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Short Report

Lutzomyia evansi, an alternate vector of *Leishmania chagasi* in a Colombian focus of visceral leishmaniasis

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It is widely accepted that *Lutzomyia longipalpis* is the proven vector of visceral leishmaniasis (VL) in the Americas (Zeledón, 1985; Deane & Grimaldi, 1985). Pifano & Romero (1964, 1973), working in a VL focus in the Venezuelan island of Margarita, collected several species of sandflies, among which *Lu. evansi* was found at times of the year when *Lu. longipalpis* was absent. In addition to the latter species, the authors considered *Lu. evansi* to be a putative vector of VL in this locality. In Costa Rica, Zeledón *et al.* (1984) also observed the association of *Lu. evansi* with *Lu. longipalpis* in an endemic focus of the disease.

During exploratory visits to the aboriginal reserve of San Andrés de Sotavento (SAS), Department of Córdoba, Colombia, preliminary entomological studies were undertaken to identify the anthropophilic phlebotomines of this area, in which VL and cutaneous leishmaniasis are endemic.

Previous sandfly collections revealed that *Lu. evansi* was the predominant species at different times of the year (Vélez *et al.*, 1988). During the present work, sandflies were caught between 1800 and 2300 h using Shannon traps and protected human bait in houses and the peridomiciliary region. In the laboratory 329 cryopreserved female sandflies were thawed and individually dissected for taxonomic purposes, and to search for promastigote infection in the digestive tract.

Lu. evansi was the predominant species (87%), followed by *Lu. gomezi* (10%) and *Lu. panamensis* (3%). In one specimen of *Lu. evansi* long promastigotes were found in the hindgut and stomodeal valve. The parasites (fewer than 200) were suspended in phosphate-buffered saline plus 1% v/v penicillin-streptomycin (Gibco), and inoculated intraperitoneally to a golden hamster. The animal was killed after 3 months, despite the absence of clinical signs of VL. Abundant amastigotes were observed in Giemsa-stained smears from spleen and liver. Triturates of these organs were inoculated to Senekjie's and Schneider's culture media, and a bone-marrow aspirate was inoculated intraperitoneally to a second hamster. Due to subculturing problems with the first isolate, the latter animal was killed 4 months later, at which time no clinical sign of disease was observed. Giemsa-stained smears from liver and spleen revealed a large number of amastigotes, which readily grew in the culture media mentioned above, yielding enough material for isoenzyme studies, as described by Saravia *et al.* (1985). In addition to the *Leishmania braziliensis* complex reference strains (Saravia *et al.*, 1985), *L. donovani* MHOM/IN/80/DD8 and *L. chagasi* MHOM/BR/74/PP75 were included in the test. Six enzymes, nucleoside hydrolase (NH; E.C.3.2.2.2), glucose phosphate isomerase (GPI; E.C.5.3.1.9), phosphogluconate dehydrogenase (GPGD; E.C.1.1.1.4.4), mannose isomerase (MPI; E.C.5.3.1.8), aspartate aminotransferase (ASAT; E.C.2.6.1.1), and superoxide dismutase (SOD; E.C.1.1.5.1.1) were examined.

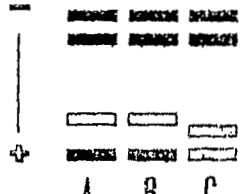

Figure Nucleoside hydrolase (E.C. 3.2.2.2.) pattern of *Leishmania chagasi* isolated from *Lutzomyia evansi* (C), compared with *L. donovani* MHOM/IN/80/DD8 (A) and *L. chagasi* MHOM/BR/74/PP75 (B) reference strains.

With the exception of NH, in which a slight difference in one of the 3 bands was observed (Figure), the isoenzyme pattern of the parasites isolated from *Lu. evansi* was identical to those of the *L. donovani* and *L. chagasi* reference strains.

The abundance of *Lu. evansi* in the SAS focus, together with its tendency to feed on man and its proven natural infection with *L. chagasi*, suggest that this sandfly species is a vector of VL. Although the presence of *Lu. longipalpis* cannot be ruled out until more extensive field studies have been carried out, our failure to collect it on this and other visits to SAS (Vélez *et al.*, 1988) demonstrates that *Lu. evansi* can be considered an alternate or even primary vector of VL in particular foci of transmission in the Americas. The secondary role of other sandfly species as vectors of VL has been previously proposed by Ryan *et al.* (1984). They observed that *Lu. antunesi* was naturally infected with promastigotes which, because of their suprapylarian location, suggested an *L. chagasi* infection, although its definitive taxonomic identification was not achieved. The recognition of *Lu. evansi* as a new vector for the disease draws attention to the importance of determining its geographical distribution, as well as its significance in VL transmission when sharing the same ecological niche with *Lu. longipalpis*. Because vector potential is the result of multiple biological factors, among them sandfly behaviour, population dynamics and host-parasite interactions, future field and laboratory studies should concentrate on *Lu. evansi* also to evaluate fully the transmission cycle of American visceral leishmaniasis.

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