



STUDIES ON THE DEVELOPMENT OF *LEISHMANIA DONOVANI* IN *PHLEBOTOMUS ARGENTIPES* AND *P. PAPATASI* AFTER BLOOD MEALS ON KALA-AZAR PATIENTS IN BIHAR, INDIA

by

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ABSTRACT

A total of 258 laboratory-bred *Phlebotomus argentipes* were let to feed on untreated parasitologically confirmed kala-azar cases. Successful development of parasites was noted in 0.54% *P. argentipes* which had fed during daytime and in 5.33% of those that had fed during the night. Of a total of 245 laboratory-bred *P. papatasi* feeding both day and night on the same patients, none was found positive for successful development of *Leishmania donovani* in the foregut.

1. INTRODUCTION

Visceral leishmaniasis or kala-azar apparently disappeared from India between 1960 to 1970, as a collateral benefit of DDT spraying under the National Malaria Control Programme, later the Malaria Eradication Programme. Sanyal et al. (1979) has mentioned that since the 1970s North Bihar has again come under the grip of kala-azar. Cases reappeared with the cessation or reduction of DDT spraying and new outbreaks occurred (100 000 cases in 1972 and 40 000 in 1978). Since 1980, approximately 15 000 new cases per year were reported but a sharp increase was notified in 1990 with 54 600 new cases and 549 deaths; 30 of 39 districts are currently endemic for visceral leishmaniasis. In this recent resurgence of kala-azar, practically no studies had been carried out to re-establish the vectorial capacity of *Phlebotomus* sandfly species prevalent in North Bihar, India. Hence, the present study was conducted to determine the vectorial capacity of two important species of North Bihar, i.e. *Phlebotomus argentipes* and *P. papatasi*, the proven and suspected vector, respectively, of kala-azar in India (WHO, 1984).

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2. MATERIALS AND METHODS

The study was conducted between March and August 1988. Adult *P. argentipes* and *P. papatasi* were caught in North Bihar. Eggs from the two species were collected in Hilton pots, brought to the laboratory, and reared to adults. Then batches of sugar-fed 3-day-old laboratory-bred *P. argentipes*¹ and *P. papatasi*² were allowed to feed on five untreated parasitologically confirmed kala-azar patients from North Bihar. Both species were allowed to feed simultaneously during the day from 11.00 to 12.00 hours and from 23.00 to 24.00 hours at night. After blood feeding, the flies were kept alive on a raisin diet up to five days, at a temperature of 27°C ± 1 and at a humidity of 65% ± 5. The guts of sandflies were dissected on the sixth day and examined under the microscope using x 400 and x 1000 magnifications. Spermathecae and cibarian armatures of the dissected flies were also observed for species identification and the position of promastigotes in the gut of flies was noted. The gut was then washed thrice with a sterile normal saline solution and punctured with a sterile pipette. A small drop of gut content was inoculated into a NNN medium and incubated at 22°C. The remaining portion of the gut was air-dried and stained by the Leishman method for final morphological examination.

3. RESULTS AND DISCUSSION

North Bihar's location in a low-lying alluvial plain and the poor socioeconomic conditions of its inhabitants have contributed to the large number of kala-azar patients since the 1970s (Sanyal et al., 1979).

Of 183 *P. argentipes* which had fed on kala-azar patients during the day, 1 (0.54%) showed development of promastigotes in the foregut; of the 75 *P. argentipes* which had been feeding during the night, 4 (5.33%) did. The promastigotes were noted in the foregut and cardia region of the five infected *P. argentipes*; they were isolated in a NNN medium.

Incrimination of *P. argentipes* as the vector of kala-azar in India is based on a long history of experimentation, which started in the mid-1920s when Knowles et al. (1924) demonstrated *Leishmania donovani* flagellates in laboratory-bred *P. argentipes* that had fed on kala-azar patients. Later, Christophers, Shortt & Barraud (1925), Shortt, Barraud & Craighead (1927) and Shortt et al. (1928 & 1931) carried out detailed studies on the vectorial capacity of *P. argentipes* in transmitting *L. donovani* in India. Swaminath et al. (1942) provided the final evidence that *P. argentipes* was the principal vector of kala-azar in the country.

Practically no work has been done on the vectorial capacity of *P. argentipes* and *P. papatasi* in the transmission of Indian kala-azar in a recent epidemic in North Bihar. Nandy (1986) demonstrated *L. donovani* bodies in 1.8% of 432 peripheral blood smears examined. All positive smears were obtained between midnight to 04.00 hours, which is the time during which sandflies bite (Hati et al., 1981). Our present observations corroborate the findings of Nandy (1986).

Adler (1947) pointed out that when *P. papatasi* were fed the Sudan strain of *L. donovani* flagellate suspensions from culture media, the flagellates behaved differently: when fed in small numbers they had the tendency to die within a few days, but in large numbers they reached the buccal cavity in three days, suggesting a breakdown of natural immunity by massive doses. Adler excluded *P. papatasi* as a carrier of kala-azar in the Sudan on epidemiological grounds. Borovsky and Schlein (1987) suggested that a specific component with trypsin-like activity in the midgut prevents the survival of *L. donovani* in *P. papatasi*. In the present study, from a total of 245 laboratory-bred *P. papatasi* none was found positive for successful development of *L. donovani* in the gut except one fly, which showed very scanty and poorly developed promastigotes only in the stomach.

Based on the above findings, it may be concluded that *P. argentipes* plays the role of vector of kala-azar in India, whereas *P. papatasi*, the prevalent phlebotomine species in North Bihar, does not.

¹ A total of 258 flies.

² A total of 245 flies.

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RESUME

Un total de 258 phlébotomes *P. argentipes*, élevés au laboratoire, ont été nourris sur des patients présentant un kala-azar confirmé parasitologiquement et avant tout traitement.

Le développement de leishmanies dans l'intestin antérieur de *P. argentipes* a été observé chez 0,54 % des spécimens nourris de jour et chez 5,33 % de ceux nourris la nuit.

Par contre, chez aucun des 245 spécimens de *P. papatasi*, nourris dans les mêmes conditions (de nuit et de jour), le développement de leishmanies dans l'intestin antérieur n'a pu être mis en évidence.

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