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RESEARCH NOTE

NATURAL INFECTION OF *LUTZOMYIA OVALLESI* (DIPTERA: PSYCHODIDAE) WITH PARASITES OF THE *LEISHMANIA BRAZILIENSIS* COMPLEX IN A RESTRICTED FOCUS OF CUTANEOUS LEISHMANIASIS IN NORTHERN VENEZUELA

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Preliminary epidemiological observations on cutaneous leishmaniasis in San Esteban, Carabobo State, Venezuela led to the conclusion that *Lutzomyia ovallesi* (Ortíz, 1952) may be implicated in transmission of this disease (Feliciangeli, 1987, *Mem. Inst. Oswaldo Cruz, 82:* 177-179). High anthropophily of this species and positive correlation between the prevalence of leishmaniasis and the population densitives of *Lu. ovallesi* were statistically proven. However since flagellates were not found in 397 females dissected from 1979 to 1980, it was assumed that a larger sample was required.

In November 1986, sandfly collections were resumed in the same locality using Shannon and CDC miniature light traps. Cryopreservation of the insects in liquid nitrogen in a solution of 10% Dimethyl-sulphoxide in PBS = 7.2 (Minter & Goedbloed 1971, *Trans. R. Soc. Trop. Med. Hyg., 65:* 175-181; Morales et al., 1981, *Biomédica, 1:* 37-41; Young & Lawyer, 1987, In: *Current Topics in Vector Research,* K. F. Harris New York *4:* 29-71) allowed continuous storage and therefore dissection of a large number of females. Parallel to the search for promastigotes, observations on the presence or absence of bloodmeals and the follicular development were made according to Christophers (1911, *Paludism, 2:* 73-89) as modified by Mer (1936, *Bull. Entomol. Res., 27:* 351-59) (Magnarelli et al., 1984, *J. Med. Entomol., 21:* 681-689). The number and percentage of dissected anthropophilic species were as follows: 4115 *Lu. ovallesi* (79.0%), 784 *Lu. panamensis* (15.1%) and 309 *Lu. gomezi* (5.9%).

Four *Lu. ovallesi* collected in Shannon traps in the following dates: 01.IX.87, 27.X.87, 4.XI. 87, 20.I.88 and cryopreserved the day after collection, were found positive for flagellates (0.08%) when the dissection was practised (07.IX.87, 12.XI.87, 4.III.88 and 19.IV.88 respectively).

In two of these specimens free forms were observed actively moving in the midgut while in the pylorus the parasites formed rosettes. Their aspect and location suggest that they were paramastigotes of the *Leishmania braziliensis* complex (Killick-Kendrick 1979, In: *Biology of the Kinetoplastida,* W.H.R. Lumsden & D.A. Evans. London/ New York, *2:* 395-460). An attempt to identify the parasites of the first isolate using monoclonal antibodies, was not successful. The other specimen, which showed heavy infection from the pylorus to the stomodeal valve, was used to inoculate a golden hamster. The Giemsa stained slide showed promastigote forms indistinguishable from *Leishmania* spp. These two females had no remnant of bloodneal in the midgut and the follicular development corresponded to stage I.

In the two remaining infected *Lu. ovallesi* the parasites were observed only in the midgut. In these specimens, however, the dissection was far from perfect and we were unable to determine the exact location of the parasites along the gut. One of them showed remnant of bloodmeal. Follicular development in both females was in stage I. Three more golden hamsters were inoculated, and are still under observation.

The dates of collection listed above show that such captures are clustered in the final months of the year, despite the regular collection of phlebotomine sandflies during the

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whole year. This observation agrees with the epidemiological records of human cases of cutaneous leishmaniasis in the same area, in the first quarter of the year (Giordanelli et al., 1978, *Bol. Dermat. Sanit., 16:* 48-52) since a period of incubation of one week to three months is commonly accepted for the appearance of leishmanial lesions.

Lu. ovallesi has previously been reported infected by flagellates. Correa (in Williams, 1970, *Trans. R. Soc. Trop. Med. Hyg., 64:* 317-368) reported finding flagellates in 1 out of 7 *Lu. ovallesi* in Panama. These were described as forming small, round, hindgut rosettes but appear not to have been seen again during the past 20 years of intensive work carried out in Panama (Christensen et al., 1983, *J. Med. Entomol. 20.* 463-484). In Belize, Williams *(loc. cit.)* described 1/627 *Lu. ovallesi* infected with flagellates in the midgut, hindgut and Malpighian tubules; the majority of which were "undoubtedly promastigotes" but which did not grow in NNN medium. The presence of a parasite of the *Le. braziliensis* complex in Belize where the vector has yet to be demonstrated has been recently confirmed (Evans et al., 1984, *Trans. R. Soc. Trop. Med. Hyg., 78:* 35-42).

The cutaneous leishmaniasis focus in San Esteban was studied in 1976 by Giordanelli et al. *(loc. cit.)* who observed the regular appearance of 10 to 20 new cases per year and an elevated rate of allergy (50%). These characteristics define the area as an ancient endemic focus (Pifano, 1960, *Arch. Ven. Med. Trop. Paras. Med., 3:* 31-61). However no typing of isolates from patients was done.

In May 1980, we isolated parasites from an ulcer on the right lower leg of a 61-year old male adult, resident of San Esteban. This isolate has been maintained since then in golden hamsters by successive passages. In July 1987, the isolate was identified (passage no. 49) in the Instituto de Biomedicina, Caracas as *Le. braziliensis* by the examination of the k-DNA and the use of monoclonal antibodies. This human parasite might be the same as the parasite found for the first time in the naturally infected *Lu. ovallesi.*

With regard to possible reservoirs, *Lu ovallesi* has been found feeding preferentially on sloths, anteaters and armadillos (Christensen et al., *loc. cit.*). However, the only *Leishmania* sp. reported in animals at San Esteban was isolated from *Heteromys anomalus.* In hamsters this parasite produced a rapid infection with lesions rich in amastigotes and numerous metastases (Torrealba et al., 1972, *Trans. R. Soc. Trop. Med. Hyg., 81:* 361) which raises the suspicion that the isolate might belong to the *Le. mexicana* complex. This suggests that more than one *Leishmania* species may be circulating in San Esteban, Venezuela.

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