

**DEVELOPMENT OF *LEISHMANIA MAJOR*  
IN THE PHLEBOTOMINE SANDFLIES,  
*PHLEBOTOMUS PAPATASI* (SCOPOLI)  
AND *PHLEBOTOMUS LANGERONI* (NITZULESCU)<sup>1</sup>**

M. G. SHEHATA, M. WAHBA, T. A. MORSY, S. EL SAID\*,  
B. M. EL SAWAF

**SUMMARY.** Laboratory bred *Phlebotomus papatasi* and *P. langeroni* were examined for their susceptibility to develop *Leishmania major* promastigotes under laboratory conditions. Promastigotes were demonstrated in the gut of both species when they were given sugar 24 hr before or after an infective blood meal and in flies offered only an infective blood. The overall infection rate was slightly higher in *P. langeroni* than *P. papatasi*.

Head promastigotes were detected in *P. papatasi* provided with sugar 24 hr before or after an infective blood meal. No head promastigotes were seen in flies offered only infective blood. In *P. langeroni*, head promastigotes were only seen in flies fed on sugar before or after an infective blood and maintained at 18° C.

Results indicate that sugar plays a major role in the migration of parasites from the gut to the head. Temperature may have a marginal effect on the migration process.

Attempts to transmit *L. major* to hamster by the bite of infected *P. papatasi* were not successful.

*Key-words:* *Phlebotomus*. Experimental infection.

**Développement de *Leishmania major* chez *Phlebotomus papatasi* (Scopoli) et *P. langeroni* Nitzulescu.**

**RÉSUMÉ.** L'expérimentation conduite sur *Phlebotomus papatasi* et *P. langeroni* élevés en laboratoire a pour but d'étudier leur aptitude à permettre le développement des promastigotes de *Leishmania major* dans l'intestin et dans la tête, avec un intérêt spécial pour le rôle des glucides. Les promastigotes sont apparus dans l'intestin moyen des deux espèces lorsqu'un repas sucré a été fourni 24 heures avant ou après le repas sanguin infestant, comme dans les Phlébotomes ayant pris un simple repas de sang. Les taux d'infestation ont été sensiblement plus élevés chez *P. langeroni* que chez *P. papatasi*. Les promastigotes sont détectés dans la tête de *P. papatasi* ayant pris un repas sucré 24 heures avant ou après le repas sanguin infestant. Aucun parasite

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\* Research and Training Center on Vectors of Diseases, Ain Shams University, Abbassia, Cairo, Egypt.

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n'a été trouvé dans la tête s'il est donné seulement un repas sanguin. Avec *P. langeroni*, les promastigotes ne sont détectés dans la tête que lorsque le repas sucré a été absorbé avant ou après le repas infestant et la température maintenue à 18° C.

Le sucre semble donc jouer un rôle important dans la migration des parasites, de l'intestin vers la tête. La température a un effet secondaire mais non négligeable sur le processus migratoire.

*Mots-clés* : *Phlebotomus*. Infection expérimentale.

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Development of *Leishmania* parasites in the sandfly has been the target of many investigators to study the transmission by bite. While some investigators were able to detect gut promastigotes in sandflies, few successfully demonstrated head parasites. The difficulties of transmitting Leishmaniasis by bite in the laboratory have led to much speculations on the factors affecting the development of the parasite in the sandfly. This study was planned to study the susceptibility of *Phlebotomus papatasi* and *P. langeroni* to develop *Leishmania major* promastigotes in the gut and head with special emphasis on the role of sugar and temperature as factors influencing the parasite activity.

### Materials and methods

*Leishmania*. The strain of *L. major* (ASc-H<sub>2</sub>) used, was originally isolated from a cutaneous lesion of an Egyptian who never travelled outside El Kusayema, south east of El Arish, Sinai Governorate. The parasites were grown in NNN medium (Adler and Theodor, 1927) and typed by isoelectrophoresis (Morsy *et al.*, 1983).

*Infecting sandflies*. Cultured parasites were washed in sterile PBS, counted and inoculated ( $2 \times 10^7$  promastigotes/ml) intradermally in the foot pads of hamsters (*Mesocricetus auratus*). Inoculated hamsters were maintained at room temperature (25-30° C) until they developed feet lesions. Hamsters were anesthetized for sandfly feeding with 0.2 ml Ketamine hydrochloride (100 mg/ml) given intramuscularly. This dosage was effective for 40 to 60 minutes, the hamster was covered with a cloth so that the flies could only feed on the feet.

*Sandflies*. Laboratory bred *P. papatasi* and *P. langeroni* were originally collected from El Agamy, Alexandria, Egypt a focus of visceral Leishmaniasis (Tewfik *et al.*, 1983). Pools of 3-day-old flies of each species, were segregated into three groups: one group was offered a 30 % sucrose solution 24 hours after an infective blood meal (blood-sugar), the second group was offered the sugar meal before the infective blood (sugar-blood) and the third group was given only an infective blood meal (blood). Engorged flies were maintained at 3 different temperatures 18, 22 or 28 ( $\pm 1^\circ$  C), and 70 % R. H. By day 3 and at daily intervals, random samples of flies from each group were dissected and their guts and heads were examined to detect and locate *Leishmania* promastigotes.

## Results

In 20 experiments, *P. papatasi* and *P. langeroni*, were tested for their susceptibility to develop gut and head *L. major* promastigotes (table I).

In the first set of experiments (exp. 1-7) sandflies were provided with sugar after the infective blood meal. *P. papatasi* developed gut infection 6-7 days after the infective meal with a rate of 9.7 % and head promastigotes were detected 10-20 days post-infection at 22° C (exp. 1-3). *P. langeroni* that were maintained at 22° C, developed gut infection by day 4 and no head promastigotes were detected (exp. 7). In experiment (4-6) *P. langeroni* were maintained at 18° C the flies developed gut infection with an overall rate of 32.5 % within 3 to 4 days, head promastigotes were also detected 8 to 9 days post-infection.

In the second set of experiments sandflies were provided with sugar before the infective blood meal (exp. 8-17). *P. papatasi* maintained at 28° C developed gut infection rate of 5.1 % by day 3 post-infection and head infection 6 days after the infective meal (exp. 8-10). When *P. papatasi* was held at 22° C both gut and head promastigotes were observed by day 7 and 16 respectively (exp. 11). *P. langeroni* maintained at 22° C developed only gut infection with an overall rate of 11.3 % by day 9 post-infection (exp. 12-14). When *P. langeroni* were kept at 18° C, females developed gut and head infection 10-20 days after infective meal (exp. 15-17).

In third set of experiments sandflies were fed on an infective blood and maintained at 22° C. *P. papatasi* developed gut infection with the rate of 17.9 % by day 3-5 and *P. langeroni* developed a gut infection rate of 11.5 % by day 8 post-infection, no head promastigotes were detected under the conditions of this experiment for both species.

In three trials of transmitting *L. major* to hamsters by the bite of infected *P. papatasi* (table II) 52 flies were fed on an infected hamster and then on sugar, 6 days later they were allowed to feed again on two noninfected hamsters. Four flies took a second blood-meal and the rest consistently refused to feed again. The four flies were dissected at death and only one had gut and head promastigotes. Hamsters on which flies had refed, were kept in separate cages and observed for 8 months. No lesions appeared.

## Discussion

*Leishmania major* can develop in the mid gut of *P. papatasi* and *P. langeroni* offered an infective blood meal before or after a sugar meal.

Parasites were demonstrated in the gut of flies that had only an infective feed. The overall gut infection rate of *P. langeroni* was slightly higher than that of *P. papatasi*. Head promastigotes were detected in *P. papatasi*, when they were

TABLE I. — Experimental infections of *Leishmania major* in *Phlebotomus papatasi* and *P. langeroni*.

Type of food	Phlebotomus species	Temperature	No.		Gut-positive			Head-positive	
			Fed	Dissected	No.	%	No. days after infective blood meal	No.	No. days after infective blood meal
I. BLOOD + SUGAR									
1	<i>papatasi</i>	22	30	30	4	13.13	7	1	10
2	<i>papatasi</i>	22	51	51	5	9.8	7	1	20
3	<i>papatasi</i>	22	42	32	2	6.2	6	1	10
4	<i>langeroni</i>	18	18	18	9	50	3	3	8
5	<i>langeroni</i>	18	10	10	2	20	3	1	9
6	<i>langeroni</i>	18	15	15	3	20	4	—	—
7	<i>langeroni</i>	22	74	74	10	13.5	4	—	—
II. SUGAR + BLOOD									
8	<i>papatasi</i>	28	33	33	2	6.1	3	2	6
9	<i>papatasi</i>	28	40	40	2	5	3	—	—
10	<i>papatasi</i>	28	6	6	—	—	—	—	—
11	<i>papatasi</i>	22	31	31	2	6.5	7	1	16
12	<i>langeroni</i>	22	7	7	1	14.3	9	—	—
13	<i>langeroni</i>	22	12	12	1	8.3	9	—	—
14	<i>langeroni</i>	22	25	25	3	12	9	—	—
15	<i>langeroni</i>	18	23	23	2	8.6	17	1	20
16	<i>langeroni</i>	18	20	20	1	5	17	—	—
17	<i>langeroni</i>	18	14	14	2	14.2	10	2	10
III. BLOOD									
18	<i>papatasi</i>	22	20	20	4	20	3	—	—
19	<i>papatasi</i>	22	28	28	5	17.9	5	—	—
20	<i>langeroni</i>	22	26	26	3	11.5	8	—	—

TABLE II. — Experimental transmission of *L. major* with *P. papatasi*.

Experiment	No. of flies allowed to feed again	No. of flies that fed	Transmission	No. of flies dissected	+ VE (head)
A	19	1	-ve	1	—
B	18	1	-ve	1	—
C	15	2	-ve	2	1
Total	52	4		4	1

offered an infective blood before or after a sugar meal but not in flies offered only infective blood.

In *P. langeroni*, head promastigotes were seen only in flies offered infective blood before or after a sugar meal and maintained at 18° C, while no head promastigotes were detected when the flies were held at 22° C or when offered an infective blood without a sugar meal.

These results add a forward step to confirm the role of sugar in the migration process of *Leishmania* parasites from the gut to the head of sandflies and consequently their ability to transmit leishmaniasis as suggested by Chaniotis (1974) and Killick-Kendrick (1979).

The effect of temperature on the development and behaviour of *Leishmania* parasites in sandflies was studied by Leaney (1977) on *Lutzomyia longipalpis* experimentally infected with *L. mexicana* and by Rioux *et al.* (1985) on *Phlebotomus ariasi* infected with *L. infantum*. The authors found that with the increase in temperature, the rate of infection and the anterior migration of the parasites in sandflies increase to reach the maximum at certain temperature. Temperatures beyond or above the optimal temperature were not in favour of the parasite development.

In the present study, *P. papatasi* experimentally infected with *L. major* showed higher rate of infection at 22° C than at 28° C and the relatively higher temperature (28° C) did not prevent the forward movement of the parasite to the proboscis. This result indicates that the temperatures tested are within the limits favourable for the development of the parasite.

In case of *P. langeroni* experimentally infected with *L. major*, promastigotes were demonstrated in the head when the flies were maintained at 18° C and not when they were maintained at 22° C, this indicates that small changes in temperature may have a marked effect on the development and behaviour of *Leishmania* in sandflies.

This result which seems to contradict the finding of Rioux *et al.* (1985) may be true for this particular sandfly/parasite combination and suggests that transmis-

sion of *L. major* with *P. langeroni* at temperature higher than 18° C would seem impossible. This beside that, in nature *P. langeroni* has a seasonal occurrence throughout May to October (Beier *et al.*, 1986) when the mean temperature is higher than 20° C and disappears when the temperature is low.

It is not clear why transmission attempts using *P. papatasi* infected with *L. major* were unsuccessful, despite the presence of the parasite within the mouthparts. Molyneux (1977) suggested that the distinctive forms found within the mouthparts of the sandfly represent a terminal infective stage which can be transmitted by bite and which can successfully initiate infection in a susceptible host.

Although it is well known that flagellates are commonly developed in sandflies (WHO 1984) it remains, however, to be seen if the parasites of *L. major* that develop in the gut of *P. papatasi* and *P. langeroni* are able to transmit the disease to the vertebrate host.

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