short life expectancy in nature (Oviedo et al. 1995, Montoya-Lerma et al. 1998) and finally, *Lu. evansi* could experimentally harbour and develop *Leishmania mexicana* but parasites did not reach its oesophagus (unpublished data). Since *Lu. evansi* has been reported coexisting in an area where *L. mexicana* occurs, all of these observations prompted us to investigate whether a second blood-meal taken by *Lu. evansi*, subsequent to an infective one, may influence the oesophageal colonization of *L. mexicana* and related parasites.

For experimental and practical reasons, Syrian hamsters were infected either with one of two WHO *L. mexicana* reference strains (i.e. *L. amazonensis* IFLA/BR/67/PHB and *L. mexicana* MHOM/VE/72/AZV) and used as source of parasites. Hamsters were anaesthetised and its posterior footpad introduced, for approximately 20 min, inside a glass devise, containing starved bred female *Lu. evansi*. Fed flies were maintained in darkness, under constant conditions (26ºC and 85% RH). Putatively infected females were fed with sucrose as food source and dissected, to evaluate parasite development in their gut, at 24 h intervals from 72 h until 168 h post-infection. Afterwards, surviving females were fed on an uninfected hamster. Refed flies were kept as indicated above, receiving sucrose solution. After 72 h and during seven days, dissections were continued, following the same 24 h schedule.

Results showed that susceptibility of *Lu. evansi* to infection ranged between 29.4% (n= 119) and 21.7% (n= 147) to *L. mexicana* and *L. amazonensis*, respectively. The typical suprapylarian development was exhibited though presence of free flagel-

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+Corresponding author. Fax: +57-2-339.3243. E-mail: jamesmon@biologia.univalle.edu.co
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late forms in the Malphigi tubules and posterior gut was frequent in those flies with massive infections.

In general terms, parasites displayed a high polymorphism, ranging from long and slender promastigotes, in the abdominal and thoracic midgut, to rounded paramastigote forms in the thoracic midgut and stomodeal valve. Flagellates were found mainly in the posterior midgut at 72 h post infection. At 96 h, a massive migration of parasites forward to the stomodeal valve was noted with the majority fully attached to this region at 120 h (Tables I, II). Surprisingly, few flies exhibited parasites attached to the anterior portion of the digestive tract. Only two specimens infected with *L. mexicana* (5%) harboured parasite forms in their oesophagus.

Refeeding was achieved in only 6.7% and 10.2% of the flies infected with *L. amazonensis* (n = 118) and *L. mexicana* (n = 49), respectively. Interestingly, at 72 h after the second blood meal, flies harbouring many parasites in their oesophaguses were observed. The estimated infection ranged between 60% for *L. amazonensis* to 75% for *L. mexicana* (Table III). Noticeably, there was not a pharyngeal aggregation of parasites.

These results suggest the existence of a physiological mechanism in *Lu. evansi*, controlling its host-seeking behaviour, similar to the hormonal "switch" reported in mosquitoes (Klowden 1990). Therefore, the sand fly hunger response should be modulated or turned off accordingly to the expansion of its gut after a bloodmeal. In addition, since the gonadotrophic cycle of *Lu. evansi* lasts on average only 3-4 days (Montoya-Lerma et al. 1998), it is likely that the full development, migration, and colonization of the *L. mexicana* in the digestive tract of *Lu. evansi* requires a second bloodmeal. Thus, it

TABLE I

Time and space distribution of *Leishmania mexicana* parasites in the digestive tract of *Lutzomyia evansi*

		Distribution of flagellates				
Time (h p.i.)[a]	No dissected/ positive flies	Malphigian tubules	Abdominal midgut	Thoracic midgut	Stomodeal valve	Esophagous
72	30/10	-	9	5	-	-
96	29/10	-	8	5	-	-
120	30/10	-	6	5	4	1
144	25/4	1/4	4	4	1	1
168	5/1	-	1	1	1	-

a: hours post infection

TABLE II

Time and space distribution of *Leishmania amazonensis* parasites in the digestive tract of *Lutzomyia evansi*

		Distribution of flagellates				
Time (h p.i.)[a]	No dissected/ positive flies	Thoracic midgut	Abdominal midgut	Thoracic midgut	Stomodeal valve	Esophagous
72	36/7	-	7	3	1	-
96	35/13	1	12	7	2	-
120	38/5	-	1	2	-	-
144	27/7	1	4	3	3	-
168	11/0	-	-	-	-	-

a: hours post infection

TABLE III

Distribution of *Leishmania mexicana* and *L. amazonensis* flagellates in refed *Lutzomyia evansi* females

				Distribution of flagellates				
Strain	1st meal	2nd meal/ positive	% refed	Thoracic midgut	Abdominal midgut	Thoracic midgut	Stomach valve	Esophagous
L. mexicana	49	5(4)	10.2	-	4/4	1/4	-	3/4
L. amazonensis	118	8(5)	6.7	1/5	4/5	2/5	-	3/5

is probable that, during this brief cycle, nutrients are rapidly digested before parasites migrate forward to the oesophagous. If so, in conjunction, these events might have important epidemiological implications in areas where *Lu. evansi* is the vector of *L. chagasi*. For instance, this might explain why gravid or semi-gravid *Lu. evansi* that were actively displaying a host-seeking behaviour were caught, very often, inside houses in the American visceral leishmaniasis (AVL) focus of San Andrés de Sotavento, Colombia (Montoya-Lerma 1996). Further, the eclectic feeding behaviour of *Lu. evansi* (Montoya-Lerma & Lane 1996) might generate multiple feeding together with a multiple probing behaviour. Hence, it is plausible to hypothesise a scenario where some partially fed flies, which were previously in contact with infected opossums or dogs [i.e. the proven reservoirs of *L. chagasi* (Travi et al. 1994)] might require a second blood ingestion, dimishing the chances for parasite transmission. Since the epidemiological implications of multiple probing or feeding are obvious we are interested to pursue studies on this particular subject, especially, to explain the paucity of AVL outbreaks in some Colombian and Venezuelan areas, where *Lu. evansi* appears as vector.

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