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Research Note

AMAZONIAN VISCERAL LEISHMANIASIS – DISTRIBUTION OF THE VECTOR *LUTZOMYIA LONGIPALPIS* (LUTZ & NEIVA) IN RELATION TO THE FOX *CERDOCYON THOUS* (LINN.) AND THE EFFICIENCY OF THIS RESERVOIR HOST AS A SOURCE OF INFECTION

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In Amazônia, the "crab-eating fox", *Cerdocyon thous*, commonly harbours *Leishmania (Leishmania) chagasi* Cunha & Chagas, the cause of American visceral leishmaniasis. Thus, the parasite has to date been isolated from 11 of 26 (42.3%) apparently healthy animals from the municipality of Cachoeira do Arari, Island of Marajó, Pará (R. Lainson et al., 1987, *Trans. R. Soc. Trop. Med. Hyg., 81:* 517 and unpublished observations). Finally, in more recent studies in the municipality of Salvaterra, Marajó, 22 locally caught foxes were radio-tagged and released for ecological observations: of these, 12 (54.6%) have been shown to be positive to the indirect fluorescent antibody test (IFAT), 2 with titres as high as 1:1,280. None of the animals has shown any abnormal behaviour or unusual appearance suggesting ill-health.

Lutzomyia longipalpis is till now the only proven vector of *L. (L.) chagasi*, and it was first recorded in the Amazon Region during epidemiological studies on 2 small, rural foci of visceral leishmaniasis in Moju and Abaetetuba, Pará, about 30 km from Marajó (C. Chagas et al., 1938, *Mem. Inst. Oswaldo Cruz, 33:* 89-229). This was at a time when there was no road system linking this region with the rest of Brazil, and it seems reasonable to suppose, therefore, that this sandfly was not introduced from the highly endemic areas of visceral leishmaniasis in the northeastern States, but that it forms a natural component of the phlebotomine fauna in certain regions of Amazônia.

Lutzomyia longipalpis is one of a number of neotropical sandflies which commonly invade man's rural habitations. This fact, together with the high rate of infection with *L. (L.) chagasi* in foxes, has led to the suggestion that there may be an enzootic maintained in these animals by a silvatic population of *Lu. longipalpis*, from which peridomestic foci of canine and human visceral leishmaniasis might be derived (R. Lainson, 1989, in M. W. Service, *Demography and Vector-Borne Diseases*, CRC Press, Boca Raton, Florida, U. S. A.). Such foci could be initiated if (a) infected sandflies migrate to human habitations; (b) if dogs acquire infection when entering an enzootic area (as, for example, when accompanying night-time hunters); or (c) if peridomestic *Lu. longipalpis* become infected after feeding on foxes when these animals move through inhabited areas at night. Whatever the route of entry, infected dogs will soon assume the rôle of principal reservoir of infection for the extra-silvatic *Lu. longipalpis*, due to the fulminating disease which *L. (L.) chagasi* produces in these animals and the vast number of parasites commonly present in their skin. At this stage of events, the movements of infected dogs are doubtless of importance in creating new foci of visceral leishmaniasis and they may also help to maintain the fox enzootic. As infected dogs may survive for some years, the peridomestic transmission cycle will persist unless steps are taken to destroy these animals and/or eliminate the sandfly vector.

This preliminary note records our attempts to test critical elements in the foregoing hypothsis, more specifically to indicate the existence

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of a silvatic *Lu. longipalpis* population, and to show that this sandfly can in fact become infected by feeding on foxes having a characte - istically occult infection with *L. (L.) chagasi.* Field observations were made in the Salvaterra study area: one of low human endemicity, but where our present observations have shown 49.0% of 247 dogs to be positive to the IFAT, and where *Lu. longipalpis* was abundant in many of the chicken-houses.

Captures of sandflies in the dry season – During 17 May – 16 July, 1988, we made 80 attempts to capture *Lu. longipalpis* (28 trapping-nights) using CDC miniature light-traps, set at a height of about 1.5 m in a patch of residual primary forest, and about 500 m from the nearest house on the outskirts of the small village of Chiquita. The traps were variously placed over cages of chicken (57), chicken + male *Lu. longipalpis* (11), a fox (8), and a box of sawdust impregnated with urine and faeces from a fox (3). One unbaited, lighted trap was set on one occasion.

The total of 47 *Lu. longipalpis* captured (22 ♂♂ and 25 ♀♀) was made up of 21 (7 ♂♂: 14 ♀♀) from the traps baited with chicken + *Lu. longipalpis;* 19 (9 ♂♂: 10 ♀♀) from those with chicken only; 5 ♂♂from those over a fox; 1 ♂ from those over the contaminated sawdust; and 1 ♀ from the unbaited, lighted trap. A total of 1,361 sandflies of other species caught included 11 different species, of which *Psychodopygus complexus* Mangabeira was by far the most common.

During this same study period, 14 captures with chicken-baited traps (7 trapping-nights) were made in nearby savanna (thorny bushes and grassland), and 2 with traps placed under roosting chicken in a tree in the back-yard of the nearest house. Both of these capture sites were about 500 m from the traps in the forest.

The savanna yielded no *Lu. longipalpis,* and a total of 28 sandflies of 3 other species. The 2 peridomestic captures produced 5 *Lu. longipalpis* (1 ♂: 4 ♀♀) and 4 sandflies of 3 other species.

Captures of sandflies in the wet season – Trapping was continued during three periods within the rainy season (which, on Marajó, usually extends from late November to late May). On these occasions only a single CDC light-trap was placed in each of the 3 trapping-sites in the forest, savanna and peridomestic habitats, and they were baited only with chickens.

(a) *Period 27 January to 3 February, 1989* – At this time the results were much more impressive, with totals of 1,119 (452 ♂♂: 667 ♀♀) *Lu. longipalpis* and 1,263 sandflies of other species in the forest (8 trapping-nights); 4 (1 ♂: 3 ♀♀) *Lu. longipalpis* and 522 sandflies of other species in the savanna (8 trapping-nights); and 756 (367 ♂♂: 389 ♀♀) *Lu. longipalpis* and 54 sandflies of other species in the back-yard of the house (2 trapping-nights).

(b) *Period 19 March to 1 April, 1989* – During this period the light-traps caught totals of 41 (11 ♂♂: 30 ♀♀) *Lu. longipalpis* and 1,015 sandflies of other species in the forest (12 trapping-nights); no *Lu. longipalpis* and 21 sandflies of other species in the savanna (6 trapping-nights); and 300 (97 ♂♂: 203 ♀♀) *Lu. longipalpis* and 12 sandflies of other species in the peridomestic site (10 trapping-nights).

(c) *Period 16 – 27 April, 1989* – Forest catches were only 1 ♀ *Lu. longipalpis* and 289 sandflies of other species (12 trapping-nights): in the savanna, no *Lu. longipalpis* and 89 sandflies of other species (12 trapping-nights): and in the back-yard of the house, 218 (108 ♂♂: 110 ♀♀) *Lu. longipalpis* and 3 sandflies of other species (12 trapping-nights).

From the results so far, the savanna would seem to be an unattractive habitat for *Lu. longipalpis.* Only 4 were caught in the Chiquita savanna during the whole of the above studies, and we failed to catch this species after 78 trapping-nights in 12 different savanna sleeping-sites of foxes elsewhere (sometimes close to chicken-houses containing abundant *Lu. longipalpis*). On the other hand, the forest trapping-site does appear to be one extra-peridomestic habitat in which the sandfly-fox contact might be made, for fox-tracking has shown one pair of *C. thous* to enter it; furthermore, both of these animals were serologically positive.

A marked association of male and female *Lu. longipalpis* in the traps on many of the positive capture nights in the forest, in both the dry and the wet seasons, strongly suggests that such forest may serve as a breeding-site for this sandfly. The future use of emergence-cones and a search for immature stages of the insect may provide more definite proof.

The fox, C. thous, *as a source of infection for* Lu. longipalpis – Laboratory-bred. *Lu. longipalpis* (Marajó colony) were infected with *L. (L.) chagasi* (fox strain from Marajó) by feeding them, through a chick-skin membrane, on a suspension of heavily infected hamster spleen suspended in rabbit blood: all of 5 flies dissected 4 days later showed large numbers of promastigotes. On the 6th day post-infection, only 4 sandflies remained alive and attempts were made to feed these on a captive fox which was serologically negative to the IFAT at the time of the experiment and 7 weeks previously. Due to difficulties in sedating the animal, only 2 of the flies were induced to feed (on the head), but subsequent dissection showed both of these insects to have massive infections.

Five weeks later the IFAT titre of the fox was 1:1,280, and on the 8th week we fed 130 clean *Lu. longipalpis* on the animal: none of these insects was infected when dissected 4 days later.

Finally, 15 weeks after the infected sandflies had fed on the fox, a further 60 clean *Lu. longipalpis* were fed on the animal. A total of 54 surviving flies was dissected, just under 4 days after the blood-meal, and 4 (7.4%) showed light to moderate promastigote infections.

Unfortunately the experimental fox died under the anaesthetic, and we were thus robbed of the opportunity of ascertaining just how long the fox might have continued serving as a source of parasites for further sandflies: plans have been made to repeat the experiment with this in mind. Although no amastigotes were detected is stained smears of tissues from the fox, the parasite was isolated in blood-agar cultures of the skin from the ears and the nose, and in pairs of hamsters inoculated with suspensions of spleen, liver and bone-marrow, respectively. The isolates were identified as *L. (L.) chagasi* by monoclonal antibodies and enzyme profiles.

In conclusion – The frequency of occult infection with *L. (L.) chagasi* in the fox *C. thous;* the experimental transmission of the parasite to a fox by the bites of only 2 *Lu. longipalpis;* the infectivity of this animal to 7.0% of sandflies fed on it nearly 4 months later, in spite of the occult nature of the infection; the isolation of *L. (L.) chagasi* from the skin of this animal (in addition to the viscera and bone-marrow); and the presence of large numbers of *Lu. longipalpis* in a silvatic habitat for at least part of the year, all add weight to the suggestion that there may be an enzootic of *L. (L.) chagasi* maintained in foxes by this sandfly. This may be of importance in the maintenance of the parasite in uninhabited or sparsely populated regions, and in serving as a source from which peridomestic foci of the canine and human disease may arise.

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