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Natural hosts of *Leishmania mexicana amazonensis* Lainson and Shaw, 1972 (Kinetoplastida: Trypanosomatidae) in French Guiana

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Abstract

The natural infection of the echimyid rodent, *Proechimys cuvieri*, and the sandfly *Lutzomyia flaviscutellata* by *Leishmania mexicana amazonensis* in French Guiana is reported.

Introduction

Cutaneous leishmaniasis due to *Leishmania braziliensis guyanensis* Floch, 1954, was first described in French Guiana (FLOCH, 1943). It extends into the northern Amazon Basin and is locally called "pian bois" or "bush yaws". Epidemiological studies carried out both in Pará State, Brazil, and in French Guiana, showed that the disease is a wild zoonosis occurring in the canopy of the primary rain forest, with the edentates *Choloepus didactylus* (the two-toed sloth) and *Tamandua tetradactyla* (the lesser anteater) as main reservoirs (LAINSON *et al.*, 1981; GENTILE *et al.*, 1981) and the sandfly *Lutzomyia umbratilis* as the vector (LAINSON *et al.*, 1976, 1979; WARD & FRAIHA, 1977; LE PONT & PAJOT, 1980).

We have recently reported the natural infection of the echimyid rodent *Proechimys cuvieri* Petter, 1978 by *Leishmania mexicana amazonensis* LAINSON & SHAW, 1972, showing the occurrence in French Guiana of a second species of anthropotropic *Leishmania* (see DEDET *et al.*, 1984).

This present paper reports the first isolation in French Guiana of *L. m. amazonensis* from the sandfly *Lutzomyia flaviscutellata* (Mangabeira, 1942), with complementary information on the epidemiology of this wild zoonosis in French Guiana.

Materials and Methods

The sandfly and mammal collections were made between January 1982 and June 1983, in a degraded area within a primary forest (FRG, Montsinery).

Trapping of mammals

Rodents and opossums were trapped in metal traps (Tomahawk Live Trap Co.) baited with various local fruits (awara, banana and mango). Edentates were purchased from local hunters.

Sandfly collections

Sandflies were captured at ground level using human bait. All flies attracted to a bared leg were collected individually in cotton-stoppered glass tubes. The collection period was during the four hours following sunset (6 to 10 p.m.) and was repeated for 15 days every month.

Isolation of Leishmania *from mammals*

Animals were examined directly for skin lesions. Blood obtained by heart puncture and bone-marrow collected by trepanning the femur were inoculated into NNN medium and RPMI medium (Gibco) supplemented with 15% foetal calf serum (Flow). Pieces of skin collected from the nose and the base of the tail, and pieces of spleen and liver were ground in mortars, the suspensions cultured in NNN and RPMI medium and inoculated into the dorsal part of the hind feet of golden hamsters.

Isolation of Leishmania *from sandflies*

Sandflies collected were identified by morphology of the genitalia. Females were dissected and their intestinal tract examined under the microscope. When found infected, the intestine tract was picked up from the slide and carefully disrupted in 0·5 ml of sterile isotonic saline. Some of the suspension was inoculated in NNN medium culture tube, and the rest into the dorsal part of the hind feet of a golden hamster.

Growth of Leishmania *stocks and cryopreservation*

The culture tubes were examined every week and discarded if negative after two months. Hamsters were examined every month and kept for more than 18 months; when the hamster inoculations were positive, the granuloma were collected, ground in a mortar and the suspension inoculated into NNN and RPMI cultures.

The stocks were routinely passaged in RPMI culture and the isolates preserved in liquid nitrogen at −196°C.

Isoenzyme characterization of the stocks

The stocks were grown on RPMI medium (Gibco) supplemented with 15% foetal calf serum (Flow). The cultures were harvested by centrifuging 2,500 rpm for 10 min, and pellets were stored, without washing, at −70°C until use. Just before electrophoresis, an equal volume of hypotonic enzyme stabilizer was added. Lysis of the parasites was achieved through freeze-thawing procedures repeated three times.

The following enzymic systems were used for characterization: malate dehydrogenase: E.C.1.1.1.37 (MDH), malate dehydrogenase (oxaloacetate decarboxylating) NADP + : E.C.1.1.1.40. (ME), isocitrate dehydrogenase: E.C.1.1.1.42 (ICD), 6 phosphogluconate dehydrogenase: E.C.1.1.1.44 (6PGDH), glucose-6-phosphate dehydrogenase: E.C.1.1.1.49 (G6PDH), phosphoglucomutase: E.C.2.7.5.1. (PGM), mannosephosphate isomerase: E.C.5.3.1.8. (MPI), glucosephosphate isomerase: E.C.5.3.1.9. (GPI), glutamate dehydrogenase: E.C.1.4.1.2. (GDH NAD and GDH NADP+), aconitate hydrolase: E.C.4.2.1.3. (ACON) and peptidase: E.C.3.4.11.1. (PEP).

Six reference strains were used: L 20 *L. mexicana pifanoi*, L 11 *L. mexicana mexicana*, PH 8 *L. mexicana amazonensis*, M 2904 *L. braziliensis braziliensis*, M 4147 *L. braziliensis guyanensis* and M 4037 *L. braziliensis panamensis*.

Electrophoresis was carried out on cellulose-acetate plates (Helena Laboratories), running at 200 volts for 30 min. All cell buffers were used with 20% sucrose, their preparation being adapted from the method of LANHAM *et al.* (1981) and as previously described (TIBAYRENC & DESJEUX, 1983).

Table I—Number of mammals collected at FRG (Montsinery) between January 1982 and June 1983, and number of trypanosomatid stocks isolated

Species	No. examined	Parasites isolated		
		T. cruzi	*L. braziliensis* group	*L. mexicana* group
MARSUPIALIA				
Didelphis marsupialis	28	7	1	0
Caluromys philander	1	1	0	0
Philander opossum	44	2	0	0
Metachirus nudicaudatus	3	0	0	0
Marmosa murina	1	0	0	0
EDENTATA				
Bradypus tridactylus	1	0	0	0
RODENTIA				
Echimys armatus	3	0	0	0
Proechimys sp.	22	0	1	2

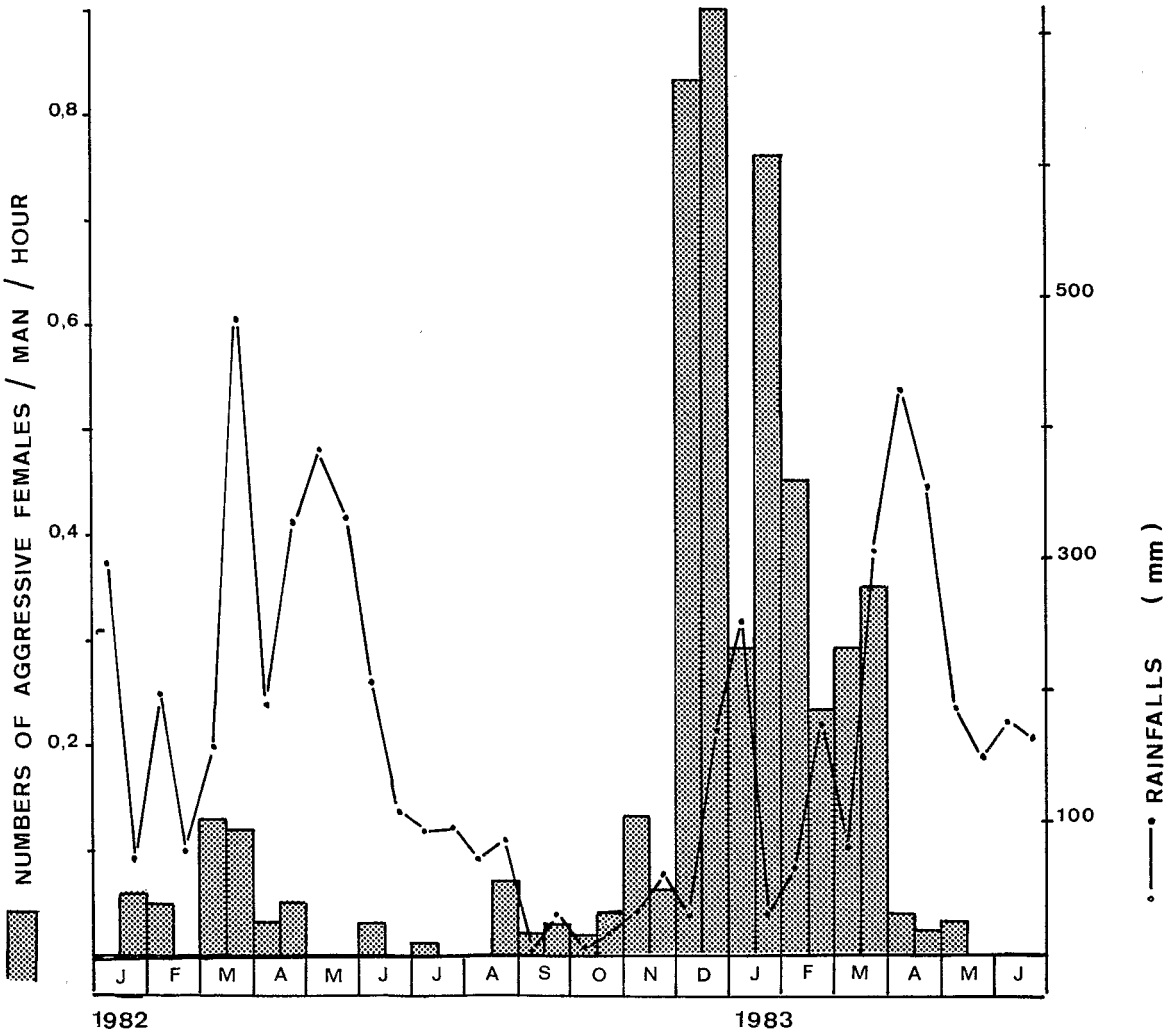

Fig. 1. Seasonal fluctuations of man-biting females of *Lutzomyia flaviscutellata*.

Results

Infection of mammals

103 mammals, mainly marsupials and rodents, were collected and examined. Out of them, several trypanosomatid stocks were isolated (Table I): 10 trypanosome strains, typed as *Trypanosoma cruzi*, zymodeme 1, and reported elsewhere (DEDET *et al.*, 1985) and three *Leishmania* stocks from *Proechimys* sp. and one from *Didelphis marsupialis*.

Two *Leishmania* stocks isolated from one *D. marsupialis* and from one *Proechimys* sp., were identified as belonging to the *L. braziliensis* complex; they will be reported elsewhere.

The other two stocks were isolated from the intact skin of *Proechimys* sp. Their cultures showed flourishing rapid growth and the infected hamsters rapidly developed (one month) a large granuloma at the point of inoculation. Compared with six reference strains by isoenzyme characterization, these two stocks were indistinguishable from the *L. mexicana amazonensis* reference strain PH 8 (MILES *et al.*, 1981). One of the *Proechimys* from which these two *L. m. amazonensis* strains were isolated was determined as *Proechimys cuvieri* Petter, 1978, and the second was lost before species determination.

Infection of Lutzomyia flaviscutellata

5,892 samples of phlebotomine sandflies were collected on man during the period of study. Of these, 254 (4·3%) were *Lu. flaviscutellata*. They were collected over a period of 16 months of the total 18-months study period. Their frequency, related to rainfall, was variable during the year (Fig. 1).

All *Lu. flaviscutellata* females (254) were dissected: only one individual showed a slight infection from which a stock was isolated. Characteristics in culture and pathogenicity were as described above for the two *Proechimys* stocks and, here again, isoenzyme characterization showed the parasite to be indistinguishable from *L. m. amazonensis* reference strain PH 8.

Discussion

Leishmania mexicana amazonensis was initially isolated from the cricetid rodent *Oryzomys capito* in Pará State, Brazil (GUIMARAES & AZEVEDO, 1964), but subsequent investigations in Belém and other foci in Brazil, revealed infection in various mammals, including a wide variety of rodents and marsupials, and foxes, and lead to the conclusion that the echimyid rodent *Proechimys guyannensis* (Geoffroy, 1803) was the major host of *L. m. amazonensis* (see LAINSON & SHAW, 1979). Other studies carried out in Belém, produced evidence that *Lu. flaviscutellata* was the major and probably the only vector of the species in the Amazon region (LAINSON & SHAW, 1968; WARD *et al.*, 1973; LAINSON & SHAW, 1979).

Investigations carried out on the mammal fauna of French Guiana showed that *Proechimys guyannensis* was in fact a complex of species, from which PETTER (1978) differentiated *P. cuvieri* on morphological criteria. Karyotypic study revealed that this new species was sympatric with, and clearly distinct from *P. guyannensis* (REIG *et al.*, 1979). In our own collections, *P. cuvieri* appears to be, in French Guiana, predominant inside the *Proechimys* complex, in the proportion of 76% *P. cuvieri* and 24% *P. guyannensis* sensu stricto (Guillotin & Petter, personal communication).

The present report of *P. cuvieri* as a local host of *L. m. amazonensis* is the first mention of the infection of this species and it raises the question as to which species of *Proechimys* is really the major reservoir of *L. m. amazonensis*, the infection of *P. guyannensis* in Pará State having been described before the separation of *P. cuvieri* as a species.

Lutzomyia flaviscutellata was reported for the first time in French Guiana by FLOCH & ABONNENC in 1943, under the name of *Phlebotomus apicalis*. It is widely distributed all over the country: neighbourhoods of Cayenne, Kaw (FLOCH & ABONNENC, 1952), High Oyapok, Saül and Maripasoula (LEGER *et al.*, 1977), Petit Inini (Le Pont, personnal communication), regions of Sinnamary and Montsinery (Pajot, personnal communication), Saint-Georges and Acarouany (Chippaux, personnal communication) and Cacao (LEGER *et al.*, 1980). In all collection sites, it appears as a scarce species, except in the neighbourhoods of Cayenne where it represented 34% of the samples collected by LEGER *et al.* (1980).

Lu. flaviscutellata is known to be a zoophilic sandfly electively attracted to rodents, principally to *Proechimys* spp.. (AITKEN *et al.*, 1975) and is only rarely taken on man (SHAW & LAINSON, 1968). These feeding habits can explain the low numbers of samples reported by the various authors in French Guiana, as sandfly collections were regularly made on human bait.

The seasonal fluctuations in the *Lu. flaviscutellata* population observed during our limited period of study, seem related to rainfall (Fig.) with peak of population at the beginning of the rainy season, as observed in the dry secondary type of forest of Pará State by SHAW & LAINSON (1972).

The infection rate of *Lu. flaviscutellata* in the present study (0·39%) is remarkably similar to those previously reported from Pará: 0·40 (LAINSON & SHAW, 1968) and 0·70% (WARD *et al.*, 1973).

Both *P. cuvieri* and *Lu. flaviscutellata* were found to harbour *L. m. amazonensis* in a primary rain forest area where concomitant isolation of *L. b. guyanensis* was made from *Lu. umbratilis*. The co-existence of these two cycles of leishmaniasis in the same biotope raises the question of the type of infection acquired by man. Up to now, human cases of leishmaniasis diagnosed in French Guiana were reported as "pian bois", the only type of leishmaniasis known to be present. As a matter of fact, during an immunological study done in 1981-82, on 26 autochthonous cases, 11 stocks were isolated and typed as *L. b. guyanensis* (BARBIER *et al.*, 1985).

While *Lu. flaviscutellata* is known to be poorly anthropophilic (SHAW & LAINSON, 1968), it is, however, probable that *L. m. amazonensis* cases occur occasionally. Local dermatologists emphasized the polymorphism of leishmaniasis lesions seen in French Guiana (PRADINAUD, 1979). Diffuse cutaneous leishmaniasis has not been reported up to now in French Guiana, but the existence of a silvatic *L. m. amazonensis* cycle in the local rain forest, draws attention to the importance of specific identification of the stocks isolated from all human cases.

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