

IMPROVED REARING CONDITIONS OF SANDFLIES (*PHLEBOTOMUS ARGENTIPES*), AS REQUIRED FOR STUDIES ON TRANSMISSION DYNAMICS OF LEISHMANIASIS

K. N. GHOSH *, A. K. GHOSH **, S. K. BASAK *, S. ADHYA **, A. BHATTACHARYA *

SUMMARY

Rearing conditions of sandflies (*Phlebotomus argentipes*) has been improved. Bloodfed females were initially maintained at 21-23° C for 5-7 days before allowing them to oviposit at 26-28° C and a proportion of flies were directly allowed to oviposit at 26-28° C just after feeding to see their oviposition survival and subsequently the refeeding rate. It was found that when the

flies were maintained following the former method gave better results. It is proposed to keep the bloodfed sandflies at a temperature lower than its suitable feeding temperature for a period longer than its oogenesis period and then to bring back the flies at slightly higher temperature, required for oviposition to get better result in oviposition survival and subsequently multiple feeding activity.

RÉSUMÉ : Amélioration des conditions d'élevage de *Phlebotomus argentipes* utile pour les études sur la dynamique de transmission des leishmanioses.

Les femelles gorgées sont — tantôt maintenues 5 à 7 jours à 21-23° avant d'être mises à 26-28° pour permettre la ponte — tantôt mises à 26-28° aussitôt après le repas pour observer leur survie après la ponte et leur attitude à prendre un second repas.

La première méthode est meilleure. Nous proposons de conserver

les phlébotomes pendant l'oogenèse à une température plus basse que celle qui est favorable au repas, puis ensuite d'élever la température. La survie après la ponte et la possibilité de repas ultérieurs sont améliorées.

INTRODUCTION

The transmission of leishmaniasis usually takes place during the second or subsequent bite of sandfly taking the first or previous feed being an infected one. But, in the laboratory, the probability of taking second blood meal is very low as most of the flies die at oviposition after first blood meal and the possibility of taking second blood meal before oviposition is not always possible as majority of sandflies are gonotrophically concordant (Killick-Kendrick, 1987). Probably, for this the successful study on the transmission of leishmaniasis is very difficult in the laboratory.

Several workers have tried to improve the oviposition survival and refeeding of sandflies in order to have successful transmission of leishmaniasis. Shortt *et al.* (1926) employed different methods for the same purpose on *Phlebotomus argentipes* but did not find any encouraging result. Later, other attempts of feeding the flies at a lower temperature (20-22° C) and then bringing back them at slightly higher temperature (24-28° C) after feeding (Killick-Kendrick *et al.*, 1977) or maintaining the flies at an uniform temperature (23-27° C) throughout (Ward, 1977) also failed to provide higher oviposition survival. We have developed a method to reduce the mortality rate of sandflies during oviposition, thus allowing studies on the transmission dynamics of Indian kala-azar.

* Immunoparasitology Research Unit, Department of Zoology, University of Calcutta, 35 Ballygunge Circular Road, Calcutta 700 019, India.

** Genetic Engineering Laboratory (*Leishmania Division*), Indian Institute of Chemical Biology, 4 Raja S.C. Mullick Road, Calcutta 700 032, India.

Address for correspondence: Pr. Amal BHATTACHARYA, P.O. Box, 10257, Calcutta 700 019, India.

Accepté le : 30 mars 1993.

MATERIALS AND METHODS

The flies used in the study were taken from a laboratory colony of *P. argentipes* (Ghosh and Bhattacharya, 1989). The females were given first blood meal either by membrane feeding method (Group A) or allowing them to bite on BALB/c mice (Group B) infected with *Leishmania donovani* (MHOM/IN/90/GE6). Simultaneously, a good number of flies were also fed on uninfected BALB/c mice or hamsters (Group C). The membrane feeding

method of Ghosh *et al.* (1989) was used for infection of *P. argentipes* and the animal feeding method was similar to that of Ghosh and Bhattacharya (1989). The flies were provided with 30 % glucose solution before and after a blood meal. A proportion of the bloodfed flies, from each group were maintained at 21-23° C (Table I). After 5-7 days, they were put in oviposition vial at 26-28° C. The rest of the flies were directly maintained at 26-28° C after blood feeding. The flies who survived oviposition were allowed to take second blood meal on BALB/c mice or hamsters.

RESULTS

Table I indicates the result of oviposition survival and refeeding rate of *P. argentipes* maintaining the bloodfed flies by two different approaches. It was found that 45.0 %, 47.0 % and 43.4 % flies survived oviposition when maintained following our new method against 19.8 %, 22.7 % and 23.7 % survivors were left by the conventional method of Group A, Group B and Group C respectively. Similarly, the success rate of giving second blood meal to the survivors of Group A, Group B and Group C by our new method was 75.0 %, 80.7 % and 71.5 % against 40.6 %, 40.7 % and 42.7 % respectively by the conventional method.

Taking all the flies of Group A, B and C into consideration, it was found that with this new method a major proportion of flies laid eggs in a scattered way (eggs were not laid in cluster) and 24.4 % (182/743) laid eggs in cluster, whereas, in the conventional method, 42.8 % (356/831) flies laid eggs in clusters. Most of the clusters of eggs were accompanied by the dead females.

The difference in oviposition survival of *P. argentipes* in the two approaches is highly significant ($z = 9.28, p < 0.01$), whereas, it is insignificant ($z = 0.77; p > 0.05$) between the infected (Group A and B) and uninfected (Group C) batches of flies maintained following the new method.

DISCUSSION

Oviposition survival and multiple feeding activity are two important parameters for the evaluation of vector potential. The result indicates that by this new method it is pos-

sible to get a higher oviposition survival and refeeding rate in sandflies (Table I). It also indicates that laying of eggs in cluster is higher in conventional approach and responsible for higher mortality rate i.e., the higher will be the clusters of eggs the lower will be the oviposition survival.

Oviposition survival and refeeding rate as observed by us are better than the results of Shortt *et al.* (1926) who found 19 % of the females to survive oviposition and 48 % of the survivors to take a second feed. Our success rate is also higher than that of Pozio *et al.* (1985), Ryan *et al.* (1987) and Knechtli and Jenni (1990) who recorded 2.2-3.2 %, 10.5 % and 2.7 % refeeding rate respectively with different sandfly species.

Although earlier workers have indicated their success in transmitting leishmaniasis but those reports lack the details of the sandfly maintenance viz., suitable temperature for higher longevity, oviposition and its oogenesis period. These phenomena usually vary at different temperatures and among species and they are vital points to be followed in maintaining sandflies while studying the transmission dynamics. The main idea which we have followed and propose for better results is that the flies after blood feeding should be maintained at temperature (21-23° C for *P. argentipes*), lower than the range of suitable temperature required for oviposition (26-28° C for *P. argentipes*) for a period longer than its oogenesis period (in case of *P. argentipes* it is 4-5 days) and then to bring back the flies to a temperature range suitable for the oviposition of the species. Immediately after oviposition these flies can be offered a 2nd or subsequent blood meals to which they show good response.

Acknowledgments. — The senior author (KNG) is indebted to Dr. R. KILLICK-KENDRICK, Dr. R. D. WARD, Dr. M. MAROLI, Dr. M. J. MUTINGA, Dr. D. G. YOUNG and Pr. J.-A. RIOUX for their help on various aspects. The membrane feeder was kindly given by Dr. R. KILLICK-KENDRICK. We are thankful to the Distributed Information Centre (DIC), Bose Institute, Calcutta, for providing computer facility in typing the manuscript. Our thanks are due to Mr. DEBASIS BANERJEE for translating the title, key words and summary into french.

TABLE I. — The effect of post blood meal maintenance on the oviposition survival and refeeding of *P. argentipes* in two different methods.

	Mode of blood feeding	No. of bloodfed flies	Initially kept for 5-7 days at	Finally kept for oviposition at	No. of females laid eggs in cluster	Oviposition survival	2nd blood meal taken
Group A	On infected animals	142	21-23° C	26-28° C	35	64	48
		161	26-28° C	26-28° C	73	32	13
Group B	Via membrane feeding	221	21-23° C	26-28° C	49	104	84
		237	26-28° C	26-28° C	101	54	22
Group C	On fresh laboratory animals	380	21-23° C	26-28° C	98	165	118
		433	26-28° C	26-28° C	182	103	44

REFERENCES

- Ghosh K. N., Bhattacharya A. : Laboratory colonization of *Phlebotomus argentipes* (Diptera: Psychodidae). *Insect Sci. Applic.*, 1989, 10, 551-555.
- Ghosh K. N., Ghosh D. K., Bhattacharya A. : Temperature difference, an useful stimulus for feeding phlebotomine sandflies (Diptera: Psychodidae) in the laboratory. *Riv. Parassitol.*, 1989, 51, 317-320.
- Killick-Kendrick R. : Methods for the study of phlebotomine sandflies. In: W. Peters and R. Killick-Kendrick (eds.). The Leishmaniasis, Vol. 1, *Academic Press*, London, 1987, 473-497.
- Killick-Kendrick R., Leaney A. J., Ready P. D., Molyneux D. H. : *Leishmania* in phlebotomid sandflies. IV. The transmission of *Leishmania mexicana amazonensis* to hamsters by the bite of experimentally infected *Lutzomyia longipalpis*. *Proc. R. Soc. Lond. B*, 1977, 196, 105-115.
- Knechtli R., Jenni L. : Experimental transmission of *Leishmania infantum* by the bite of *Phlebotomus perniciosus* from Switzerland. *Acta Trop.*, 1990, 47, 213-216.
- Pozio E., Maroli M., Gradoni L., Gramiccia M. : Laboratory transmission of *Leishmania infantum* to *Rattus rattus* by the bite of experimentally infected *Phlebotomus perniciosus*. *Trans. R. Soc. Trop. Med. Hyg.*, 1985, 79, 524-526.
- Ryan L., Lainson R., Shaw J. J., Wallbanks K. R. : The transmission of suprapylarian *Leishmania* by the bite of experimentally infected sandflies (Diptera: Psychodidae). *Mem. Inst. Oswaldo Cruz.*, 1987, 82, 425-430.
- Shortt H. E., Barraud P. J., Craighead A. C. : An account of methods employed on feeding and refeeding sandflies, *P. argentipes*, for the second and third time, on man and animals. *Indian J. Med. Res.*, 1926, 13, 923-942.
- Ward R. D. : The colonization of *Lutzomyia flaviscutellata* (Diptera: Psychodidae), a vector of *Leishmania mexicana amazonensis* in Brazil. *J. Med. Entomol.*, 1977, 14, 469-476.