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# DEVELOPMENT OF *LEISHMANIA (VIANNIA) BRAZILIENSIS VIANNA*, 1911 IN *LUTZOMYIA INTERMEDIA* (LUTZ & NEIVA, 1912) (DIPTERA: PSYCHODIDAE: PHLEBOTOMINAE) UNDER EXPERIMENTAL CONDITIONS

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*The development of* Leishmania (Viannia) braziliensis *in experimentally infected* Lutzomyia intermedia, *showed colonization of the hindgut from 48 h after the infective blood-meal, and the migration of flagellates to the foregut, with a massive infection of the cardia at the 5th day post infection. Up to 10 days following the infective blood-meal, very few parasites were seen in the pharynx and cibarium.*

*The role of* L. intermedia *as a vector of cutaneous leishmaniasis is discussed according to the established criteria.*

Key words: *Leishmania (Viannia) braziliensis* – *Lutzomyia intermedia* – experimental infection

Studies on the behaviour of *Leishmania* in sandflies have been made in attempts to clarify the mechanism of transmission the parasite and to increase our knowledge on the morphology of the parasite in the sandfly gut. They have brought to light some interesting new findings (Killick-Kendrick, 1979; 1986; 1987a; Sacks & Perkins, 1985; Lainson et al., 1987; Lainson & Shaw, 1988; Killick-Kendrick et al., 1988; Walters et al., 1989).

Such studies acquired more significance after Lainson & Shaw (1972; 1973) had classified the mammalian leishmanias into two groups according to the developmental patterns in the sandfly gut: the section Peripylaria (development in the hindgut with anterior migration to the foregut of the invertebrate host) and Section Suprapylaria (only midgut and foregut development). More recently, Lainson & Shaw (1987) suggested division of the genus *Leishmania* into the subgenera *Viannia* (perypilarian parasites) and the subgenus *Leishmania* (suprapylarian parasites).

Others workers have considered the criteria necessary to incriminate sandfly vectors (Killick-Kendrick, 1987b; 1990). Among the more important ones are the degree of anthropophily of the sandfly and its natural infection by the same species of *Leishmania* as that infecting man.

Supporting observations are the production of flourishing infections in experimentally infected sandflies with migration of flagellates to the foregut, suggesting that these insects can support the development of the *Leishmania* species in question after digestion of the blood-meal.

Since the observations of Aragão (1922) in a focus of cutaneous leishmaniasis in Rio de Janeiro city, epidemiological evidence has been accumulating to incriminate *Lutzomyia intermedia* as the vector of *Leishmania (Viannia) braziliensis* in several endemic areas in Southeast Brazil.

According to the criteria for incriminating a sandfly species as vector, mentioned above we report here on the development of *L. (V.) braziliensis* in experimentally infected *L. intermedia,* regarded as the most likely major natural vector of this parasite in Rio de Janeiro State.

## MATERIALS AND METHODS

*Experimental infection* – Batches of 100 females of *L. intermedia,* from a closed laboratory colony from Rio de Janeiro State in its

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39th generation, were infected with two strains of *L. (V.) braziliensis,* both from Rio de Janeiro State: MCAN/BR/87/DOG4 and MHOM/BR/86/HAS3.

The sandflies were infected by feeding them on hamster skin lesions and maintained at approximately 25 ± 1 °C, according to the technique described by Rangel et al. (1985). Some sandflies were infected through a chick-skin membrane using suspension of promastigotes (Ward et al., 1978).

*Examination of sandflies* – These were dissected at intervals between 12 h and 10 days after the infective blood-meal and the gut examined by phase-contrast microscopy.

Observations at 12, 24 and 36 h were made after fixing the infected midugt smear with methanol and staining with Giemsa.

## RESULTS

The percentage of infected sandflies and development of *L. (V.) braziliensis* in the gut of *L. intermedia,* at each period of observation, are showed in the Figure.

No significant differences were seen in the behaviour of the two strains, or after using the two feeding methods.

The results are presented following observations on the digestive tract at different periods after infective blood-meal. No parasites were seen in the midgut 12 h following the infective bloodmeal.

24 h – some oval amastigotes were seen in the midgut, among the round forms;

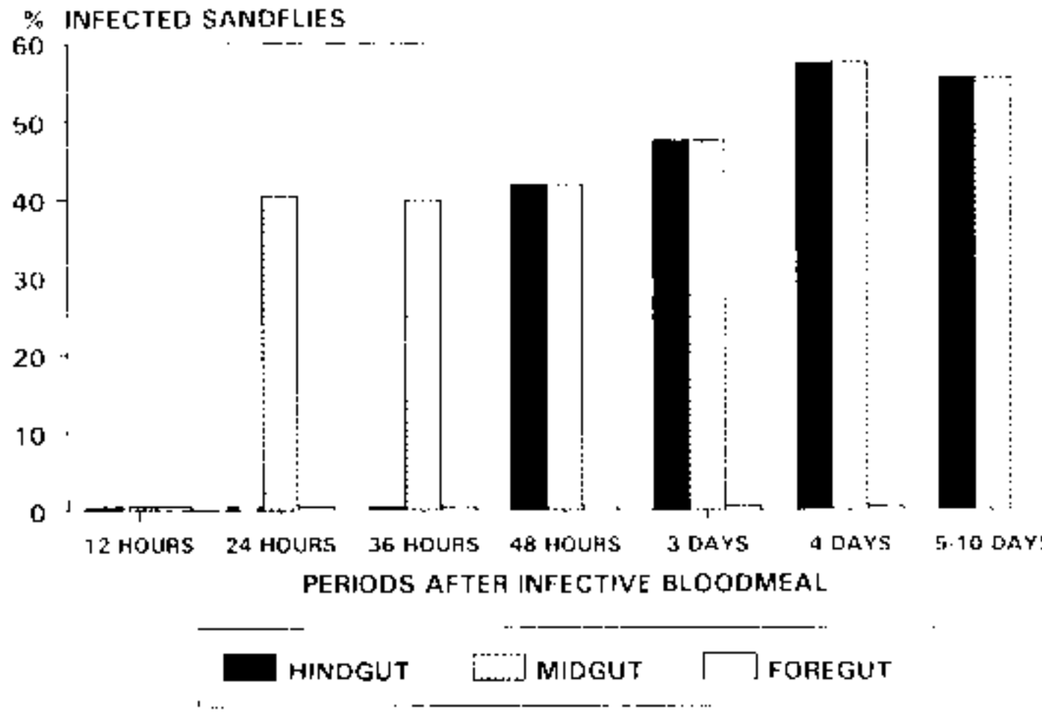
36 h – round and oval amastigotes and a few promastigotes in the midgut;

48 h – promastigotes and paramastigotes present in the pylorus and a few promastigotes in the midgut;

3 days – increasing number of paramastigotes in the pylorus and ileum, and of promastigotes in the midgut;

4 days – large numbers of paramastigotes in the ileum and pylorus, and an increasing number of promastigotes in the midgut;

5-10 days – individual paramastigotes and clusters of paramastigotes in the pylorus and ileum, a large number of promastigotes in the midgut and a massive infection of the stomodeal valve, after digestion of the blood.




Growth of *Leishmania (V.) braziliensis* in experimentally infected *Lutzomyia intermedia.*

Although, a search for flagellates in the probocis was not made systematically, in six specimens a few parasites were seen there.

## DISCUSSION

Aragão (1922) produced strong evidence suggesting *L. intermedia* as a vector of cutaneous leishmaniasis in Rio de Janeiro city and later studies have supported this view in all foci of the disease in Southeast Brazil.

In several endemic areas the distribution of *L. intermedia* coincides with that of the human disease. This species is highly anthropophilic and predominant inside houses and in peridomestic habitats (Aragão, 1922; Guimarães, 1955; Araújo-Filho, 1979; Rangel et al., 1986; 1990). It has shown to be attracted to dogs and equines infected with *L. (V.) braziliensis* (Araújo-Filho, 1979; Rangel et al., 1986; 1990), and has been found infected with *Leishmania* probably of the *braziliensis* complex in the States of São Paulo (Forattini & Santos, 1952) and Rio de Janeiro (Rangel et al., 1984).

The study of the development of *L. (V.) braziliensis* in *L. intermedia* in experimental infections helps incriminate this sandfly as a vector of cutaneous leishmaniasis. It shows the ability of *L. intermedia* to harbour *L. (V.) braziliensis* with migration of the flagellates to the foregut and massive infection of the stomodeal valve, after digestion of the infective bloodmeal.

From the above studies and existing literature, we suggest that there is no doubt about the role of *L. intermedia* as the vector of cutaneous leishmaniasis in Rio de Janeiro State.

In some specimens, flagellates were observed in the proboscis. Recently, several studies have discussed the importance of the presence of flagellates in the proboscis for the proper mechanism of transmission, of *Leishmania* species, but some workers have reported on the experimental transmission without parasites in the proboscis (Lainson et al., 1977, 1987; Pozio et al., 1985; Killick-Kendrick, 1986).

With our own results we are inclined to agree with these authors that transmission may take place after the regurgitation of parasites from the midgut to the mouth parts.

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