PHLEBOTOMINE SAND FLIES (DIPTERA:PSYCHODIDAE) AND DIFFUSE CUTANEOUS LEISHMANIASIS IN THE DOMINICAN REPUBLIC

BY

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To my parents,

Robert and Mary Johnson

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A survey for phlebotomine sand flies (Diptera:Psychodidae) in the Dominican Republic revealed that Lutzomyia cayennensis hispaniolae (Fairchild and Trapido) was widely distributed and fairly common. Lutzomyia christophei (Fairchild and Trapido) was more limited in geographic distribution. Specimens of the latter species were obtained by light traps, flight traps, and aspirator collection from human bait and resting sites. Laboratory colonies of both species were established and life-cycle data were obtained. Lutzomyia cayennensis females readily fed on Anolis lizards. Female Lu. christophei readily fed on rodents and were capable of experimentally transmitting a Dominican strain (Isabel-WR336) of Leishmania to BALB/c mice seven to ten days after biting infected mice. Development of the

parasite occurred in the anterior midgut in both Lu. christophei and Lu. anthophora (Addis), a species that was also experimentally infected. The course of development in the sand fly was observed by dissecting 15 infected Lu. anthophora on days 1-7 post-feeding. Development in this species was parallel to that observed in the 17 Lu. christophei. Promastigotes from flies four and five days post-feeding were infective to hamsters, as determined by xenodiagnosis with sand flies and spleen culture.

In culture medium, Leishmania-Isabel strain grew at a much slower rate than either of two strains of L. mexicana. Hamsters and IRC mice, experimentally inoculated from culture, showed no outward sign of infection until at least 2.5 months after inoculation with the Isabel strain.

In the Dominican Republic, 10 of the 21 known case sites were visited. Coffee and cacao groves were characteristic of these sites. Two female Lu. christophei were captured while biting a patient. Four species of mammals (170 specimens) were trapped from five of the case sites and examined for leishmaniasis using various methods. None was found to be infected, though 4 of 44 Rattus rattus from one site were seropositive (1:16), as determined by indirect fluorescent antibody test.

Lutzomyia christophei is most probably the vector of diffuse cutaneous leishmaniasis in the Dominican Republic. The identity of the reservoir remains unknown, but R. rattus is the most likely suspect.

CHAPTER 1

INTRODUCTION

Literature Review

Phlebotomine sand flies* are biting members of the dipteran family Psychodidae (Quate and Vockeroth, 1981). They are suspected or confirmed vectors of various parasitic agents including phleboviruses, such as sand fly fever virus; bartonellosis (Adler and Theodor, 1957); saurian malaria (Ayala and Lee, 1970); trypanosomes of amphibians, lizards (Anderson and Ayala, 1968), and mammals (McConnell and Correa, 1964); and leishmaniasis (Bray, 1974).

Leishmaniasis is a complex of diseases which is caused by Leishmania spp. occurring in many parts of the world. In 1981, the World Health Organization estimated that there were 400,000 new cases of leishmaniasis in the world, annually. The disease occurs in the Americas, Europe, Africa, and Asia. It has been considered the second most important protozoan disease of man after malaria (Anonymous, 1981). The only known biological vectors are phlebotomine sand flies (Bray, 1974). In the Americas, leishmaniasis

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* In this presentation, sand fly will refer to members of Diptera: Psychodidae: Phlebotominae.

occurs mainly among persons living in rural or forested areas (Herrer et al., 1966). Leishmaniasis appears in three basic clinical forms-visceral, mucocutaneous, and cutaneous.

In 1948, a subform of cutaneous leishmaniasis was reported independently in Venezuela (Convit and Lapenta, 1948) and in Bolivia (Barrientos, 1948). At first, it was considered a new form of the disease because of its characteristic features (Convit et al., 1962; Convit and Kerdel-Vegas, 1965). Bryceson (1969, p. 709) summarizes these features as follows:

1. There is an initial lesion which spreads locally, and from which the disease disseminates to other parts of the skin, often involving large areas.
2. The lesions are nodules and do not ulcerate.
3. There is a superabundance of parasites in the lesion.
4. The histology is characteristic in that macrophages full of amastigotes predominate in the lesion.
5. Internal organs are not invaded and there is no history of visceral leishmaniasis.
6. The leishmanin (Montenegro) test is negative.
7. The disease progresses slowly and becomes chronic.
8. Treatment with antimony produces only slight and temporary improvement.

By these criteria, other cases have been reported from the USA in Texas (Simpson et al., 1968), Brazil, Ecuador, Mexico, Ethiopia, and Tanzania (Bryceson, 1969). The appearance of the lesions gave rise to the name diffuse (or disseminated) cutaneous leishmaniasis (DCL). The causative agent of the Venezuelan cases was first named Leishmania pifanoi (Medina and Romero, 1962). Areas where cases occurred were endemic for cutaneous leishmaniasis, but not the visceral disease (Convit and Kerdel-Vegas, 1965). Further work determined that rather than being a new parasite, DCL was the result of a deficiency in the host's cell-mediated immunity (Bryceson, 1970b; Convit et al., 1971). The disease is caused by the same species of Leishmania that causes ulcerative cutaneous leishmaniasis in the same area, e.g., L. aethiopica in Ethiopia (Lemma et al., 1970; Bray et al., 1973) and L. mexicana mexicana, L. m. amazonensis, and L. m. pifanoi in Central and South America (Lainson and Shaw, 1978).

In 1975, a new focus of DCL was reported in the Dominican Republic (Bogaert-Diaz et al., 1975). Three humans, siblings, were found infected. There have been 22 additional cases, none of which had ulcerating lesions, supporting the concept that DCL is determined both by parasite characteristics and a defect in host immunocompetence (Walton, pers. comm.). Studies of some of the patients from the Dominican Republic showed that this defect is a specific

inhibition of lymphocyte-proliferation responses by adherent suppressor T-cells, which has a genetic basis (Petersen et al., 1982). Many of the Dominican DCL patients have been symptomatically cured using hot (45°C) water treatment (Neva, pers. comm.). The Leishmania causing DCL in the Dominican Republic remains unnamed, but it appears to be different from strains in the L. braziliensis complex and in the L. mexicana complex based on enzyme electrophoretic mobility assays (Kreutzer et al., 1983), excreted factor serotype, growth in artificial media, and infectivity and pathogenicity in laboratory rodents (Schnur et al., 1983).

Although leishmanaisis may be mechanically transmitted under lab conditions by Stomoxys calcitrans, stable flies (Lainson and Southgate, 1965), Rhipicephalus sanguineus, brown dogs ticks (Sherlock, 1964), and Glossina morsitans, tsetse flies (Lightner and Roberts, 1984), sand flies are the only known biological vectors (Lainson and Shaw, 1978). In the New World, 21 species of sand flies have been reported as natural hosts of Leishmania spp. infecting man, but only 6 species have been determined, at present, to be natural vectors (Table 1-1).

Experimentally, New World Leishmania are capable of infecting a number of sand fly species, most of which are then capable of transmitting the infection to a susceptible mammalian species (Killick-Kendrick, 1979). The amastigote stage is parasitic in vertebrate macrophages (Bray, 1974).

Table 1-1. Sand fly species (*Lutzomyia*) which are known or suspected vectors of leishmaniasis in the New World, by country.

Country	Suspected or Proven *Lutzomyia* Vectors	*Leishmania* Strain[1]
Belize	*olmeca*[2]	*L.m.m.*
Bolivia	*longipalpis*[3]	*L.d.c.*
Brazil	*longipalpis*[2]	*L.d.c.*
	amazonensis[3]	*L.b.b.*
	intermedia[2]	*L.b.b.*
	migonei[3]	*L.b.b.*
	paraensis[3]	*L.b.b.*
	pessoai[3]	*L.b.b.*
	wellcomei[2]	*L.b.b.*
	whitmani[3]	*L.b.b.*
	anduzei[3]	*L.b.g.*
	umbratilis[2]	*L.b.g.*
	flaviscutellata[3]	*L.m.*
Colombia	*longipalpis*[3]	*L.d.c.*
	trapidoi[3]	*L.b.*

Table 1-1. Continued

Country	Suspected or Proven *Lutzomyia* Vectors	*Leishmania* Strain[1]
Costa Rica	*shannoni*[3]	*L.b.*
	ylephiletor[3]	*L.b.*
El Salvador	*longipalpis*[3]	*L.d.c.*
French Guiana	*umbratilis*[3]	*L.b.g.*
Guatemala	*longipalpis*[3]	*L.d.c.*
	olmeca[3]	*L.m.*
Honduras	*longipalpis*[3]	*L.d.c.*
Mexico	*longipalpis*[3]	*L.d.c.*
	olmeca[2]	*L.m.*
Nicaragua	*longipalpis*[3]	*L.d.c.*
Panama	*gomezi*[3]	*L.b.p.*
	panamensis[3]	*L.b.p.*
	trapidoi[2]	*L.b.p.*
	ylephiletor[3]	*L.b.p.*

Table 1-1. Continued

Country	Suspected or Proven *Lutzomyia* Vectors	*Leishmania* Strain[1]
Paraguay	*longipalpis*[3]	*L.d.c.*
Peru	*peruensis*[3]	*L.p.*
	verrucarum	*L.p.*
Surinam	*umbratilis*	*L.b.g.*
USA	*diabolica*	*L.m.*
Venezuela	*longipalpis*[3]	*L.d.c.*
	flaviscutellata[3]	*L.m.a.*
	townsendi[3]	*L.m.g.*

Source: World Health Organization, 1984, pp. 56-61.

[1]*Leishmania* strains: *L.m.m.* = *L. mexicana mexicana*, *L.d.c.* = *L. donovani chagasi*, *L.b.b.* = *L. braziliensis braziliensis*, *L.b.g.* = *L.b. guyanensis*, *L.b.p.* = *panamensis*, *L.p.* = *L. peruviana*, *L.m.a.* = *L.m. amazonensis*, *L.m.g.* = *L.m. garnhami*.

[2]Proven vector.

[3]Suspected vector.

Following ingestion by the sand fly, a telmophagic feeder, the parasite exsheathes its flagellum to become the promastigote, which multiplies initially in the hind or midgut, depending on the parasite species. Members of the L. braziliensis complex (Section Peripylaria) develop in the posterior midgut and anterior hindgut before moving anteriorly to effect transmission. Leishmania donovani and subspecies in the L. mexicana complex (Section Suprapylaria) multiply initially in the anterior midgut (Killick-Kendrick, 1979). Transmission to a vertebrete occurs during the sand fly bite, but the actual mechanism is unknown (Killick-Kendrick, 1978).

Two extant species of sand flies are known from the Dominican Republic (Fairchild and Trapido, 1950). Two fossil species have been discovered recently in Dominican amber (Johnson and Young, in preparation), reported to be from the Oligocene Period, 40 to 60 million years old (Sanderson and Farr, 1960). Prior to this study, practically nothing was known abut the distribution or biology of the living species. One of these, Lutzomyia christophei (Fairchild and Trapido, 1950), belongs to the Lu. verrucarum species group (Lewis, 1968), which also contains a number of man-biting species (Young, 1979) and at least one vector of leishmaniasis in Peru (Lainson and Shaw, 1979). Contrary to Lainson's (1983) statement, nothing was known about the feeding habits of Lu. christophei, prior to the author's study. The other species, Lu. cayennensis hispaniolae (Fairchild and Trapido,

1950), is an endemic subspecies of a species that occurs from Mexico south to Ecuador and French Guiana (Young, 1979). This species is known to feed on poikilothermic vertebrates, though there is one report of females feeding on bats in Venezuela (Deane et al., 1978). The specific feeding habits of Lu. c. hispaniolae were not known prior to this study.

Laboratory rearing of sand flies is a necessary part of studying disease transmission, because it is an assured method of obtaining uninfected flies. Colonization can also lead to a better understanding of the life cycle of the vector and parasites (Killick-Kendrick, 1978). Successful rearing has been accomplished using various techniques and several different formulations of larval food (Chaniotis, 1967; Endris et al., 1982; Gemetchu, 1976; Young et al., 1981).

In the New World, leishmaniasis is a zoonotic disease, with rodents serving as reservoir hosts for the L. mexicana complex, canids for L. donovani, a variety of rodents, procyonids, sloths, dogs, and primates for the L. braziliensis complex (Table 1-2) (Lainson and Shaw, 1978). In the Dominican Republic, ten species of rodents, two primates, four to five insectivores, and four to six sloths are known from fossils (Varona, 1974; Woods, pers. comm.). Some survived until the time of Columbus (1492 AD), but only two endemic terrestrial species exist today: an insectivore, Solenodon paradoxus Brandt; and a capromyid rodent,

Table 1-2. Other Mammalian hosts of *Leishmania* strains which cause human leishmaniasis in the New World, by country.

Country	*Leishmania* Strain[1]	Nonhuman Mammalian Hosts
Belize	*L.m.*	rodents: *Heteromys*, *Nyctomys*, *Ototylomys*, *Sigmodon*(R)[2]
Brazil	*L.d.c.*	dog(R), foxes: *Cerdocyon*(R), *Lyalopex*
	L.b.b.	(rodents: *Akodon*, *Oryzomys*, *Proechimys*?)[3]
	L.b.g.	sloth: *Choelopus*; anteater(R), tamandua(R)
	L.m.	rodents: *Proechimys*(R), (*Dasyprocta*, *Heteromys*, *Neacomys*, *Nectomys*, *Oryzomys*, oppossums, *Marmosa*, *Caluromys*, *Metachirus*?)
Colombia	*L.d.c.*	dog(R)
Costa Rica	*L.b.*	sloths: *Bradypus*(R), *Choelopus*
French Guiana	*L.b.g.*	sloths: *Choelopus*
Guatemala	*L.m.*	*Ototylomys*
Mexico	*L.m.*	rodents: *Heteromys*, *Nyctomys*, *Ototylomys*, *Sigmodon*

Table 1-2. Continued

Country	*Leishmania* Strain[1]	Nonhuman Mammalian Hosts
Panama	*L.b.p.*	sloths: *Bradypus*(R), *Choelopus*; (primates: *Aotus*, *Sanguinus*; procyonids: *Bassaricyon*, *Nasua*, *Potos*?)
Peru	*L.p.*	(dog?)
Venezuela	*L.m.*	(rodents: *Heteromys*, *Proechimys*, *Zygodontomys*?)

Source: World Health Organization, 1984, pp. 56-61.

[1] *Leishmania* strains: *L.m.m.* = *L. mexicana mexicana*, *L.d.c.* = *L. donovani chagasi*, *L.b.b* = *L. braziliensis braziliensis*, *L.b.g.* = *L.b. guyanensis*, *L.b.p.* = *L.b. panamensis*, *L.p.* = *L. peruviana*.

[2] R = Proven reservoir.

[3] (Mammal?)--animal found infected in nature, extent of infection not determined.

Plagiodontia aedium Cuvier, both of which are extremely uncommon. Both animals are secretive and are found in relatively undisturbed habitat (Woods, 1981). Introduced mammals have replaced the endemic mammals in most areas. Three species of Old World rodents now occur througout the island in cities, tropical rain forests, and deserts. These are Rattus rattus alexandrinus (Geoffroy), the roof or black rat; R. norvegicus (Berkenhout), the Norway or brown rat; and Mus musculus brevirostris Waterhouse, the house mouse.

Geography of the Dominican Republic

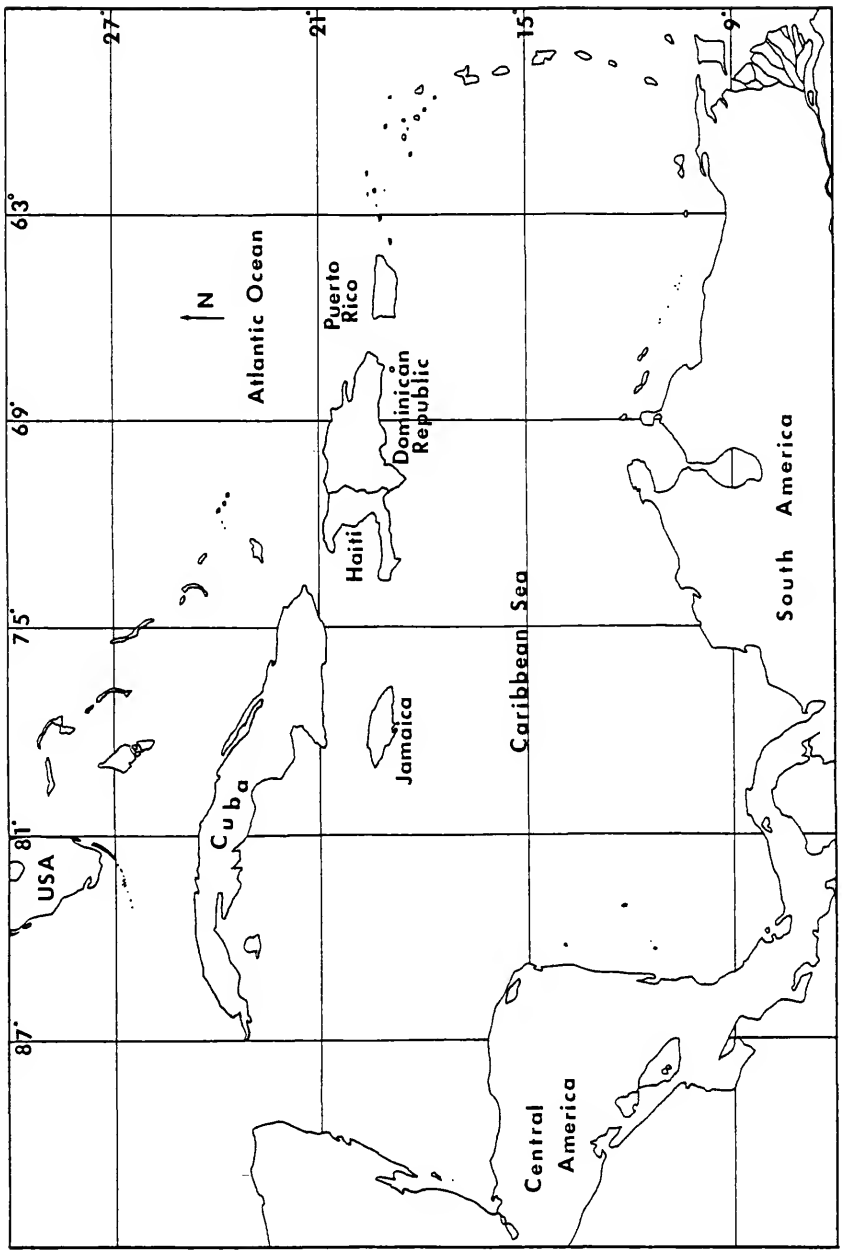
The Dominican Republic occupies the eastern two-thirds of the island of Hispaniola, with Haiti occupying the western side. The island is a member of the Greater Antilles, lying between latitudes 17°30' to 20°00' with the Atlantic Ocean to the north and the Caribbean Sea to the south (Fig. 1-1). The Republic has an area of 43,230km^2, of which roughly two-thirds is mountainous. Geographically the country is broken up into four regions, based, in part, on the mountain ranges (sierras and cordilleras). The Eastern region includes the Cordillera Oriental with the Llano Oriental (Eastern Plains) to the south. Directly west lies the Cibao with the Cordillera Septentrional to the north, just inland from the coast, the Cibao Valley, and the Cordillera Central to the west and south. To the northwest lies the Linea Noroeste, bordering Haiti. El Sur is the region to the south west of the Cordillera Central; it also

borders Haiti, and contains two smaller mountain chains Sierra de Neiba to the north of Sierra de Baoruco (Fig. 1-2). Most of the 6.2 million inhabitants (1982 census) live on the valley floors that separate the cordilleras, or on the rolling Eastern Plains. About 60% of the Dominicans live in rural and agricultural areas. The majority are small land owners. Much of the land has been cleared for agriculture. The main agricultural products include sugar cane, rice, coffee, cacao, cotton, tobacco, corn, and beef. The country has a tropical maritime climate; in the lower elevations, temperature average 22° to 28°C with a range during July 1981 to August 1982 at Pedro Sanchez in El Seibo Province, of 16° to 33°C. Annual rainfall averages 1397 to 1524mm with a range of 508 to 2413mm, depending on location. The highest rainfall usually occurs in the eastern portion where the rainy season lasts from May to November (Anonymous, 1977; Sholdt and Manning, 1979).

Current Status of Diffuse Cutaneous Leishmaniasis in the Dominican Republic

Since the discovery of the first three cases of DCL in the Dominican Republic in 1974 (Bogaert-Diaz et al., 1975), an additional 22 cases have been diagnosed (Bogaert-Diaz, unpublished data). Precise life histories are known only for a few of the patients who have not moved to different localities during the presumed evolution of the disease. Personal data, when recorded, often did not denote the exact

Figure 1-1. The Caribbean region, showing the location of the Dominican Republic, on the island of Hispaniola.


87°
81°
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69°
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27°
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USA
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Dominican Republic
Puerto Rico
Atlantic Ocean
Caribbean Sea
Central America
South America

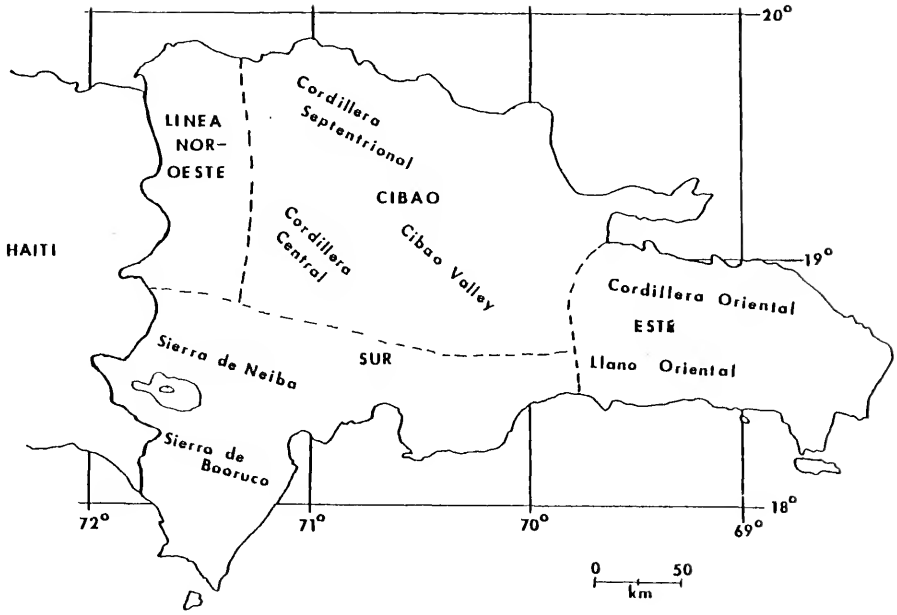


Figure 1-2. The geographic regions of the Dominican Republic (Cordilleras and Sierras are mountain ranges).

location of residence. Many patients had moved from the country to towns before being diagnosed. Others have lived in several different areas for various periods of time; thus, current place of residence may not have been where the disease was contracted. Presumed site visitations and interviews led to the confirmation of the probable locality of infection for 15 patients. Brief descriptions of these sites are given below and are shown as confirmed sites in Figure 1-3; the remaining sites are shown as presumed. The unknown incubation period and the relative benignancy of the infection make it difficult to determine the probably date of infection. Eight of the patients had had nodules or plaques for four or more years before the disease was diagnosed. At least 13 of the patients were under four years of age when signs of the infection first appeared, so epidemiological data must be based upon recollections of parents or other relatives. The most outstanding feature of the epidemiology of the disease is its positive correlation to the Cordillera Oriental, as 19 patients (76%) resided in or near this region of the country (Fig. 1-4). Most of the patients have been symptomatically cured of the disease with hot (46°C) water treatment (Neva, pers. comm.) (Fig. 1-5).

During the period March to September 1982, a serological survey for leishmaniasis was undertaken by personnel of the Instituto Dermatologico, in which the author assisted. In the survey, blood samples were taken from residents

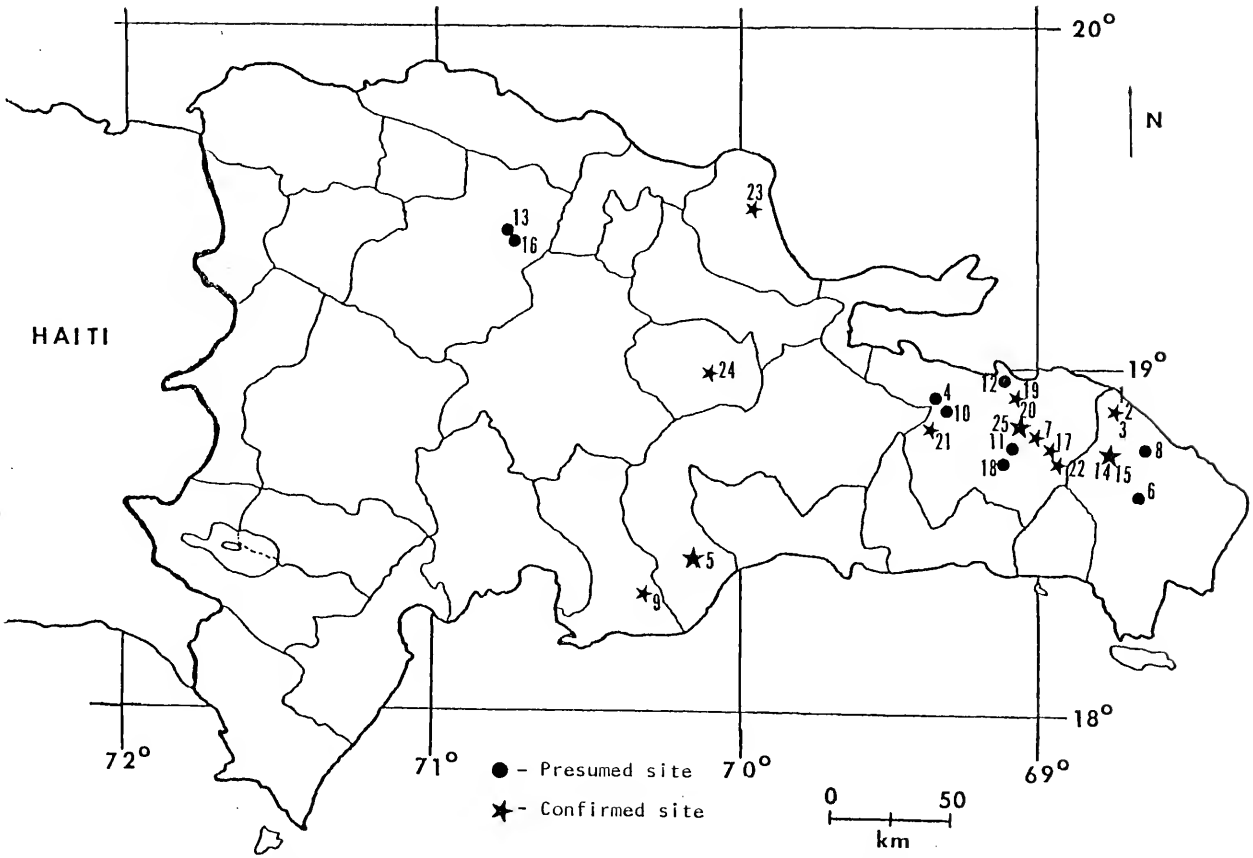


Figure 1-3. Case sites for patients with diffuse cutaneous leishmaniasis (DCL) in the Dominican Republic (see Appendix 1-1 for name of numbered localities).

Figure 1-4. Typical terrain in the Cordillera Oriental, Dominican Republic.

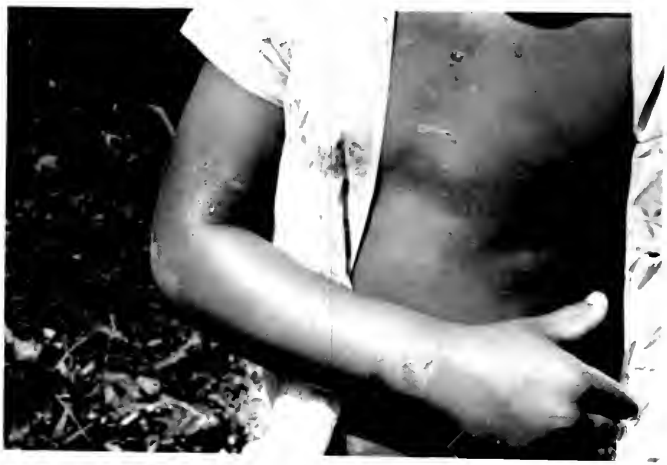


Figure 1-5. A young Dominican DCL patient with healed lesions on wrist and upper arm.

living in the area of 7 of the 21 case sites (Fig. 1-3; cases 1-3, 7, 10, 17, 19 and 20, 21, and 22) and two control sites (sites in regions where no cases of leishmaniasis had been diagnosed). Site selection was based on accessibility of the site to vehicular transport and on the probability of obtaining an adequate number of volunteers from the local residents. Indirect fluorescent antibody test (IFAT) was performed on the sera, revealing that 26.0-48.0% of the samples from case sites were seropositive for leishmanial antibody, at 1:8 or higher titer. At the two control sites 0% and 14% were determined to be seropositive (de Quinones, unpublished data). The pertinent patient history data are given in Appendix 1. Cases 1-3 are siblings, cases 14 and 15 are son and father, and cases 19 and 20 are neighbors, but unrelated.

Study Site Selection

Four principal study sites were visited regularly during the study. One, Pedro Sanchez, was not a leishmaniasis case site, but was used as a control site, i.e. a site with sand fly habitat but no known cases of leishmaniasis. Pedro Sanchez was selected as a typical nonagricultural wooded area. Due to extensive land clearing for agricultural purposes, pasture, sugar cane planting, and coffee/cacao groves, very little natural forest habitat exists in the Dominican Republic. The Pedro Sanchez site was representative of wooded river bank habitat in the

leishmaniasis endemic region of the Cordillera Oriental. The field station (and author's residence) for the study was located less than 0.5km distant, thus the site was convenient at all times of year. The other three principal sites were leishmaniasis case sites, in all instances the DCL patients were living at or near the sites. All four patients (patients 19 and 20 were neighbors) were young and had not lived elsewhere at the time the disease was diagnosed. Interviews with the patients' parents led the author to believe that the disease was contracted at the sites. Very few changes had occurred at the sites in the intervening years between the appearance of the first signs of the disease and the beginning of the author's research, this was not the case with several other presumed case sites.

The system of roads in the Dominican Republic is generally poor, particularly in mountainous regions and the Eastern region, thus accessibility of the site was an important criterion in study site selection. Certainty of the site of disease contraction was another important criterion. Many of the older DCL patients have lived in various localities throughout their lives and do not remember clearly where they were living when signs of the disease first appeared, 20 years or more previous. Another important factor was whether or not personnel of the Instituto Dermatologico were familiar with the location of the case site. In a few cases, the patients had visited

rural public clinics where they had been diagnosed, their place of residence at time of infection was not known except in rather general terms. Eleven of the 21 DCL case sites were never visited by the author, primarily due to the factors mentioned above.

The three principal study case sites were approximately 35km west (Loma Pena Alta), 18km north (Morro de Miches), and 13km east (Trepada de Jabilla) of the field station and control study site at Pedro Sanchez. All four sites were visited in excess of 100 man-hours during the study. The remaining sites listed below were DCL case sites and were visited one or more times, depending on proximity and accessibility. Due to the concentration of cases in the Cordillera Oriental, this area received the greatest amount of attention by the author.

Study Site Description

Pedro Sanchez, El Seibo Province

Altitude: 76m

This site consists of a gallery forest along the banks of the Rio Seibo on the southern outskirts of the village of Pedro Sanchez. The Rio Seibo is 0.5 to 1.5m deep, depending on the exact location and time of year, and 4 to 6m wide in this region. The wooded border on the northwest bank was 20 to 50m wide and extended for several kilometers. It contained several trees with diameter up to 1m, a moderately thick shrub understory. A section of forest, with a length

of 40m, was used for this study, through August 1982; when the site was revisited in May 1983, it had been extensively altered for agricultural use.

The remaining study sites, listed alphabetically, all are DCL case sites where a patient is living, or was living at the presumed time of infection.

Altos de Peguero (5km E by 5km N of El Seibo), El Seibo Province (Fig. 1-3, #17)

Altitude: 72m

The case site is on the northern side of an isolated ridge on the southern edge of the Cordillera Oriental. Several coffee and cacao groves are present in the area, the largest of which is a 2ha cacao grove about 50m from the former residence of the DCL patient. Coffee and cacao groves also contain scattered larger hardwood trees to provide light shade. There are no major streams or rivers in the immediate vicinity, the closest being 1.5km distant.

Carrasco (10km S of Rio San Juan), Maria Trinidad Sanchez Province (Fig. 1-3, #23)

Altitude: 15m

The area surrounding the patient's former residence is flat pasture for at lease 2km, with the exception of a 1.5ha cacao grove, 400m distant from the residence. A small stream runs next to the grove and forms a small pool (7m diameter) where the children of the area are said to swim.

La Culatica (10km S of Nisibon), Altagracia Province (Fig. 1-3), #1-3)

Altitude: 150m

The patients' (three siblings) residence was located on a small hill above a small (0.5ha) coffee grove. Other coffee and cacao groves are in proximity to the house site, some of which are owned by the patients' father. A small wooded stream runs below the site, through parts of the coffee grove. This locality is situated in the northeast portion of the Cordillera Oriental.

Iguana Arriba (23km NE of Bani), Peravia Province (Fig. 1-3, #9)

Altitude: 152m

The exact locality of the DCL patient's residence was not known, but the area consists of extensive coffee plantings throughout low mountain terrain. A few small streams are present in the low areas between ridges. Larger trees are found along the streams and in the coffee groves, providing shade.

Loma Pena Alta (13km NW of Hato Mayor), El Seibo Province (Fig. 1-3, #21)

Atltitude: 442m

The DCL patient lived at the top of the ridge, adjacent to a 0.5ha coffee grove. The eastern side of the ridge was shrub-overgrown pasture. Some coffee groves were present on

the western side, which was mostly lightly forested with numerous rocky outcroppings.

Monte Claro (16km NE of Cotui), Sanchez Ramirez Province (Fig. 1-3, #24)

Altitude: 76m

A 0.5ha coffee grove is 10m distance from the site where the DCL patient lived at the time of infection. The area consists of rolling hill pasture, with the coffee grove being the only wooded area in a radius of 1km from the house. The Rio Chacuey has a lightly wooded border, and is about 1km distant from the site.

Morro de Miches (10km S of Miches), El Seibo Province (Fig. 1-3, #19, 20; Fig. 1-4)

Altitude: 305m

Two unrelated patients live at this site, approximately 100m apart and separated by the peak of the ridge. One patient lived besided a 0.25ha coffee grove which contained several large shade trees. The grove is separated from nearby wooded areas by pasture and cultivated plots. A small stream runs down the ridge, 100m from the house site. The other patient's residence was located on the northern edge of a rather extensive mixed planting of coffee and cacao which had a length of about 0.5km and a width of 10-30m.

Najayo Arriba (20km NW of San Cristobal), San Cristobal Province (Fig. 1-3, #5)

Altitude: 400m

The patient's residence is located on the side of a ridge immediately above a small (0.5ha) coffee grove. A small stream runs along the bottom of the ridge, about 75m from the house. The site is located on the southern edge of the Cordillera Central.

Rio Llano (10km W by 30km N of Higuey), Altagracia Province (Fig. 1-3, #14 and 15)

Altitude: 250km

The patients (father and son) lived in a house 15m from a small stream. A small (0.5ha) coffee grove is situated on the opposite side of the stream. Rio Guancho is less than 0.5km distant. A gallery forest, 10-30m wide, runs along the banks of the river. This site is in the eastern portion of the Cordillera Oriental, about 10-15km (straight line distance) south of La Culatica.

Trepada de Jabilla (2.8km N of Las Cuchillas), El Seibo Province (Fig. 1-3, #7)

Altitude: 130m

The patient's residence was approximately 10m from the edge of a rather extensive coffee grove (± 3ha). As with other coffee groves, there are some larger shade trees present. The terrain is rolling hills, primarily pasture,

south of the Cordillera Oriental. The Rio Soco borders on side of the coffee grove and is 0.5 to 2.0m deep, depending on location and season, and generally 10 to 12m wide. Several other coffee groves exist in the area.

Objectives

In 1975, Bogaert-Diaz et al. reported the discovery of three human cases of diffuse cutaneous leishmaniasis (DCL) in the Dominican Republic, the first autochthonous cases of cutaneous leishmaniasis known in the country or in the entire West Indies, except Trinidad, a continental island. Over the succeeding four years, a concentrated search for additional cases, carried out under the National Leprosy Program, revealed 15 more cases of DCL. As of March 1984, 25 cases of DCL have been diagnosed. Surprisingly, none of the patients had ulcerating lesions. Interest in this unique situation led to grant support from the World Health Organization to the Instituto Dermatologico Dominicano for epidemiological studies of DCL in the Dominican Republic, beginning in 1981. Some objectives of the author's research, listed below, were included in the project. Field studies were performed in the Dominican Republic during the periods 19-24 May 1981, 27 July 1981 to 2 August 1982, and 18 May to 3 August 1983. Laboratory studies were performed at a field station (Pedro Sanchez) and at the Instituto Dermatologico in Santo Domingo, the University of Florida, and Walter Reed Army Institute of Research.

A. Field Studies:

1. To survey for the presence of phlebotomine sand flies at selected locations in the Dominican Republic.
2. To study the ecology of sand flies including host preference, resting sites, population dynamics, and seasonal and geographic distribution for species located in the survey.
3. To identify the wild and/or peridomestic reservoir host(s) of leishmaniasis.

B. Laboratory Studies:

1. To establish laboratory colonies of indigenous phlebotomine sand flies.
2. To study the life cycle of Leishmania in the sand fly.
3. To determine the vector potential of these flies.
4. To further elucidate some of the biological differences between the Dominican Leishmania and other known Leishmania spp.

CHAPTER 2

SURVEY FOR, AND COLONIZATION OF, *LUTZOMYIA CAYENNENSIS HISPANIOLAE* AND *LUTZOMYIA CHRISTOPHEI*

Introduction

Phlebotomine sand flies, the only known biological vectors of leishmaniasis, also transmit other parasitic agents of man and other vertebrates (Adler and Theodor, 1957). A new focus of leishmaniasis was discovered in 1974 in the Dominican Republic (Bogaert-Diaz et al., 1975), the eastern portion of the islnd of Hispaniola. Only two extant species of sand flies are known from Hispaniola, *Lutzomyia cayennensis hispaniolae* (Fairchild and Trapido, 1950) and *Lu. christophei* (Fairchild and Trapido, 1950). Both species were collected from tree trunks and buttresses, primarily, but little was known about the habits and range of these species. *Lutzomyia cayennensis hispaniolae* is an endemic subspecies of *Lu. cayennensis* (Floch and Abonnenc) that ranges from Ecuador and French Guiana north to Mexico. Most members of the *Lu. cayennensis* species group are reported as reptile feeders (Young, 1979). *Lutzomyia christophei* is a member of the *Lu. verrucarum* species group that contains an number of man-biting species (Lewis, 1968) including one suspected vector of leishmaniasis (Lainson and Shaw, 1979).

This study was conducted to determine the geographic range, the habits, and life cycle of the two species.

Methods and Materials

Field Studies

Chaniotis (1978) and Young (1979) reviewed techniques used for sampling phlebotomine sand flies. The methods used in the present study were aspirator collections at resting sites, man-biting collections, sticky paper traps, flight traps, CDC light traps (Sudia and Chamberlain, 1962), and Disney traps (Disney, 1966). Specific equipment preparation and field techniques for aspirator collections were given by Endris et al. (1982) (Fig. 2-1). Aspirator collections were made at various sites around the country, mostly from tree trunks, but also from tree holes and rock crevices. Cigarette smoke was occassionally used to disturb resting sand flies from the latter two types of resting sites. Live wild-caught sand flies were transported to the field station at Pedro Sanchez where they were either held in a feeding cage (Fig. 2-2) for host preference studies or kept individually for oviposition and later dissected for parasites.

The other techniques were used at five leishmaniasis case sites or the study site at Pedro Sanchez (see Chapter 1). Attempts to collect sand flies from human bait at

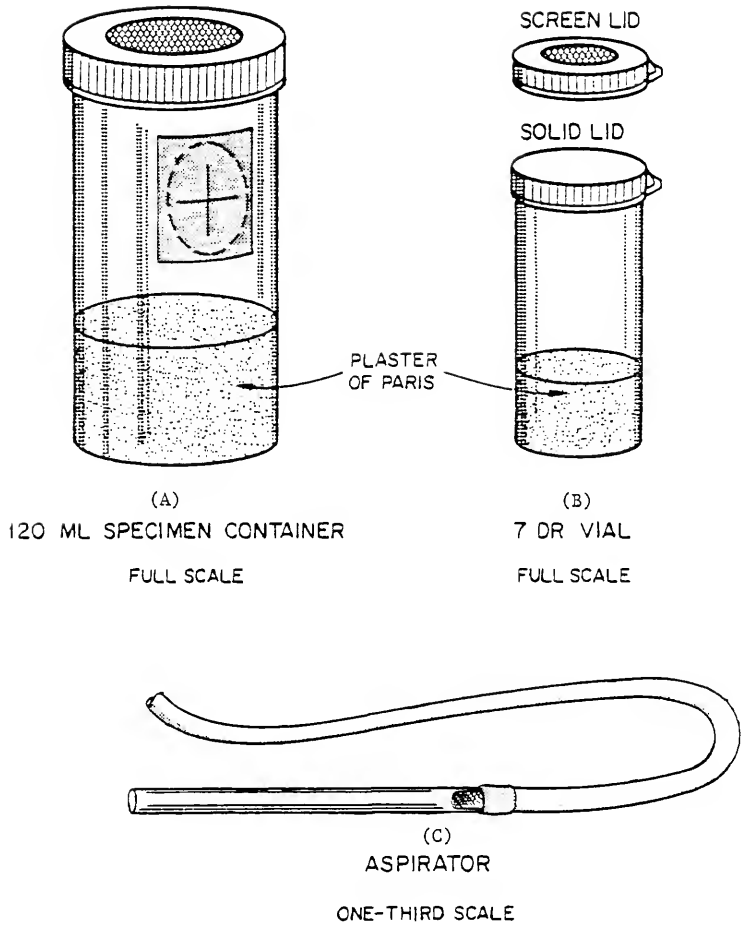


Figure 2-1. Field collecting equipment and rearing containers for phlebotomine sand flies (from Endris et al., 1982). (A) Collecting containers. (B) Oviposition/larval rearing vial. (C) Aspirator.

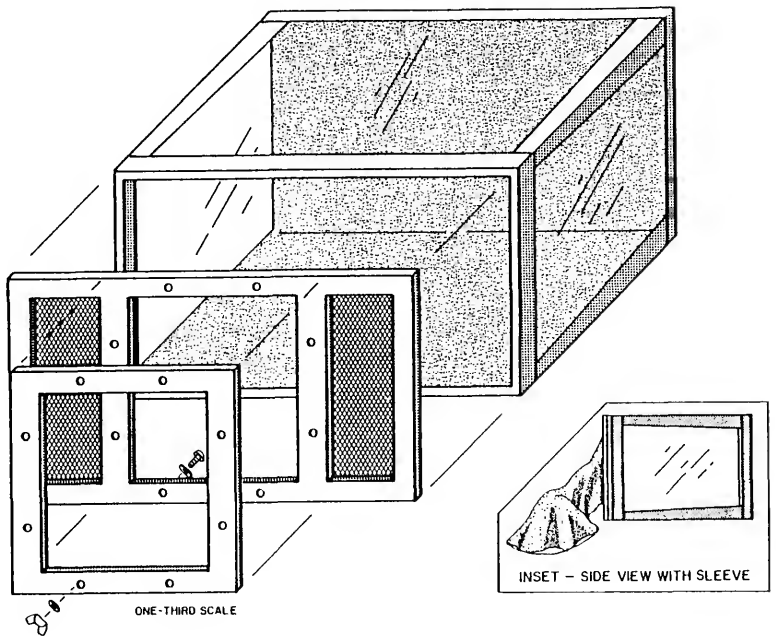


Figure 2-2. Sandy fly feeding cage--a modified aquarium with plaster of paris bottom and back (from Endris et al., 1982).

Trepada de Jabilla were made various times during the day and at dusk during November 1981 to July 1982 and May 1983. Similar attempts were also made at Loma Pena Alta at dusk on various days in June and July 1983. A sticky-paper trap of double-sided tape on a 25cm square wooden frame was used at Pedro Sanchez from 21-23 May 1981. The trap was placed in the crotch of a tree (1m high) known to harbor resting sand flies. Flight traps (Fig. 2-3) and CDC light traps (Fig. 2-4) were set at various sites (Tables 2-1, 2-2). Two modified CDC light traps were also employed. In one, a UV light source was substituted for the normal light source; the second type had no light source, instead a cage containing a hamster, Mesocricetus auretus, was suspended above the trap (Fig. 2-4). The light traps were run on nights when the moon was less than one-half full, or when the sky was mostly overcast. CDC traps were turned on in late afternoon and taken down the following morning. Flight traps were set up in coffee or cacao groves, with one exception (lightly wooded stream bank at Hato Mayor). The flight traps were emptied every two to four days. The trap collections were checked for sand flies with the aid of a dissecting microscope (7-30X). Disney traps (Fig. 2-5) were used at two sites with hamsters serving as bait (Table 2-3). The bottom of cake pans (22.5 x 30.0cm) or cookie sheets (27.0 x 38.0cm) were covered with a thin layer of mineral oil or castor oil. The condition of the hamsters was

Figure 2-3. The author in front of a flight trap in a cacao grove at Altos de Peguero, El Seibo Prov.

Figure 2-4. A CDC trap and modified traps (from left - CDC with UV light source, normal CDC, and hamster-baited trap).

Table 2-1. Location and dates for flight trap samples in the Dominican Republic.

Location	Date
Altos de Peguero - cacao grove	9-25 Mar, 1-28 May 1982
Carrasco - cacao grove	3-10 Jul 1982
Hato Mayor - wooded stream bank	17 Aug 1981
Loma Pena Alta - coffee grove	24-27 Jul 1982 9 Jun-3 Aug 1983
Monte Claro - coffee grove	19-26 June 1982
Morro de Miches - coffee grove	7-14 Aug 1981 5 Oct-3 Nov 1981
Pedro Sanchez - wooded river bank	20-22 May 1981
Trepada de Jabilla - coffee grove	6 Nov-14 Dec 1981 18 Jan-26 Jul 1981 23 May-18 Jun 1983

Table 2-2. Location, trap type, and dates for CDC in the Dominican Republic.

Location	# And Trap Type[1]	Date[2]
Altos de Peguero	3 CDC	2-3 Apr 1982 27-28 May 1982
Carrasco	2 CDC	9-10 Jul 1982
La Culatica	1 CDC 1 CDC-UV	7-8 Jun 1983
Loma Pena Alta	2 CDC	26-27 Jul 1982
	2 CDC 2 CDC-UV 2 CDC-HB	2-3 Jun 1983
	1 CDC-UV 1 CDC-HB	23-24 Jun 1983
	1 CDC-UV	9-10 Jul 1983
	2 CDC-UV	17-18 Jul 1983
	1 CDC-UV	23-24 Jul 1983
	3 CDC-UV	30-31 Jul 1983
Monte Claro	2 CDC	25-26 Jul 1982
	4 CDC 2 CDC-HB	21-22 May 1983
Morro de Miches	1 CDC	22-23 Sep 1981
	2 CDC	18-19 Jul 1982
	3 CDC 2 CDC-UV 2 CDC-HB	29-30 May 1983

Table 2-2. Continued

Location	# And Trap Type[1]	Date[2]
Pedro Sanchez	2 CDC	20-21 May 1981
	1 CDC	7-8 Aug 1981
	2 CDC	5-6 Jun 1982
Trepada de Jabilla	2 CDC	18-19 Jan 1982
	1 CDC	16-17 Apr 1982 21-22 May 1982 30-31 May 1982 9-10 Jun 1982 30 Jun- 1 Jul 1982 14-15 Jul 1982
	2 CDC 2 CDC-UV 2 CDC-HB	26-27 May 1983
	1 CDC 1 CDC-UV	30-31 May 1983
	1 CDC	18-19, 19-20 Jul 1983

[1]CDC-UV - with blacklight source, CDC-HB - hamster baited trap, no light (see Fig. 2-4).

[2]From approximately 18^{30} hrs Day 1 - 10^{00} hrs Day 2

Figure 2-5. A Disney trap, used for collecting rodent-feeding sand flies.

Table 2-3. The sites and dates for Disney traps used for phlebotomine sand fly sampling in the Dominican Republic.

Site	# traps	Date
Loma Pena Alta	2	8 July - 3 August 1983
Trepada de Jabilla	2	28 May - 15 June 1982
	4	23 May - 2 June 1983
	2	7 - 9 June 1983

checked twice a day by local personnel and food and water were provided at all times. Hamsters were exposed for three to four days and then replaced by others.

Soil samples (dirt, humus, and leaf litter) were collected from tree buttresses and tree holes at five sites where sand flies were common (Table 2-4). Samples (1-4 liters in volume) were placed in plastic bags for transport back to the field station. For observation, a sample was transferred to a white plastic tray, and the material was examined with the aid of a dissecting microscope (7-30X). Larger leaf matter was checked for the presence of larvae or pupae and then discarded. Recovered larvae were held in larval rearing vials (Fig. 2-1b) with a small amount of larval food (Young et al., 1981), and held until adult emergence for species identification. The rest of the sample was returned to the plastic bag and periodically checked for emerged adults or immature stages. The bags were held at ambient temperature (19-30°C) for up to two months.

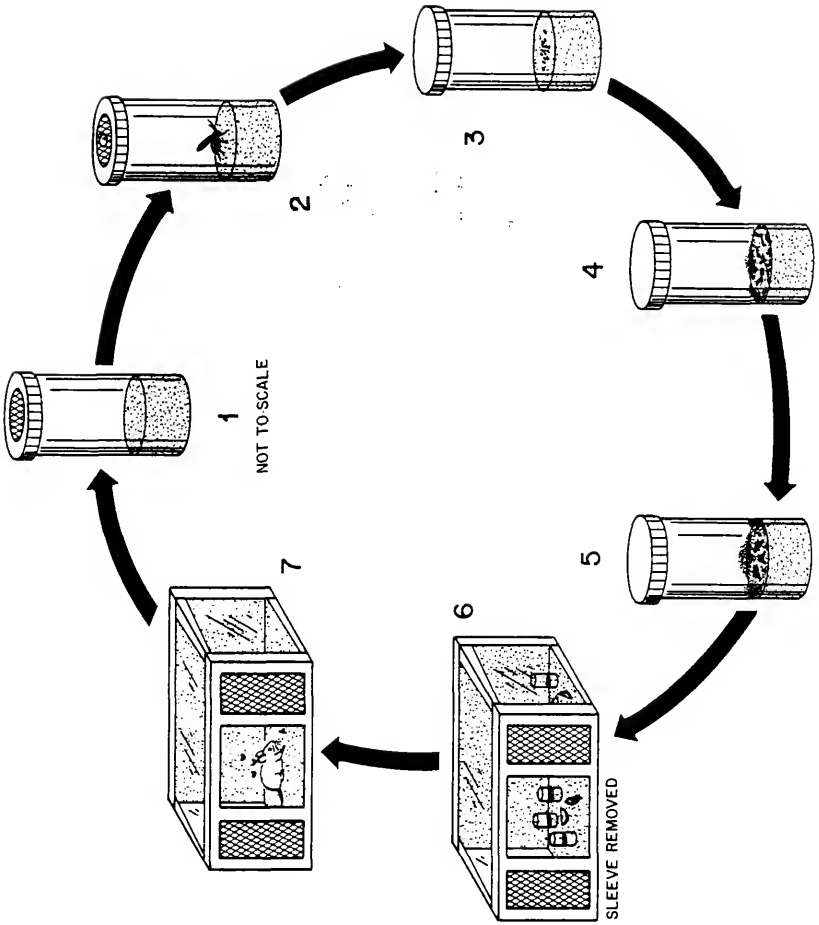
Laboratory Rearing

Techniques used for laboratory rearing of Dominican sand flies (Fig. 2-6) were those described by Endris et al. (1982). Individuals from an egg batch were raised together or individually, as described by Perkins (1982), in a modified tissue culture plate (Fig. 2-7). Newly hatched (within 6 hrs) first instar larvae were transferred from

Table 2-4. Collection sites and dates for soil samples examined for the presence of sand fly larvae.

Site	Date	# Samples	Microhabitat
Altos de Peguero	28 May 1982	3	leaf matter at tree base-cacao grove
	2 Jul 1982	2	as above
Loma Pena Alta	20 Jul 1982	2	leaves and humus at tree base-coffee grove
Monte Claro	25 Jul 1982	5	as above
Pedro Sanchez	15 Aug 1981	3	leaves and humus at tree base-gallery forest
	27 Aug 1981	4	as above
	22 Sep 1981	3	as above
	30 Oct 1981	4	leaves and humus at tree base-upland woods
	25 May 1982	2	as above
	15 Jun 1982	3	as above
Trepada de Jabilla	2 Dec 1981	3	leaves and humus at tree base-coffee grove
	18 May 1982	5	leaves and humus at tree base and in tree hole-coffee grove
	8 Jun 1982	3	as above

Figure 2-6. Schematic diagram of laboratory techniques for rearing of phlebotomine sand flies. (1) Plaster of Paris at bottom of rearing vial in moistened with tap water. (2) Wild-caught or lab-reared gravid females are placed individually in vials and drop of sugar solution is placed on each screen lid. (3) A solid lid replaces the screen lid after eggs are deposited. (4) Larval food is sprinkled on the plaster of Paris anytime before the larvae hatch. (5) Additional larval food is added as larvae grow. (6) Lidless vials containing pupae are placed in the feeding cage; fruit slices provide a sugar source for emerging adults. (7) For a bloodmeal source, a vertebrate is placed in the feeding cage (Endris et al., 1982).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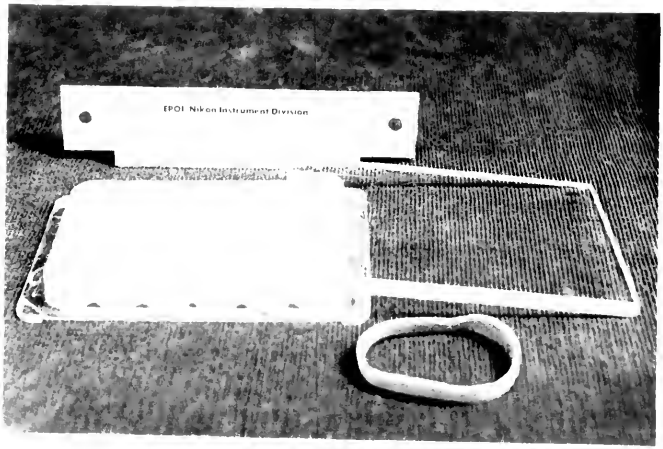


Figure 2-7. A modified microtiter plate for rearing individual sand fly larvae (approximately 0.5cm layer of plaster of Paris in each well).

oviposition vials to wells in the tissue culture plate (one larva per well). The larvae were checked at approximately the same time every day. Individual rearing was performed under different temperature-humidity regimens for both species of sand flies. Lutzomyia christophei larvae were held in a Hotpack chamber (Hotpack, Inc., Philadelphia, PA) at 23°C or 28°C. High humidity was maintained by placing the plates with moistened plaster of Paris in tightly sealed plastic boxes lined with moist paper towels. Lutzomyia cayennensis were reared under three sets of conditions. The first group was reared in a Hotpack chamber at 28°C, as above. The second and third groups were held at ambient conditions in the Dominican Republic of 24 to 33°C and 60 to 95%RH (August-September), and 16 to 28°C with 30 to 90%RH (January-February), respectively.

Eclosion was checked in the vials or plates once per day. Adults were released into the feeding chamber. At the field station, various types of locally available fruit were provided as a sugar source. These included grapefruit, orange, or sweet lemon sections (skin removed); cashew fruit; and peeled mango skin. Lutzomyia christophei reared at Walter Reed Army Institute of Research (WRAIR) were provided with apple slices. For Lu. christophei females, an anesthetized Rattus rattus, Syrian hamster (Mesocricetus auretus), or BALB/c mouse (Mus musculus) was provided as a blood source. An Anolis sp. lizard was regularly provided as a blood source for female Lu. cayennensis; occasionally a

human hand or hamster was offered. The lizard was either restrained in a small cylinder of hardware cloth or was allowed to be free. If free, the mouth was taped shut to prevent the ingestion of sand flies.

To determine the age at first feeding for either species, all flies emerging in a six hour period were held separate by species. For Lu. cayennensis, an Anolis lizard was provided until all females were fed or dead. If necessary, the lizard was exchanged every two to three days for a similar sized, conspecific lizard. The lizards were not restrained. For Lu. christophei females, an anesthetized hamster was provided for one hour two times per day, approximately 0903 hrs and 1530 hrs.

Sand Fly Dissections

A sample of both male and female sand flies from various survey sites was routinely dissected for species determination, using the methods given by Young (1979), or by simply cutting off the head and the last few abdominal segments and mounting them in a drop of Hoyer's mounting medium (Young, pers. comm.). In addition, wild-caught and lab-fed sand flies were dissected upon death to examine the digestive tract for parasites. The dissections were done in normal saline or in Medium 199 (GIBCO, Grand Island, NY) on a microscope slide and observed with the aid of a compound microscope (200X or 450x). Permanent slides were made by removing the cover slip and allowing the liquid to dry, then

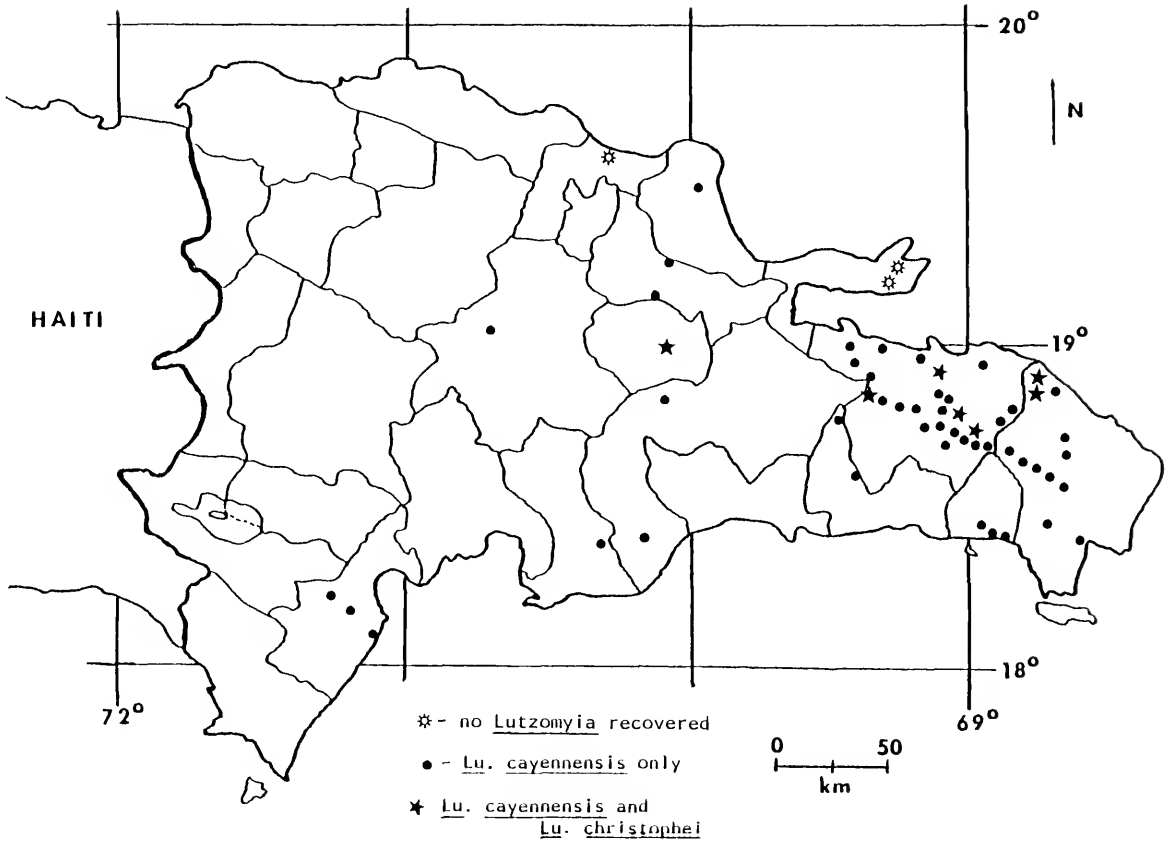
fixing with absolute methanol and staining with Giemsa for 20 minutes. After drying, a drop of Euparal mounting medium was added and a cover slip placed over it.

Results

Field Studies

Adult Lu. cayennensis were aspirator-collected from tree trunks (10 cm diameter and larger) at many localities in the Dominican Republic (Fig. 2-8, Appendix 2). At all sites, there was sufficient tree and shrub vegetation to provide a "forest-floor" type habitat, with little ground cover vegetation. The sites were shaded, to varying degrees. Many sites in El Seibo Province were visited more than once. This species was also recovered from flight trap samples at three sites (Appendix 2). None was recovered by any other method. Adult Lu. christophei were recovered at seven sites by flight trap, CDC trap, or aspirator collection from rock and tree crevices, primarily of ceiba trees (Ceiba pentandra) (Fig. 2-8, Appendix 2). The use of cigarette smoke facilitated their capture from deeper crevices. Six of these sites were leishmaniasis case sites; the seventh was about 5km distance from a case site. No Lu. christophei were collected by hamster-baited CDC traps, Disney traps, or man-biting collections. At Loma Pena Alta, both Disney traps were within 1m of resting sites of Lu. christophei females. The man-biting collections attempted

Figure 2-8. Collection sites for Lu. cayennensis hispaniolae and Lu. christophei in the Dominican Repuglic from May 1981 - August 1983 (See Appendix 2-1, 2-2).


20°
N
HAITI
19°
18°
72°
69°
✲ - no Lutzomyia recovered
● - Lu. cayennensis only
★ Lu. cayennensis and Lu. christophei
0
50
km

at this site were performed within 2 to 3m of tree crevices known to harbor resting sand flies. Two female sand flies were recovered while biting DCL patient #25, in September 1983; these were later identified as Lu. christophei, by the author.

Populations of Lu. cayennensis at Pedro Sanchez varied throughout the year and the population at Trepada de Jabilla followed the same trends from January through July 1982 (Fig. 2-9). The population sample was based on the total number of flies aspirator-collected from 10 marked trees. The sample from Pedro Sanchez was always larger than the same week's sample from Trepada. At Pedro Sanchez, the samples varied from a high of 67 flies to a low of 2 flies; the total for the 39 samples was 986 flies (536 males and 449 females). At Trepada, the high was 39 flies and the low was 0 flies; the total for the 22 samples was 269 flies (156 males and 113 females). Fewer flies were collected during the period February to mid-May 1982, which corresponds to the dry season and the first two weeks of the rainy season. The population level began to rise approximately two and one-half weeks after the beginning of the rainy season (3 May 1982). The male:female ratio, on dates when females were collected, ranged from 0.90 to 3.00 ($\bar{x}$ = 1.90) males/female (8 out of 22 samples had no females). Blood-fed or gravid females comprised 0 to 100% ($\bar{x}$ = 31.3%) of the females at Pedro Sanchez and 0 to 75%

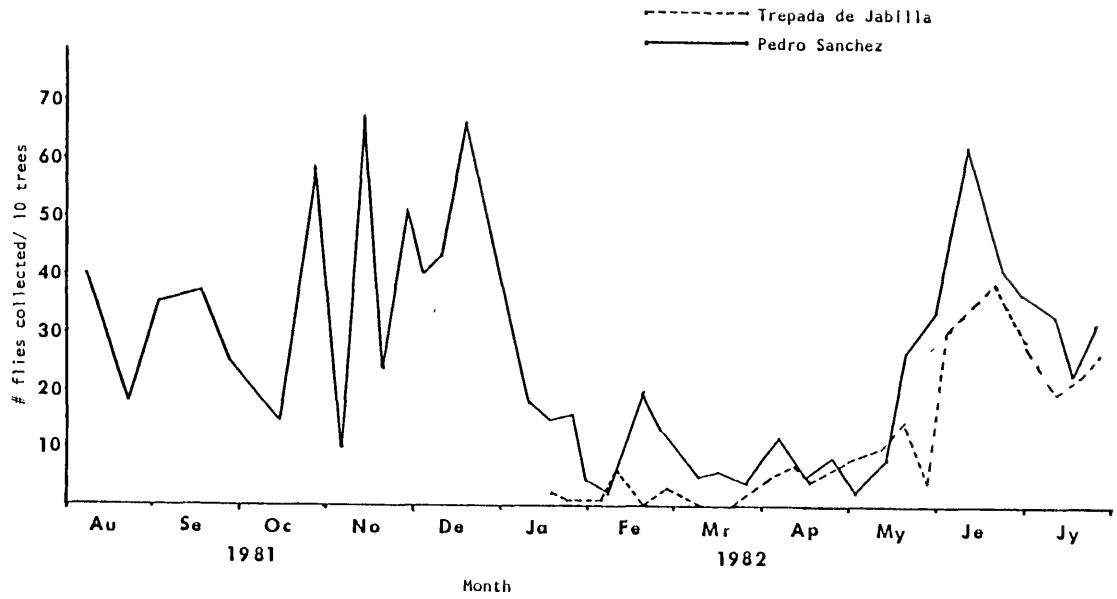


Figure 2-9. Weekly sample populations of Lu. cayennensis at two study sites in the Dominican Republic (based on tree trunk resting collections).

($\bar{x}$ = 48.9%) at Trepada. Due to the scarcity of Lu. christophei adults, seasonal abundance could not be monitored for this species.

During the wettest months (May to December), Lu. cayennensis adults could be found on tree trunks up to a height of 2m. Normally when disturbed, the sand flies would fly only a short distance (less than 10cm); however, on rain-wetted tree trunks the sand flies readily flew off the trees. During the dry season, the flies were generally found at the tree base, often where the ground had separated from the tree trunk in fissures, or in the moss at the base. Smaller tree holes also harbored Lu. cayennensis at this time of year. Female Lu. cayennensis were commonly observed feeding on Anolis lizards during the day, principally on Anolis distichus, but also on A. cybotes. Lizard identification was based on Cochran (1941).

Adult Lu. christophei were usually recovered from deep tree crevices and ground level tree holes in large shade trees in coffee groves. Two types of trees provide crevices which could serve as resting sites for Lu. christophei, the ceiba and the strangler fig (Ficus spp.). The former may have very large buttresses and the latter usually has numerous crevices of various sizes as it entwines its host tree. Cigarette smoke puffed into the crevices forced the sand flies to the entrance, where they were more easily captured. Most of the Lu. christophei collected were obtained at Loma Pena Alta, where a total of 81 flies

(51 males and 22 females) were captured by aspirator, 7 flies (2 males and 5 females) were captured in a flight trap, and 23 flies (10 males and 13 females) were captured in CDC or CDC-UV traps. In one tree hole, 14 sand flies were found in association with a rat nest constructed of leaves. Of eight females, three were blood-fed or gravid and were the only fed Lu. christophei females recovered in aspirator collections. A common characteristic of the other resting sites was the presence of land snails and millipede feces. On rare occasions at Loma Pena Alta, male Lu. christophei were found resting on tree buttresses near the entrance to tree crevices, in association with Lu. cayennensis. Lutzomyia christophei was the more active of the two species.

Five fourth-instar larvae were recovered from a soil sample at Monte Claro. The microhabitat was humus and leaf litter which had accumulated in the buttress of a large shade tree in a coffee grove. The larvae were reared to the adult stage and were Lu. cayennensis. No other phlebotomine larvae were seen or recovered from the other soil samples (Table 2-3).

Laboratory Studies

Wild-caught and lab-reared Lu. cayennensis adults behaved similarly. Females fed readily on Anolis lizards in the feeding chamber, but showed no interest in feeding on human, hamster, or rat (R. rattus). Over 50 wild-caught

females were exposed to a skink, Mabuya mabouya, in a feeding chamber, but none fed or was seen probing. Twenty lab-reared females were exposed to tree frogs, Hyla sp., and toads, Bufo marinus, but the flies showed no interest in feeding. The preferred feeding site on lizards was on the mid-dorsal region to the tail base. Some females were occasionally observed feeding on the top of the head and the shoulder region. Females rarely probed more than one spot before feeding to repletion. Feeding time ranged from 62.5min to 82.5min (n = 26, $\bar{x}$ = 73.0min±6.25min (S.D.)). Feeding only occurred under lighted conditions. Females would feed at any time of day in the feeding chamber, provided that the chamber was left in a well lighted room. Generally, females first fed at age 3.0 to 4.5 days (n = 50) posteclosion; however, on various occasions lab-reared flies, less than 48hrs old, fed on lizards. Mating was observed before, during, or after bloodfeeding, with the pair remaining in copula for up to 21min. Females began oviposition four to five days post-bloodfeeding. Complete oviposition usually required less than one day. Wild-caught females generally died within 24hrs after laying eggs. Holding the flies in a Hotpack environmental chamber helped to increase the survivorship of lab-reared females. Approximately 17 to 33% of the females of every generation survived to take a second blood meal; 50 to 75% of these survivors subsequently laid a second batch of eggs. No males or unfed females lived more than six days.

Female Lu. cayennensis were anautogenous, each female laying up to 60 eggs. Most unmated bloodfed females died without ovipositing (20 out of 23 flies), but three laid partial egg batches of 5 to 9 eggs. The size of a full egg batch for wild-caught females was 38 to 60 eggs (n = 50, $\bar{x}$ = 47.7±6.6 (S.D.)), based on females collected with a full blood meal that had no eggs retained at death. For lab-reared females, a full egg batch contained 39 to 60 eggs (n = 50, $\bar{x}$ = 46.6±6.3 (S.D.)). Of 103 F_1 females held singly in vials, only 19 laid full egg batches, 61 females laid partial egg batches of 7 to 29 eggs, and 23 females died without laying eggs. The percent egg hatch, for full egg batches from wild-caught and lab-reared females ranged from 20.4-100% (n = 100, $\bar{x}$ = 68.4%±29.7% (S.D.)). Percent egg hatch for partial egg batches ranged from 0 to 100% (n = 50, $\bar{x}$ = 61.2%±33.2 (S.D.)). Percent egg hatch was not statistically different between the two groups (t-test, p = 0.05). When three or fewer eggs were laid by a female, none hatched.

All hatching from an egg batch occurred within a 12hr period. Under ambient conditions at the field station, the incubation time for eggs was 8 to 12 days, depending on time of year (ambient temperature). Longer incubation times were associated with cooler temperatures, especially in January and February (Table 2-5).

Larvae of Lu. cayennensis exhibited burrowing behavior. Larvae tended to stay under their food, except when the food

was very damp. When individually reared, male sand fly larvae developed faster than did females, under all three temperature-humidity regimes (t-test, $p = 0.05$) (Table 2-5). Males developed faster under ambient conditions in August-September (24-33°C, 60-95%RH) than males under constant conditions (27°C, 85%RH). Both groups developed faster than those under ambient conditions in January-February in the Dominican Republic (16-28°C, 30-90%RH) (t-test, $p = 0.05$). The time from oviposition to adult eclosion, for males and females, is presented in Table 2-5 and Figures 2-10, 2-11, 2-12.

A closed colony of Lu. cayennensis was maintained for six generations, or for almost one year.

Wild-caught and lab-reared Lu. christophei behaved similarly in the laboratory. Females readily fed on anesthetized rodents, including a wild-caught R. rattus, laboratory mice and hamsters. They readily probed on a human hand. The females showed no feeding site preference, feeding equally well on the ears, paws, or eyelids of the offered rodent host. Females often probed more than one spot and after initiating feeding, many females moved to a second site to complete feeding. Females of this species fed equally well under light or dark conditions. Feeding time ranged from 2.75min to 4.95min ($n = 25$, $\bar{x} = 3.35$min ±0.50min (S.D.)). Some lab-reared females fed as early as 24hrs after emergence, though most did not feed until 48 to 72hrs after eclosion. Females took a very large bloodmeal

Table 2-5. Mean duration (± S.D.) in days of immature stages of Lu. cayennensis hispaniolae at three temperature regimes, according to sex of sand fly.

Condition	Sex	Egg	Larval Instars 1	2	3	4	Pupa	Total (egg-adult)	n
Constant (28°C)	male	8	5.1±1.0	3.6±0.8	3.0±0.4	6.5±1.6	7.1±0.3	33.7±1.9	12
	female	8	5.0±1.0	3.2±0.8	4.4±1.3	6.5±2.3	7.5±0.8	36.1±2.5	11
		(48)	(31)	(28)	(28)	(25)	(23)		
Ambient (24-33°C)	male	8	4.7±0.5	2.3±0.5	2.7±0.5	6.0±0.7	6.7±0.6	30.3±1.3	23
	female	8	5.0±0.4	2.4±0.5	3.1±0.3	6.4±0.7	7.7±0.7	32.5±1.0	24
		(48)	(48)	(48)	(48)	(48)	(47)		
Ambient (16-28°C)	male	12	5.0±0.0	3.6±0.6	3.1±0.7	8.3±0.6	9.1±0.3	43.6±1.6	17
	female	12	5.4±1.0	3.3±0.8	3.6±0.7	8.9±0.8	9.4±0.5	45.5±1.6	24
		(48)	(47)	(46)	(46)	(42)	(41)		

Note: Number in ( ) indicates individuals surviving each stage.

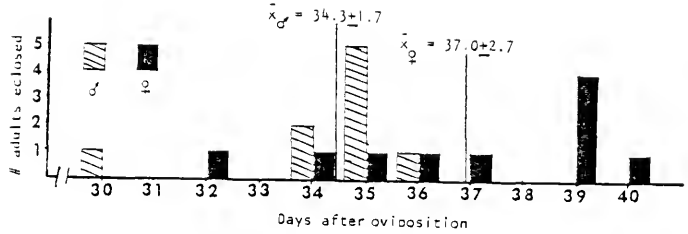


Figure 2-10. Eclosion time in days after egg deposition for Lu. cayennensis reared under constant conditions (28°C, 90%RH).

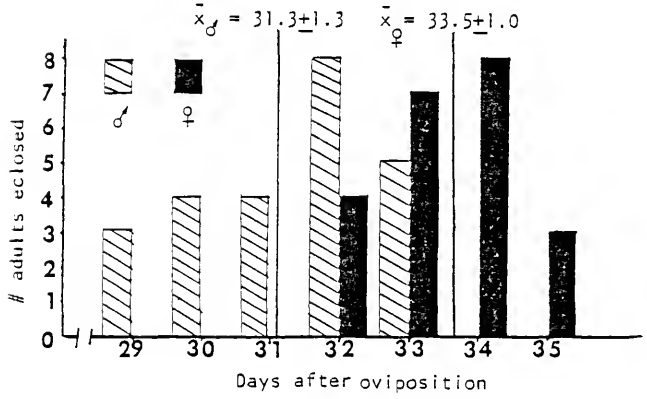


Figure 2-11. Eclosion time in days after egg deposition for Lu. cayennensis reared under ambient conditions August-September (24-33°C, 60-90%RH).

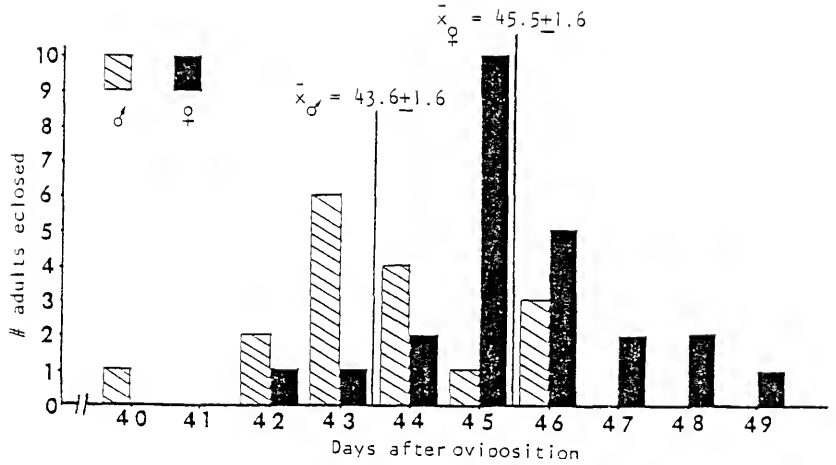


Figure 2-12. Eclosion time, in days, after egg deposition, for Lu. cayennensis reared under ambient conditions, January-February (16 to 28°C, 40 to 85%RH).

(Fig. 2-13), but were very active afterwards. Mating, only rarely observed, occurred before or after feeding, with coupling lasting up to 28.5min. Oviposition began at least five days after blood-feeding, but for a few females, it was delayed up to ten days post-feeding; it was usually completed in less than one day. Approximately 17% of the females that survived five or more days post-feeding (8 of 46 F_1 and F_2 females) did not exhibit ovarian development after their first blood meal. By seven days however, they had excreted the blood meal remnants in their feces and were capable of refeeding and developing eggs. In the F_3 generation, 8 of 52 females (15.8%) developed partial egg batches of eight or fewer eggs before refeeding. All eight died without ovipositing.

Only 2 of the 11 lab-fed wild-caught females laid full egg batches of 39 and 49 eggs. The nine other flies laid 0 to 35 eggs ($\bar{x}$ = 7.3±11.3 (S.D.)). The size of a full egg batch for lab-reared females was 35 to 87 eggs (n = 10, $\bar{x}$ = 50.7±12.9 (S.D.)). Of 64 F_1 to F_3 females which survived five or more days post-feeding, only 14 (21.9%) laid full egg batches, 24 females (37.5%) laid partial egg batches of 5 to 27 eggs, and 26 females (40.6%) died without ovipositing. The number of eggs laid, plus the number retained at death (a measure of reproductive potential in the above 64 females) ranged 36-88 eggs and/or ovarioles/female ($\bar{x}$ = 64.1%±24.3% (S.D.)). The percent egg hatch for full egg batches ranged from 12.6% (11/87 eggs) to 100% (36/36 eggs)

Figure 2-13. Female Lu. christophei feeding on BALB/c mouse.

(n = 12, $\bar{x}$ = 64.4%±31.1% (S.D.)). Percent egg hatch for partial egg batches ranged from 0% (0/18 eggs) to 100% (28/28 eggs) (n = 20, $\bar{x}$ = 56.8%±38.1% (S.D.)). Percent egg hatch was not statistically different between the two groups (t-test, p = 0.05). All hatching from an egg batch occurred within a 24hr period. The time period for egg incubation was 10 to 17 days, depending, in part on the temperature maintained (Table 2-6).

Lutzomyia christophei larvae exhibited burrowing behavior, prefering to remain under the food in the larval rearing containers. In the individual and group rearing experiments, development time from egg to adult ranged from 51 to 69 days for the F_1 generation (27°C) and from 57 to 73 days for the F_2 and F_3 generations (23°C). Males developed at a faster rate than did females (t-test, p = 0.05). The data from these rearing experiments are presented in Table 2-6 and Figures 2-14 and 2-15.

The closed colony of Lu. christophei is being maintained at Walter Reed Army Institute of Research, Washington, D.C.

Sand Fly Dissections

Dissections of 319 wild-caught parous Lu. cayennensis were made. The flies came principally from five sites, of which 46.4% (148 flies) of the total were from leishmaniasis case sites. Most females had a blood meal evident at capture and did not die, or were not killed, until the blood

Table 2-6. Mean duration (±S.D.), in days, of immature stages of Lu. christophei at constant temperature and humidity, according to sex of sand fly.

Generation/ Condition	Sex	Larval Instars						Total (egg-adult)	n
		Egg	1	2	3	4	Pupa		
F_1 Constant (28°C, 85%RH)	male	10	9.5±0.9	6.1±2.3	5.3±1.1	13.4±3.1	13.2±0.8	57.7±6.3	14
	female	10	9.9±1.2	6.2±2.3	6.2±1.4	17.5±3.3	13.4±1.1	63.5±6.5	26
		(46)	(46)	(43)	(42)	(41)	(40)		
F_2 & F_3 Constant (23°C, 85%RH)	male	15.2±1.7	----------	N.D.	----------		15.3±2.4	62.6±4.2	78
	female	15.2±1.7 (203)	----------	N.D.	----------		16.7±2.2 (173)	65.7±3.0	95

Note: Numbers in ( ) indicate individuals surviving each stage.

N.D. = Not Determined

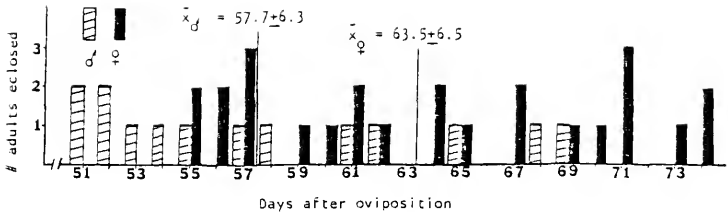


Figure 2-14. Eclosion time, in days after oviposition, for F_1 Lu. christophei reared under constant conditions.

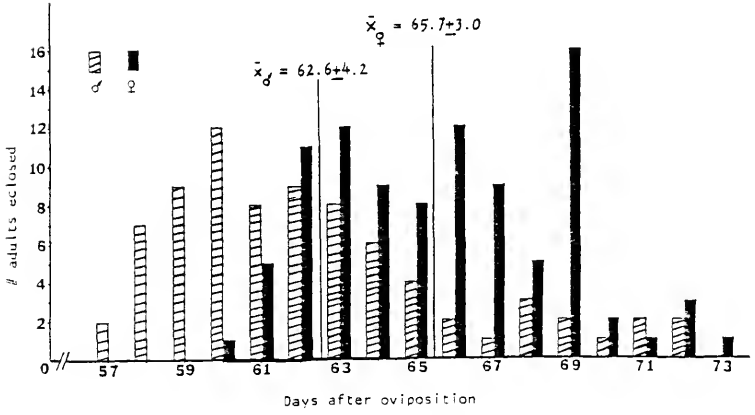


Figure 2-15. Eclosion time, in days after oviposition, for F_2 and F_3 Lu. christophei reared under constant conditions.

had been digested and the residue excreted. The totals for the sites, the percent blood-fed or gravid, and the time of year collected are presented in Table 2-7.

Dissections were examined of 23 wild-caught Lu. christophei from Loma Pena Alta. Nine of these took bloodmeals in the laboratory, two on a R. rattus captured at the site and seven on a hamster. None of the 23 lab-fed females was positive for parasites. The results of feeding lab-reared Lu. christophei on leishmanial-infected BALB/c mice are presented in Chapter 3.

Discussion

Lutzomyia cayennensis hispaniolae has a widespread geographic distribution in the Dominican Republic. It occurs in wooded areas within 100m of the coast and in coffee groves in the interior (Loma Pena Alta, elevation 427m). Throughout the sugarcane growing regions, Lu. cayennensis was found along streams and rivers wherever there was a sufficient number and density of trees to support a "forest-floor" type habitat (i.e. leaf and other organic matter present). This species was found at or near eight of the DCL case sites visited during the survey. In the wild, Lu. cayennensis appears to be very sedentary, as very few were collected by the flight traps, often despite close proximity of the trap to known resting sites. At Monte Claro, where 13 flies were recovered from the flight

Table 2-7. Site of collection for female Lu. cayennensis dissected.

Site	# Blood-Fed or Gravid	#Parous	Total	Time of year
Pedro Sanchez	93	34	127	May 1981, Aug 1981-Jul 1982
Trepada de Jabilla	50	21	71	Nov 1981-Jul 1982
Altos de Peguero	25	9	34	Mar-Jul 1982
Loma Pena Alta	13	7	20	Jul 1982
Monte Claro	12	8	20	June 1982
Morro de Miches	1	2	3	Aug 1981
Miscellaneous	37	7	44	Aug 1981-Jul 1982

trap collection, the trap was set so that the side of one of the end panels was in contact with a tree on which were found over 70 resting sand flies. The absence of Lu. cayennensis in light trap collections suggests that this species was not attracted to light. Both wild-caught and lab-reared flies exhibited a positive phototaxic response in the feeding chamber, which was noted by changing the position of a desk lamp used to illuminate the chamber. Later, as sand flies were collected from small tree holes, it became clear that this was a probable response to light as an escape attempt, with the path towards the light replacing the path out of a tree hole. In the lab and in the wild, this species was only diurnally active, as were its hosts, Anolis lizards. Because these lizards are very common, there is no great need for extensive host-seeking behavior.

Although there may be some wind-borne dispersal of adults, most of the distance an individual female travels probably occurs during the 60+min that it is feeding on a lizard. This length of time is much longer than that of similar-sized mammalian feeding species, such as Lu. anthophora (Endris, 1982) and Lu. christophei; however, the lizards are not capable of reaching the sand flies to dislodge them. Lutzomyia vexator (Coq.), also a lizard-feeder that occurs in the USA and is about twice the size of Lu. cayennensis, feeds to repletion in only 10min (Perkins,

unpublished data). Chaniotis (1967) reported 60+min feeding times for some lizard-feeding sand flies in California.

The collection of larvae at Monte Claro represents the first recovery of larvae of Lu. cayennensis in the field. Hanson (1968) was unsuccessful in locating Lu. cayennensis larvae in Panama. Monte Claro supported a very large population of sand flies in a very small area. Many of the trees sampled in the coffee grove had 50+ sand flies resting on them; thus it does not seem surprising to find the larvae in such a situation. Hanson (1968) noted that larvae burrowed in culture, but to what depth they occur in nature is unknown. In the current study, the soil samples were jostled in transport back to the field station, so the depth of the five recovered larvae was not known. The microsite where they were collected had an unusually deep layer of humus, 4 to 6cm deep. An injury to the tree trunk may have been the cause of the ooze that started 0.5m above ground level and continued to the ground, perhaps enriching the soil at this spot. The soil samples taken from both Pedron Sanchez and Trepada had very little humus.

The population levels of Lu. cayennensis are related to wet and dry seasonal periods. Ambient temperature probably influences the population level as well. In the eastern region, the dry season usually lasts from late February to the beginning of May. May is the wettest month, but from June through December the ground remains fairly well saturated. The population level of Lu. cayennensis, although

somewhat erratic on the weekly basis, remained fairly high from August to December 1981 and from late May to July 1982 (Fig. 2-9).

The short lifespan of wild caught and lab-reared flies at the field station was probably due to low humidity. Other sand flies reared in the laboratory, and kept in the environmental chamber at ±85%RH, often lived 13 to 15 days after eclosion. Early female death was the probable cause when only partial egg batches were laid. Sugar feeding was never observed in nature and only rarely in captivity. Lack of carbohydrate may have been a contributing cause to early mortality. As Lu. cayennensis is a very sedentary species, the natural sugar source must be very close to the resting sites, perhaps secretions from the trees.

The rearing times observed for Lu. cayennensis were similar to those reported for Lu. anthopora, a similar sized species (Endris, 1982), but much shorter than those for the sympatric Lu. christophei. The individual rearing gave somewhat misleading results, in that most flies emerged within a seven day period. When larvae from a single egg batch were reared together in a vial, emergence of adults occurred during a period of up to 22 days. This delay could be the effect of overcrowding or interference, as has been reported among mosquito larvae (Ikeshoji and Mulla, 1970), but not previously for sand flies.

Lutzomyia christophei appears to have a more limited distribution than Lu. c. hispaniolae, as Lu. christophei

were recovered at seven sites, all of which were leishmaniasis case sites, or in close proximity to such sites. As resting Lu. christophei were secreted in tree crevices, their apparent scarcity may have been partially an artifact of the amount of time spent at the survey sites and the collection methods used. It was not until cigarette smoke was used to flush the flies from the crevices, that specimens of this species were collected with any regularity. With the exception of various locations in the Pedro Sanchez area, including the study site and a small 15h woods, few non-case sites received more than 10hrs of searching during the study period. The habitat of this species, crevices and ground-level tree holes in large shade trees (primarly Ceiba pentandra) in coffee groves and rock crevices in forested areas, also may be a factor limiting its distribution. In the Dominican Republic, very few virgin rain forests remain, coffee and cacao groves, however, may simulate forest conditions, as heavy leaf litter and much shade are characteristic of these areas. Typically, land snails and millipede feces were observed in the Lu. christophei-inhibited crevices; it is quite likely that snail and millipede byproducts enrich the soil of these crevices and possibly provide sand fly larvae with an adequate diet. Lutzomyia christophei may have evolved as a nest inhabiting species with one or more of the tree hole-inhabiting mammals that were present before the arrival of the Spaniards (1492 AD), much the way Lu. anthophora is associated with woodrat

(Neotoma) nests in the USA (Young, 1972). Plagiodontia aedium, the only endemic rodent in the D.R., is a tree hole inhabitant (Woods, 1981). As the introduced rats replaced the endemic rodents, the sand fly may have moved into the tree hole nest of R. rattus or Mus musculus. Along with this move may have been the development of a new reservoir host for an endemic Leishmania; although there is discussion as to whether the Dominican Leishmania is native to the country or introduced (Walton, pers. comm., Zeledon, pers. comm.).

Although sand flies were never encountered in large numbers in crevice resting sites, residents at several case and non-case sites reported them to be a major annoyance at times. On Cuba, man-biting sand flies have been reported from caves (Avila et al., 1969); although the species was not determined, these flies were probably Lu. orestes (Young, pers. comm.), a very close relative of Lu. christophei. In the Dominican Republic, very few of the visited DCL case sites had caves or rock outcroppings. "Erisos" were reported to be common in June and July, at Altos de Peguero, Loma Pena Alta, and Trepada de Jabilla, though much more so in past years than in the three summers (1981-1983) that the author was present. "Erisos" were originally described to the author as pale-colored flies, smaller than a mosquito, which start biting around dusk and continue into the late evening, inside the house as well as outside. The bite was described as being as painful as a

mosquito's. "Erisos" also hold their wings aloft, and tend to hop around on the person before biting. All descriptions were similar and all described typical sand fly behavior. In September 1983, two "erisos" were collected while feeding, on one of the leishmaniasis patients. These were confirmed to be female Lu. christophei by the author and Dr. D. G. Young.

Thus Lu. christophei is regarded as the probable vector of leishmaniasis in the Dominican Republic. It fulfills the requirements of readily feeing on man and rodents, the probably reservior hosts, and is capable of experimentally transmitting the Dominican Leishmania (see Chapter 3). One of the difficulties of in studying this sand fly is based on its long life cycle of more than two months. Futher work needs to be done on determining the relationship of the long cycle to the natural habitat. Much also remains to be determined on the bionomics of this species. The epidemiological data, such as vector efficiency and infection rates in the field, need to be studied further. The sample of 23 wild-caught female flies available for examination during this study was insufficient. A long-range program, performed by personnel who could visit the sites at various times over a two or three year period, is needed.

CHAPTER 3

GROWTH OF *LEISHMANIA*-ISABEL STRAIN IN CULTURE MEDIUM, LABORATORY RODENTS, AND SAND FLIES

Introduction

New World cutaneous leishmaniasis is caused by members of the *Leishmania braziliensis* and *L. mexicana* complexes (Bray, 1974), but diffuse cutaneous leishmaniasis (DCL) has been associated only with the *L. mexicana* complex in the Americas (Schnur et al., 1983) and with *L. aethiopica* in Africa (Bray and Bryceson, 1969). Current knowledge of DCL is based primarily on studies of *L. aethopica* in Ethiopia (Bryceson, 1969, 1970a, b, c) and *L. mexicana pifanoi* in Venezuela (Convit et al., 1971). The focus of DCL in the Dominican Republic is unique owing to the complete absence of human cases with ulcerating lesions (Bogaert-Diaz, unpublished data). This is one of the features that has sparked interest in the indentity of this *Leishmania*. Schnur et al. (1983) and Kreutzer et al. (1983) believe that the Dominican parasite differs from other known subspecies in the *L. braziliensis* and *L. mexicana* complexes, but appears to be closer to strains in the *L. mexicana* complex. Lainson (1983) reported that the Dominican parasite developed in the

anterior midgut (Suprapylaria) of experimentally infected Lutzomyia longipalpis from Brazil. Schnur et al. (1983) described some of the biological characters of the parasite in culture and lab animals, but only in subjective terms. Since the identity of the Dominican Leishmania remains undetermined, the commonly used strain, isolated from a 14-year-old female patient from the Dominican Republic, is designated Leishmania-Isabel strain (Petersen et al., 1982).

The purpose of this study was to quantitate the growth of the Dominican parasite in comparison to other strains of Leishmania, to determine the course of infection in susceptible laboratory rodents, to describe the course of infection in susceptible sand flies, and to effect transmission with the probable natural vector species.

Methods and Materials

Comparison of the Growth of Three Strains of Leishmania

Stock culture of L. mexicana amazonensis was obtained from Dr. K. P. Chang, Rockefeller University, inoculated into a Syrian hamster, Mesocricetus auretus, and later reisolated and passaged one time on Schneider's Drosophila Medium (GIBCO Laboratories, Grand Island, New York) supplemented with 20% (v/v) heat-inactivated (56°C, 30min) fetal bovine serum (FBS) (Hendricks and Wright, 1979). The stock culture of L. mexicana-Texas (WR-411) was provided by Dr. Larry Hendricks, Walter Reed Army Institute of Research

(WRAIR) and was handled as above. The culture of Leishmania-Isabel strain (WR-336) was provided by Dr. Eileen Franke, WRAIR, but was not passaged through animals. For each strain, 15 tubes of enriched Schneider's medium were inoculated so that the Day 0 populations were approximately 2.50×10^5 log phase promastigotes/ml. The tubes were held in a Hotpack environmental chamber (Hotpack, Inc., Philadelphia, PA) at 24.0°±0.5°C. The populations were checked daily for 16 days using a hemacytometer with the aid of a compound microscope (200X)

Growth of Leishmania-Isabel Strain in Laboratory Rodents

Prior to inoculation in rodents, the Isabel strain was passaged one time on NNN medium (Mansour et al., 1974). Subadult or young Syrian hamsters and IRC strain mice (5-7 weeks old) were inoculated via intracardial (IC), intraperitoneal (IP), or subcutaneous (sub Q) routes. the dosages used are given in Table 3-1. The animals were maintained by inoculation group. Two animals from each species/inoculation group were examined at 30 and 60 days post-inoculation. Four subcutaneously inoculated hamsters were examined via xenodiagnosis with sand flies, Lutzomyia anthophora, at 3, 7, and 11 months post-inoculation; at 15 months, they were killed and assayed, as outlined below, using Schneider's medium for cultures.

Before killing the animal, 0.2-0.5ml of blood was taken via heart puncture and added to 4ml RPMI medium (80% RPMI

Table 3-1 Method of inoculation and number of promastigotes used to infect laboratory rodents with Leishmania-Isabel strain.

Species	Method of Inoculation	Size of Inoculum
Hamster	subcutaneous	9.00×10^4 promastigotes
Hamster	intraperitoneal	2.25×10^5 promastigotes
Hamster	intracardial	9.00×10^4 promastigotes
Mouse	subcutaneous	1.13×10^5 promastigotes
Mouse	intraperitoneal	5.65×10^5 promastigotes

Medium 1640 (GIBCO) + 20% FBS (v/v)). A subcutaneous aspirate was taken from the left hind paw and placed in another tube of RPMI. After death, tissue samples were taken of hind paw skin, liver, and spleen. Impression smears were made of each on microscope slides. The tissue samples were then placed in a Petri dish of normal saline which contained 4000U Penicillin G Sodium (U.S. Biochemical Corporation, Cleveland, OH) and 1.5mg/ml Streptomycin Sulfate (U.S. Biochemical Corp.), with one Petri dish/ animal. The Petri dishes were left in a refrigerator (4°C) for 24hrs. The method was adapted from Herrer and Christensen (1975). The following day the Petri dishes were removed and uncovered in a biological hood. Each tissue sample was washed twice with normal saline. A small portion of tissue

($9mm^2$ skin, $27mm^3$ liver or spleen) was then placed in 2ml sterile normal saline in a sterile mortar and ground by pestle until macerated. The solution was allowed to settle for 1 min then 1ml of supernant was drawn off and added to a tube of RPMI Medium. The culture tubes were held at room temperature for eight days then checked for the presence of promastigotes, with the aid of a compound microscope (200X). Impression smears were fixed in absolute methanol, stained with Geimsa for 30 minutes, then observed with a microscope (1000X).

The Growth of Leishmania-Isabel Strain in the Sand Fly

Four-month-old BALB/c mice were inoculted in the hind foot pads with approximately 2.5×10^5 promastigotes of Leishmania-Isabel strain. The mice were held for four to six weeks to allow development of the histiocytoma, during which time the foot pad grew to two to three times normal size. Four to seven-day-old laboratory-reared Lutzomyia anthophora, a species from Texas and Mexico, were allowed to feed on the infected hind foot pads of the mice. The body and tail of the mouse was covered so that only the hind feet were exposed to the sand flies. After feeding, the sand flies were held in groups of 20 to 50 flies, in 40dr rearing vials, and held in a Hotpack environment chamber at 23.°±0.5°C and 70±5%RH. A sample of flies were dissected on each day (Day 1 to 7 post-feeding) so that a total of 15 infected flies were observed for each day. After

dissecting out the digestive tract in Medium 199 (GIBCO), the mouthparts, head, and digestive tract were examined for the presence of leishmanial promastigotes, with the aid of a microscope (100X, 200X). The number of infected flies was noted along with location, number and shape of the parasites. To determine if the parasites observed were infective, a sample (pooled by for Day 3, 4, and 5) was inoculated into the hind foot pads of two six-week-old hamsters.

Transmission of Leishmania-Isabel Strain by Lutzomyia christophei

Female F_3 generation Lu. christophei were allowed to feed on the swollen hind foot pads of Leishmania-Isabel (WR-336) infected BALB/c mice. The sand flies fed on the mice five to seven weeks post-inoculation. The flies were then held individually in 7dr vials in a Hotpack chamber, 23.0°±0.5°C and 80±5%RH, until death or until they were ready to refeed, usually seven or more days after the first bloodmeal. Females that died one to seven days post-feeding were dissected and examined, to correlate the infection in Lu. christophei with that in Lu. anthophora, performed as above. For their second bloodmeal, the flies were released into a feeding chamber with an anesthetized noninfected BALB/c mouse, covered with a cloth sleeve except for the hind feet and tail. The refed flies were then recaptured and held individually in 7dr vials until death. Upon death, the flies were dissected to determine the state of

infection. The mice used for refeeding the sand flies were maintained in the laboratory for three weeks before attempting to xenodiagnose leishmanial infection with Lu. anthophora and/or diagnosing through culturing of spleen and liver tissue sample and subcutaneous aspirate in Schneider's medium, performed as above. Female Lu. anthophora were used for xenodiagnosis due to the unavailability of female Lu. christophei at the time.

Results

Comparison of the Growth of Three Strains of Leishmania

The two strains of Leishmania mexicana, L. mexicana-Texas and L. m. amazonensis grew at rates that were not statistically different, but their growth rates were much faster than that of the Leishmania-Isabel (t-test, $p = 0.05$) (Fig. 3-1). The L. mexicana strains maintained log phase growth until Day 6, stayed in a stationary phase until Day 10, and then decreased rapidly. Cultures of the Isabel strain did not achieve peak population growth until Day 12, after which they declined slowly. Post-peak populations were difficult to estimate due to the large number of dead or inactive promastigotes present in the samples, only motile promastigotes were counted.

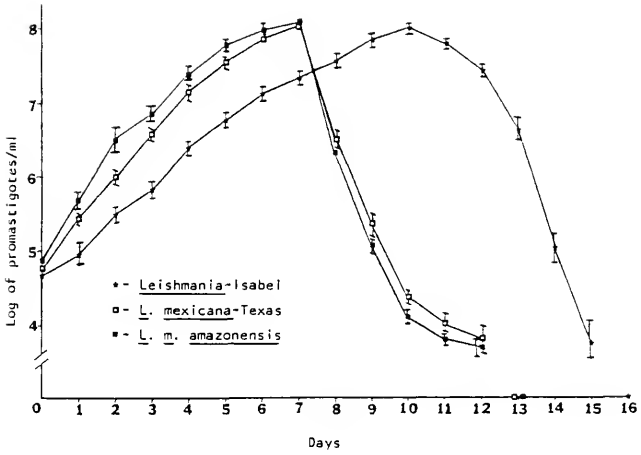


Figure 3-1. Daily estimated mean population of three strains of Leishmania grown in Schneider's medium, at 25.0°C.

Growth of Leishmania-Isabel Strain in Laboratory Rodents

Leishmania-Isabel-inoculated hamsters and IRC strain mice examined at 30 and 60 days post-inoculation showed no externally visible signs of infection. Four hamsters were inoculated via subcutaneous route and maintained for over 60 days, by 75 days post-inoculation, only one hamster exhibited a very slight swelling of the hind paw. All four were xenodiagnosed using lab-reared Lu. anthophora and were infected at this time. By 7 months post-inoculation, the cutaneous infection had apparently self-cured because none of 30 sand flies feeding on the "infected" foot of each hamster developed promastigote infections. Aspirate cultures taken at seven months were also negative. When the four hamsters were killed at 15 months, no parasites were isolated by aspirate, liver, or spleen culture.

The most sensitive method for determination of infection, besides xenodiagnosis, was by culturing of splenic tissue. No parasites were observed in the cultures of heart blood or in skin impression smears. Active infections were recovered from all animals killed at 30 days, by spleen culture and from all, but one mouse, via liver culture. The results were much more variable at 60 days (Table 3-2). At 60 days, no leishmaniae were observed or isolated from one mouse (IP) and two hamsters (1IP, 1IC). Animals inoculated via subcutaneous injection were the most readily confirmed as infected by the different methods of culturing and culturing and impression smears (Table 3-2, Fig. 3-2).

Table 3-2 Number of animals examined determined to be infected with Leishmania-Isabel strain via different isolation methods.

Species	Inoculation Method	Day Examined	Cultures					Impression Smears		
			Heart Blood	Foot Aspirate	Skin	Spleen	Liver	Skin	Spleen	Liver
Mouse	sub Q	30	0	0	0	2	1	0	1	0
		60	0	0	1	1	1	0	1	1
	IP	30	0	0	0	2	0	0	1	1
		60	0	0	0	2	0	0	2	0
Hamster	sub Q	30	0	0	1	2	2	0	2	2
		60	0	1	1	2	2	0	2	2
	IP	30	0	0	0	2	2	0	2	2
		60	0	0	0	1	0	0	0	0
	IC	30	0	0	0	2	2	0	2	1
		60	0	0	0	1	0	0	1	1

Note: n = 2, except Mouse-sub Q-60 days where n = 1.

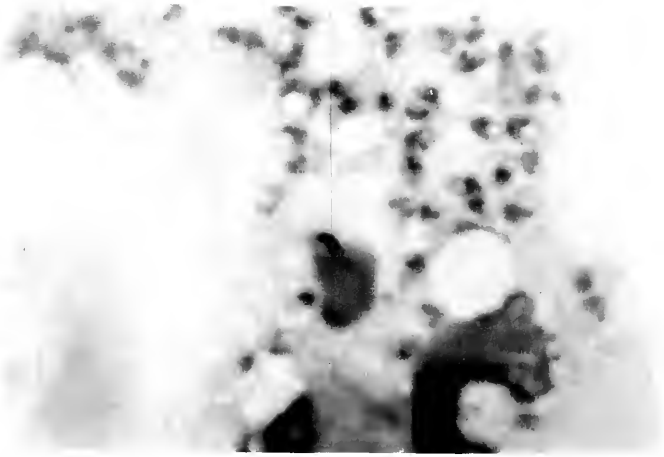


Figure 3-2. Leishmania-Isabel strain amastigotes in a spleen impression smear stained with Giemsa (1000X).

Growth of Leishmania-Isabel Strain in the Sand Fly

Promastigotes were observed in some of the Lu. anthophora females in each sample from Day 1 to Day 7 post-feeding on a Leishmania-Isabel infected BALB/c mouse. The percent infected increased with time, but the percent with bacterial contamination (Leishmania infection undeterminable) decreased with time, because of the high death rate in these flies. In the sample from Day 1, only one promastiogote was observed in 30 fly dissections; however, a few amastigote-infected macrophages were observed in the blood meals in five of the dissections which were later stained and examined with the aid of microscope (1000X). For the remaining days, infected flies represented 53.6% to 75.0% of the sample dissected each day. The infections were of several hundred to several thousand promastigotes/fly (Table 3-3). Promastigotes were first observed only in the anterior midgut, (Fig. 3-3) near the stomodael valve in Day 2 and Day 3 flies. By Day 4, parasites were observed in the posterior pharynx, as well. On Day 5, promastigotes were observed in the mouthparts in 4 of the 15 infected flies. Mouthpart infections were observed 12 of 15 and 13 of 15 infected Day 6 and Day 7 flies, respectively (Fig. 3-4). The shape of the promastigotes was highly variable in young infections (Day 2 and 3); however, only elongate forms were observed anterior of the stomodael valve

Table 3-3. The course of development of Leishmania-Isabel strain in the sand fly, Lutzomyia anthophora, based on daily dissections, Days 1 to 7 post-feeding.

Day post-feeding	Status of blood meal	Ovarian status	# infected flies/sample	Location, number & shape of parasites
1	RBC's intact, meal dark red	undeveloped	*/30	anterior MG^{+} w/ blood meal, only 1 promastigote observed in dissections, stumpy form, amastigotes observed in macrophages in 5 dissections
2	RBC's intact meal black	undeveloped	15/28	anterior MG w/ blood meal, 100's observed in all infected flies, primarily stumpy forms
3	Blood remnants present	slightly developed	15/27	anterior MG, forward to stomodaeal valve, 100's observed, stumpy and some elongate forms
4	Meal totally excreted in most flies	well developed	15/25	±1000 in anterior MG, 50-100 in pharynx, elongate forms
5	Meal excreted in all flies	eggs fully developed	15/22	1000's in anterior MG, 50-100 in pharynx, 10-20 in cibarial region, 5-10 in mouthparts, elongate forms

Table 3-3. Continued.

Day post-feeding	Status of blood meal	Ovarian status	# infected flies/sample	Location, number & shape of parasites
6	as Day 5	commenced oviposition	15/21	as Day 5
7	as Day 5	finished oviposition	15/20	1000's in anterior MG, 100's in pharynx, 20-50 in mouthparts, elongate forms

* parasites probably still in amastigote stage, infection not determinable

\+ MG = midgut

Figure 3-3. Promastigotes of Leishmania-Isabel strain from the anterior midgut of the sand fly, Lu. anthophora (1000X).

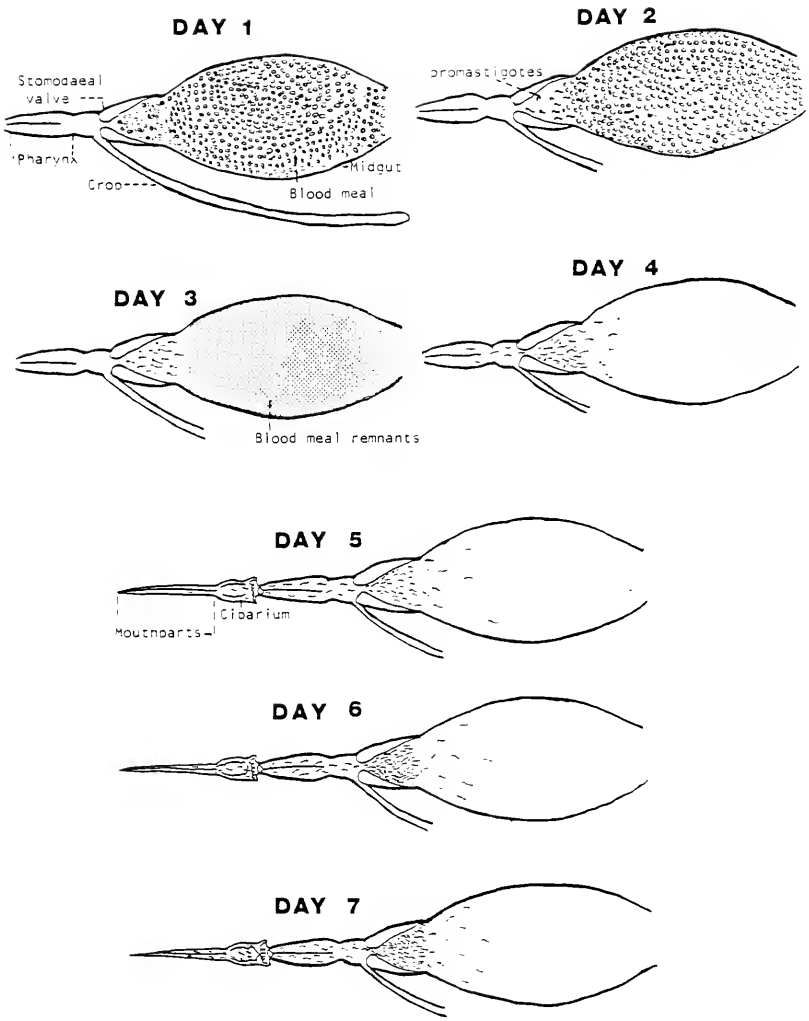


Figure 3-4. The course of Leishmania-Isabel strain infection in the sand fly, Lutzomyia anthophora.

in Day 4-7 flies. Parasite development in 17 Lu. christophei, dissected between Days 3-6 post-feeding, was parallel to that observed in Lu. anthophora. These data are summarized in Table 3-3. Promastigotes from flies, both four and five days post-feeding, were infective to hamsters, as determined by xenodiagnosis using Lu. anthophora, three weeks after inoculation and/or by spleen and skin tissue culture five weeks after inoculation. Day 3 promastigotes were not infective, as determined by the same methods. The estimated inoculation dose for the two hamsters in each of the three groups was 2.0×10^4 promastigotes/hind food pad.

Transmission of Leishmania-Isabel Strain by Lutzomyia christophei

A total of 8 out of 52 (15.4%) F_3 Lu. christophei females survived seven to twelve days post-feeding to refeed on noninfected BALB/c mice. All of the flies subsequently died within 24hrs of their second bloodmeal. One fly each fed on each hind foot and tail on two mice (#1 and 3); one fly fed on a hind foot, another on the tail of a third mouse (#2). All eight flies had leishmanial infections when examined. All three mice were accidently killed, by overdose of anesthetic, approximately four weeks after being fed upon. Only one mouse had any externally obvious signs of leishmaniasis. Mouse #2 had a slight, but noticeable, swelling of the sand fly-bitten hind foot. Xenodiagnosis with Lu. anthophora and culturing of a subcutaneous aspirate

revealed that the swelling was due to leishmanial infection. Spleen cultures from all three mice were positive by seven days, cultures of liver tissue were negative for all the mice. A subcutaneous aspirate from the tail of mouse #3 was positive; however, aspirate cultures from the hind feet of mice #1 and #3 were negative, as was the aspirate from the tail of #1. A few amastigote-infected macrophages were observed in a spleen impression smear from mouse #2.

Discussion

Due to the recent discovery of leishmaniasis in the Dominican Republic, relatively little comparative work has been done to characterize the parasite. The Leishmania still does not have a specific name; therefore, the most commonly used strain is referred to as Leishmania-Isabel (WR-336), after the patient from whom the primary isolate was made in the Dominican Republic. Strain determination must be made on the basis of a variety of biological and biochemical characteristics, as compared to those characteristics of other known strains. The differences between L. mexicana and L. braziliensis complexes are often matters of degrees, rather than absolute contrasts. In laboratory cultures, the Dominican parasite did not grow as rapidly as the two L. mexicana ssp., although it did achieve a maximum concentration (promastigotes/ml) very near that of the L. mexicana strains. Hendricks et al. (1978) and Childs et al. (1978) reported similar results with various L.

mexicana strains, though the maximum yields for L. braziliensis strains were much lower. After discovering the slower growth rate of the Dominican parasite, cultures of tissues from potential reservoir hosts (Chapter 4) were checked on Day 7, rather than Day 3 or 4, as would have been the case with a L. mexicana strain (Hendricks and Wright, 1979). Other biochemical characters of the Dominican Leishmania are more typical of L. mexicana strains (Schnur et al., 1983). The almost inapparent lesions produced in hamsters were not typical of the growth exhibited by most strains of Leishmania in this species of rodent (Zeledon et al., 1982), nor were hamsters known to self-cure leishmanial infections. The low pathogenicity of DCL-producing parasites in laboratory rodents has also been noted for L. aethiopica, the causative agent of DCL in Ethiopia. Inapparent lesions are also characteristic in wild reservoir hosts for cutaneous leishmaniasis in the Americas (Herrer and Christensen, 1975).

Perhaps the most outstanding result from the animal inoculations was determining the relative sensitivities of the methods for detecting infection. The purpose of this was to use the infection of laboratory rodents as a model for determining which techniques might be best applicable to the survey for the reservoir host in the Dominican Republic (see Chapter 4). The two most successful methods for detecting infection were the culturing of liver tissue and

of spleen tissue. These two methods require the use of sterile facilities and were not used in the reservoir study until June 1983. They should be used in any further studies. Liver and spleen impression smears also often confirmed infection, but may be adequate for laboratory studies only, as these may result in a much greater number of amastigote parasitized macrophages than would be found in a wild host (Hoogstraal and Heyneman, 1969). The cutaneous mode of inoculation, either subcutaneous or intradermal, would be the normal route when the promastigotes are transmitted by the sand fly. In laboratory animals, this also proved to be the most successful in establishing the infection. Intracardial and intraperitoneal inoculation were successful in establishing leishmanial infections in some animals. It is unknown if these resulted in inapparent cutaneous infections. Other strains of Leishmania causing DCL have not been found to infect laboratory rodents via IP or IC inoculation (Bray et al., 1973; Convit and Kerdel-Vegas, 1965). Both hamsters and mice were susceptible to the Isabel strain, which is not true of L. aethiopica, to which mice are refractory (Bray et al., 1973). These results might have been due to the strain of mouse used rather than the parasite itself. The BALB/c mice obtained for infecting the sand flies developed very large lesions in less than four weeks after inoculation (2.5×10^5 promastigotes/foot pad) which worsened to virtual amputation

by six to eight weeks post-inoculation (Berman, pers.comm.). The IRC strain mice used by the author did not develop obvious infections, despite an inoculum half as large as that used for the BALB/c mice. Hamsters fit in between the two mice strains, in terms of acceptable laboratory hosts for the Dominican Leishmania. The loss of infection, by seven months post-inoculation, was a startling discovery as hamsters are not known to be able to self-cure from leishmanial infections. This further suggests the low pathogenicity of Leishmania-Isabel strain.

Growth of the parasite in sand flies can also be used to assist in species determination of the Leishmania. In the classification of the New World Leishmania, those that develop in the anterior midgut are considered to belong in the L. mexicana complex or L. donovani (Section Suprapylaria). Members of the L. braziliensis complex develop in the posterior midgut and subsequently move forward (Section Peripylaria) (Killick-Kendrick, 1979). On the basis of site of development, the Dominican Leishmania would be classified as L. mexicana ssp., although it differs from other members of the group in other respects. Strains of L. mexicana develop rapidly in the sand fly Lu. anthophora, producing infections of promastigotes which often number in the tends of thousands (Young, pers. comm.). The Isabel strain developed fewer parasites in both Lu. anthophora and Lu. christophei; however, the sand fly promastigotes appeared to be highly infective, as the inoculation and transmission

experiments indicated. Sacks and Perkins (1984) recently reported the phenomenon of increased promastigote infectivity with time. In the author's study, promastigotes were infective as early as four days after ingestion by the sand fly. Both Lu. anthophora and Lu. christophei lay their eggs as early as five days post-feeding (Endris, 1982; see Chapter 2). Thus, if the first bloodmeal was from an infected host, the sand fly could transmit infective promastigotes as soon as it refeeds, after oviposition on Day 5. While Old World leishmaniases are very vector species specific, those of the New World usually exhibit normal development in a wide range of sand fly species (Killick-Kendrick and Ward, 1981). Thus, it should be possible to extrapolate between development of Leishmania-Isabel in Lu. anthophora to that in Lu. christophei. The 17 infected Lu. christophei females that died three to six days post-feeding, exhibited parallel parasite development to that observed in detail in Lu. anthophora. The Dominican sand fly, Lu. christophei, proved to be a very good host for the Dominican Leishmania, being capable of maintaining an infection at least 15 days post-feeding. Further work should be done to determine the effect of the promastigote infection on this species of sand fly once the laboratory colony becomes more firmly established and adapted to captivity. Transmission of the parasite by the sand fly is further evidence that it may be the natural vector in the Dominican Republic.

Field studies must be continued so as to give further support to the laboratory studies with the sand fly. The laboratory models established in this work must now be extended to field studies, using the appropriate post-mortem Leishmania-isolation techniques on potential reservoir hosts, taking into account that longer incubation periods may be necessary for cultures. The question of the identity of the Dominican Leishmania also remains to be answered, whether it is a new species or a new subspecies of L. mexicana.

CHAPTER 4

SURVEY FOR RESERVOIR HOSTS OF HUMAN LEISHMANIASIS IN THE DOMINICAN REPUBLIC

Introduction

American cutaneous leishmaniasis is a zoonotic disease caused by the members of the Leishmania braziliensis and L. mexicana complexes. A variety of wild mammals have been recorded as hosts for these parasites, primarily rodents for the L. mexicana complex; however, members of the L. braziliensis complex are known from a more diverse grouping which includes rodents, edentates, procyonids, marsupials, and canids (Lainson and Shaw, 1979). The potential host fauna for leishmaniasis in the Dominican Republic is very limited. Only two native terrestrial mammal species are known: a capromyid rodent, Plagiodontia aedium Cuvier, and a large insectivore, Solenodon paradoxus Brandt. Both species are rare and inhabit only relatively undisturbed areas (Woods, 1981). Introduced species have replaced the native fauna and include the murid rodents Rattus rattus alexandrinus (Geoffroy), R. norvegicus (Berkenhout), and Mus musculus brevirostris Waterhouse; the mongoose, Herpestes auropunctatus Hodgson; and feral cats, Felis catus L. (Woods, pers. comm.; Garcia M., pers. comm.). Many people keep dogs which

are somewhat free-ranging. The reservoir host of human diffuse cutaneous leishmaniasis in the Dominican Republic is unknown, but as the parasite is more similar to members of the L. mexicana complex (Schnur et al., 1983), the murid rodents are the most likely suspects.

The American leishmaniases very rarely produce obvious lesions in most of their wild hosts (Herrer and Christensen, 1975). However, the parasite may be recovered from culture of skin, liver, or spleen biopsies (Herrer et al., 1966) or from subcutaneous aspirates of infected animals. Serodiagnosis, which is routinely performed for detecting visceral leishmaniasis, and occasionally cutaneous leishmaniasis, in man (Walton et al., 1972), is generally not used for the detection of wild hosts of cutaneous leishmaniasis. No other trypanosomatid parasites of mammals, including man, are known to occur in the Dominican Republic (Walton, pers. comm.). The prevalence of subclinical human leishmaniasis is not known, although this could be an important factor in maintenance of the disease and its distribution.

Materials and Methods

Tomahawk live traps and Sherman collapsible traps (Fig. 4-1) for mammals were set at six leishmaniasis case sites (Table 4-1). Traps were baited with various foods such as: peanut butter-corn flour mixture, avocado pieces, fried plantain, banana, coconut, or sausage for rodents and

Figure 4-1. Sherman (on left) and Tomahawk live traps for small mammals.

Table 4-1. Mammal specimens collected at six leishmaniasis case sites in the Dominican Republic.

Site	# Trap-Nights	*Rattus rattus*	*R. norvegicus*	*Mus musculus*	*Herpestes*	Total
Carrasco	30	0	0	0	0	0
La Culatica	4	3	0	0	0	3
Loma Pena Alta	757	44	1	6	3	54
Monte Claro	15	0	0	2	0	2
Morro de Miches	730	10	0	6	0	16
Trepada de Jabilla	5689	63 (38+25)*	5 (5+0)*	27 (22+5)*	0	95
Total	7266	120	6	41	3	170

* # collected 1981-82 and # collected 1983.

raw egg in shell or chicken leg for mongoose. When possible, local personnel checked the traps daily and were paid on a per animal basis. If known in advance that the case site would not be visited according to schedule, then trapping was only done on the three nights preceding the next visit. Captured animals were removed from the field to nearby residences and later to the field station; there they were given water and food. They were transported to the Dominican Dermatology Institute (Instituto Dermatologico) laboratory in Santo Domingo for examination, usually once per week during April to July 1982 and June to August 1983. Animals that died at the station, and all animals trapped prior to April 1982, were examined at the station.

Various methods were used to check for potentially infected animals. These included visual examination for suspicious lesions; culturing of subcutaneous aspirates, liver, splien, and skin tissue (Herrer et al., 1966) in NNN medium (Mansour et al., 1983) or Schneider's Drosophila medium (Grand Island Biological Co. (GIBCO), Grand Island, NY) (Hendricks and Wright, 1979); indirect fluorescent antibody test (IFAT) with fluorescein (FITC)-labelled conjugate (SIGMA Chemical Co., St. Lousi, MO); impression slides of skin, liver, and spleen tissue; and histological section slides of liver and spleen tissue. Animals alive at time of examination were humanely killed with ether or by suffocation through compression of the thorax. Blood samples for IFAT were taken through cardiac puncture

technique prior to killing. Aspirates were taken by injecting 0.03 to 0.10ml of normal saline of Medium 1640 (GIBCO) subcutaneously in the nose, foot, or tail base with a tuberculin syringe and 26 gauge needle. The liquid was then withdrawn, along with any blood that appeared, and injected into a culture tube. Prior to injection, the skin surface was cleaned with 100% ethanol or 95% isopropanol and allowed to dry. The culture tubes were maintained at ambient temperature and checked for growth at seven to ten days. Contaminated tubes were checked on the first day that contamination was noted. Impression and section slides were stained with Giemsa and hematoxylin, respectively, for 20min. The slides were later examined with the aid of a compound microscope (400X, 1000X) for the presence of amastigote-infected macrophages. Other domestic or feral mammals were visually examined live, when available.

Results

A total of 167 specimens of three species of rodents (Fig. 4-2) and three Herpestes (mongoose) were collected. Mammals were collected during two time periods: October 1981 to July 1982 (5721 trap-nights producing 83 rodents (0.015 animals/trap-night)) and May to August 1983 (1545 trap-nights producing 87 animals). (During the latter period animals/trap-night was not applicable as an unknown number were collected by hand.) Seventy-two (42.6%) rodents were trapped at Morro de Miches or Trepada de Jabilla from

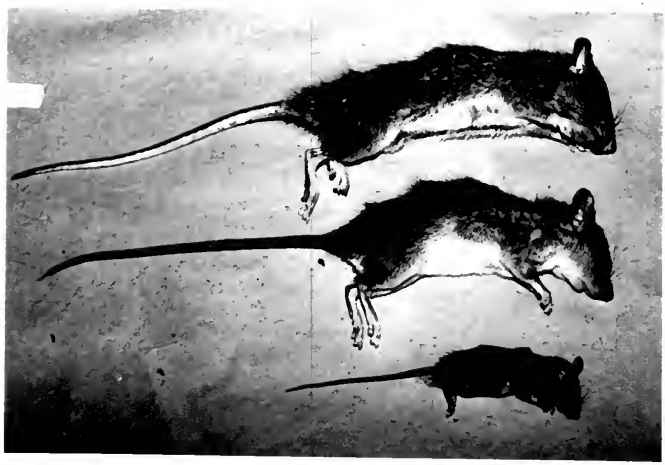


Figure 4-2. The three species of rodents trapped during the survey (from top- *Rattus norvegicus*, *R. rattus*, and *Mus musculus*).

April to July 1982. Thirty (17.8%) rodents came from Trepada during May and June 1983. Fifty-four (32.0%) were collected at Loma Pena Alta in July 1983 (Table 4-1).

The six Rattus norvegicus (four females and two males, range of body length 175 to 225mm, $\bar{x}$ = 214.2mm) were examined by various methods (Table 4-2), but were not found to be infected with Leishmania. Rattus rattus was the most commonly collected mammal species. However, no Leishmania were isolated from the 120 specimens examined (61 females, 56 males, and 3 not identified due to degree of decomposition, body length range 105 to 22mm, $\bar{x}$ = 180.0mm). Aspirates or tissue samples of some specimens were combined in culture tubes according to sex and tissue type. Tubes for the 21 specimens examined at the field station were contaminated by bacteria and/or mold. The IFAT showed that 4 (9.1%) of the 44 R. rattus from Loma Pena Alta examined were seropositive for antibodies against the Dominican Leishmania. One rat had severe edema of the feet, but only bacteria were recovered from the affected limbs. Another had a large (20mm diameter) spot on the hind dorsal region which was almost hairless, resembling a leishmanial lesion, but no parasites were revealed by skin impression or cultured subcutaneous aspirate.

Forty-one Mus musculus (house mouse) were trapped. There were 25 females and 16 males; body length ranged 71-93mm ($\bar{x}$ = 81.0mm). As above, some aspirates or tissue

Table 4-2. Examination techniques used for mammals collected during survey for reservoir hosts of leishmaniasis in the Dominican Republic, October 1981 to August 1983.

Species	# Collected	# Visually Examined Only	# Examined by Post-Mortem Technique				
			Aspirate Culture*	Tissue Culture	IFAT	Impression Smears	Histo-logical Sections
Rattus rattus	120	18	41	78	44	39	90
R. norvegicus	6	0	5	5	0	5	6
Mus musculus	41	4	22	33	0	28	21
Herpestes auropunctatus	3	0	3	3	0	0	3

* Tissue-skin (ear, foot, and/or tail), spleen, and liver.

samples were combined according to sex and tissue type. No mice were found to be infected.

The three Herpestes collected were females; body length ranged 270-320mm ($\bar{x}$ = 300mm). None was infected. The examination data for the above four species are summarized in Table 4-2.

In addition, one aspirate was taken from a wild-caught Plagiodontia aedium female being held at the University of Florida. This animal was assumed to be approximately six years old when captured in 1974 from a locality in the Cordillera Oriental. It showed no suspicious lesions and the culture was also negative. One feral cat was trapped at Morro de Miches, but no lesions were observed. Five dogs, all less than one year old, were visually examined at Trepada; none showed any suspicious lesions.

Contamination with bacteria and/or mold was a frequent problem in culture tubes during the first survey period, October 1981 to July 1982. During the second period, contamination affected only the dermal samples.

Discussion

Leishmanial infections were not detected in any of the 170 mammals trapped; however, it is possible that some infections were not revealed by the methods used. Impression smears may be positive only at very high infection levels, as observed in laboratory infections (Hoogstraal and Heyneman, 1969). The aspirate and tissue

samples cultured at the field station, invariably developed bacterial and/or fungal contamination due to a lack of sterile facilities. The tissue (spleen and liver) samples cultured at the Institute were not contaminated, but demonstrated no Leishmania as well. Due to the lack of standardized treatment and contamination of samples, it is not known if some infection were missed, however few in numbers.

The animals from Loma Pena Alta were collected in the same time period as the Lu. christophei from that site. Unfortunately, it is not known exactly how many of the animals were from the forests and coffee groves. At this site, leaf nests, 2 to 3m high, were common in the shrubs in the pasture lands surrounding the coffee groves. Many of the 18 subadult R. rattus from the site were probably collected by hand from these nests. Contact with sand flies was probably limited for these young rats. The serological analysis indicated that some of the R. rattus from Pena Alta had been exposed to the leishmanial parasite; however, the status of the infection was not known, as no parasite were recovered. Research by Zovein et al. (1984) suggested a high correlation between seropositive results and infection for Old World rodent leishmaniasis. It is also possible that a seropositive individual in the Dominican study might not have had a current infection (Zuckerman and Lainson, 1977).

The native mammalian fauna of the Dominican Republic is very depauperate today consisting of 13 species of bats, 1 species of rodent, and 1 species of insectivore (Woods, 1981). The latter two exist in low numbers and definitely were not present at several of the DCL case sites, as they require relatively undisturbed habitat (Woods, 1981). The hutia, Plagiodontia aedium, makes its dens in partially decayed large trees or rock crevices. It is more prevalent in areas where Rattus is less prevalent (Sullivan, 1983). Most of the case sites visited had neither rock outcroppings or large trees with crevices, the exception being Loma Pena Alta. Introduced species have replaced the native mammals and the four most common are Rattus rattus R. norvegicus, Mus musculus and Herpestes auropunctatus, all of which occur throughout the island (Woods, pers. comm.). As rodents are the known reservoir hosts for members of the L. mexicana complex (Lainson and Shaw, 1979), it is possible that one or more of the introduced rodents is acting in that capacity in the Dominican Republic. Due to the presumed intimate relationship between R. rattus and the probably vector, Lu. christophei (see Chapter 2), this species of rodent is the most likely reservoir suspect, with M. musculus the second most likely due to its abundance and habitat. Rattus norvegicus has different habits from R. rattus and may be much less common in wooded areas.

Of the nine species of endemic rodents (Varona, 1974) existent on Hispaniola at the arrival of the Spaniards, all

were tree cavity or ground hole dwellers (Woods, pers. comm.). As the Rattus and Mus invaded these habitats, they might have come to represent an increasingly important role in the epidemiology of leishmaniasis in the Dominican Republic. Other species of mammals, including dog, cat and mongoose, are present at many of the case sites and may have some role in the epidemiology of the disease. Several dogs were visually examined at Trepada de Jabilla, but none showed any suspicious lesions; however these dogs were all young, less than one year old, and may not have been around during a period of abundance of sand flies. There were no dogs present at several of the case sites. Cats live primarily in the coffee groves and forested areas where they were present only as feral animals. They might serve as an additional blood source to a potential vector, but felids are not know to support infections by Leishmania spp. in the Americas. The mongoose live in more open areas and the possibility of sand fly contact is much less, viverids are also not known to support Leishmania infections in the New World. The three mongoose obtained in the study were very carefully examined, due to difficulty of capture, but were uninfected. These three larger species are probably not present in the high populations that would be necessary to maintain the disease.

Another possibility is that man could be serving as the reservoir, as is the case with L. tropica and L. donovani in India (Bray, 1974). A serological survey performed by

that the percent seropositive at seven leishmaniasis case sites ranged from 26.0 to 48.0% of the individuals tested. The observation that children as young as one year old were demonstrated as seropositives (de Quinones, unpublished data) may indicate that transmission was actively occurring during the author's study period. It has been suggested that the seropositive individuals may have subclinical leishmaniasis (Zeledon, pers. comm.), or perhaps uncomplicated skin lesions, as is suspected with L. mexicana pifanoi infections in Venezuela (Lainson, 1983); but man is probably not the reservoir in the Dominican Republic (Zeledon, pers. comm.).

The high prevalence of seropositives at the various case sites suggests that the leishmanial parasite should be abundant in the reservoir host as well. Of all the suspect species, only the murid rodents are present at high population levels, as would be necessary to maintain the parasite in the absence of man. It is curious as to how the Dominican Leishmania came to occur in the Dominican Republic (and possibly Haiti), whether endemic for millenia, imported with the Indians of the Caribbean basin, or imported with the Europeans and Africans. Determining the reservoir host fauna might shed some light on this perplexity. It is important to determine the relationship between seropositive (human or rat) and the state of infection. Xenodiagnosis, using laboratory-reared Lu. christophei would be an excellent way to determine the prevalence of subclinical

infections in man and other mammals. Only very intensive trapping of all possible wild reservoir hosts will lead to a more definitive answer as to whether leishmaniasis is indeed a zoonotic disease in the Dominican Republic.

CHAPTER 5

SUMMARY

During the field and laboratory investigations, the following were achieved or determined:

1. A survey of phlebotomine sand flies was conducted in the Dominican Republic between May 1981 and August 1983. Two species, Lutzomyia christophei and Lu. cayennensis hispaniolae were recovered.

2. Laboratory colonies of Lutzomyia cayennensis hispaniolae and Lu. christophei were established. Of 319 wild caught Lu. cayennensis and 23 Lu. christophei dissected, none was found infected with Leishmania or other vertebrate parasites.

3. Leishmania-Isabel strain developed in the anterior midgut of both Lu. christophei and Lu. anthophora.

4. Leishmanial promastigotes from experimentally-infected sand flies were infective to hamsters four days post-blood meal. Lutzomyia christophei females were

capable of supporting a hamster-infective leishmanial infection up to at least 15 days post-feeding.

5. Laboratory-reared Lu. christophei were capable of transmitting Leishmania-Isabel strain from infected BALB/c mice to uninfected mice.

6. In culture medium, Leishmania-Isabel strain grew at slower rate than did two strains of L. mexicana.

7. Of 170 wild mammals examined for leishmaniasis, none was positive; however 4 of 47 Rattus rattus from Loma Pena Alta were positive for anti-Leishmania antibodies.

APPENDICES

APPENDIX 1.

DOMINICAN LEISHMANIASIS PATIENT PARTIAL CASE HISTORIES.

	Site	Age at Diagnosis	Time of Evolution	Presumed Age at Infection	Site of Lesions
1.	Nisibon	9y	6y	3y	arms, face
2.	Nisibon	4y	3y	1y	face, arm, leg
3.	Nisibon	8y	3y	5y	extremities
4.	El Valle	40y	1y	39y	ear
5.	San Cristobal	4y	3y	1y	cheek, arm
6.	Higuey	8y	4mo	7.5y	arm
7.	Las Cuchillas	12y	4y	8y	arms, cheek
8.	Nisibon	5y	2y	3y	cheek, arm
9.	Bani	13y	2y	11y	thigh
10.	El Valle	29y	?	young	ears
11.	El Seibo	57y	6mo	56.5y	leg, arms
12.	Miches	32y	8y	24y	forearm
13.	Santiago	6y	--	--	--
14.	Higuey	11mo	3mo	8mo	face, arm
15.	Higuey	32y	8mo	31y	knee, hand
16.	Navarrete	42y	7y	35y	thigh
17.	El Cuey	60y	--	--	arm, leg

	Site	Age at Diagnosis	Time of Evolution	Presumed Age at Infection	Site of Lesions
18.	El Seibo	43y	15y	28y	arm, leg
19.	Miches	5y	9mo	4y	arm, face, thigh
20.	Miches	4y	--	--	arm
21.	Yerba Buena	1.5y	1y	3mo	cheek
22.	Santa Lucia	4y	3.5y	6mo	face, forearm
23.	Carrasco	20y	4.5y	5.5y	arm, leg
24.	Monte Claro	3y	2.5y	3mo	cheek, legs
25.	Las Cuchillas	40y	20y	20y	arms, shoulders, knees

Source: Bogaert-Diaz, unpublished data.

Note: y = year, mo = months.

APPENDIX 2.

COLLECTION SITES AND DATES FOR LUTZOMYIA CAYENNESIS HISPANIOLAE IN THE DOMINICAN REPUBLIC, MAY 1981 - AUGUST 1983.

Altagracia Province. Bayahibe: 29 Aug 81, 6km W, 2 females, 8km W, 1 male, 14km W, 2 males. Boca de Yuma: 15 Jun 82, 1km W, 2 females, 3km N, female, 2 males. Higuey: 30 Aug 81, Rio Yuma 2km S, 1 female, 1 male, Rio Sanate 8km S, 1 male, 13 km W, 1 female, 23km W 1 male, 28km W, 1 male; 5 Oct 81, 10km N, 3 females, 3 males; 26 May 82, 15km N, 2 females, 3 males. Nisibon: 5 Oct 81, 1km S, 1 female, 3 males.

Barahona Province. Cienaga: 7 Jul 82, 1km S, 11 flies. Polo: 7 Jul 82, 8km E, 6 flies; 4km N, 8 flies.

Duarte Province. Castillo: 10 Jul 82, 5km E, 3 flies.

El Seibo Province. El Cuay: 5km S (Altos de Peguero), Mar 82, 5 visits, May 82, 4 visits, Jun 82, 3 visits, Jul 82, 3 visits. El Seibo: 28 Aug 81, 10km W, 1 male, 14km W, 1 male, 18 km W, 1 female, 3.5km N, 1 female, 5.2km N, 1 male; 16 Oct 81, 8km N; 15 Dec 81, 11km W. Hato Mayor: 23 Sep 81, 11km N, 5 flies; 28 Sep 81, 11km N, 6 females, 15 males, 16km N, 3 females, 5 males; 20 Oct 81, 8km N, 1 female, 4 males, 11km N, 5 females, 9 males; 21 Nov 81, 11km. N. Las Cuchillas: 6 Nov 81, 5km S, 10km S, 16km S; 2.8km N (Trepada de Jabilla) Nov 81, 7 visits, Dec 81, 5 visits, Jan 82, 5 visits, Feb 82, 5 visits, Mar 82, 6 visits, Apr 82, 5 visits, May 82, 6 visits, Jun 82, 9 visits, Jul 82, 8 visits. Loma Pena Alta: 22 Jun 82, 24 July 82. Miches: 28 Aug 81, 9km S (Morro de Miches, 2 females, 2 males; 4 Nov 81, 3km W, 2 males. Pedro Sanchez: 19-21 May 81, Aug 81, 10 visits, Sep 81, 6 visits, Oct 81, 6 visits, Nov 81, 5 visits, Dec 81, 4 visits, Jan 82, 5 visits, Feb 82, 7 visits, Mar 82, 6 visits, Apr 82, 6 visits, May 82, 9 visits,

Jun 82, 10 visits, Jun 82, 9 visits, 25 Sep 81, 7km S, 1 male. Sabana de la Mar: 4 Nov 81, 6km S, 5km E. Santa Lucia: 17 Aug 81; 31 Aug 81, 47 flies, 12km N, 3 flies; 19 Sep 81, 7 females, 5 males; 22 Oct 81 20km N, 1 male; 4 Dec 81. Yerba Buena: 22 Jun 82, 1km S, 3km NW, 7km NW.

La Vega Province: Bonao: 10 Jul 82, 1km SW, 2 males.

La Romana Province. Guerrero: 28 Aug 81, 1km N, 1 female, 1 male. La Romana: 29 Aug 81, 10km E (Rio Chavon), 1 male. Rio Cumayasa: 28 Aug 81, 2 males.

Maria Trinidad Sanchez Province. Carrasco (Rio San Juan): 3 July 82, 1 female, 1 male.

Peravia Province. Iguana Arriba (20 km NE Bani): 8 July 82, 13 flies.

San Cristobal Province. Villa Altagracia: 19 June 82, 10 km NW, 5 flies.

San Pedro de Macoris Province. San Pedro de Macoris: 14 Sep 82, 16 km N, 4 females, 3 males.

Sanchez Ramirez Province. Monte Claro (Cotui): 19, 16 Jun 82. Pimentel: 27 Apr 82, 5 km S, 1 female, 1 male.

APPENDIX 3.

COLLECTION SITES AND DATES FOR LUTZOMYIA CHRISTOPHEI IN THE DOMINICAN REPUBLIC, MAY 1981 - AUGUST 1983.

Altagracia Province. La Culatica: 7 June 83, 1 female (CDC-UV) Nisibon: 7-8 June 83, 5 km SW, 2 females, 4 males.

El Seibo Privince. Altos de Peguero: 22-25 Mar 82, 1 female (flight trap). Loma Pena Alta: 31 May 83, 5 females, 2 males (CDC, CDC-UV); Jun 83, 4 visits; Jul 83, 10 visits (CDC-UV, flight trap, aspirator); 3 Aug 83, 1 female (flight trap). Morro de Miches: 19 May 81, 1 male. Trepada de Jabilla: 15-20 Nov 81, 1 female (flight trap); 20 Nov 81, 2 males; 14 Dec 81, 2 males; 1-5 Feb 82, 1 male (flight trap); 5-10 Feb 82, 1 male (flight trap).

Sanchez Ramirez Province. Monte Claro: 9 Jun 83, 1 male.

Note: Aspirator was used as the method of collection unless otherwise specified.

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Richard N. Johnson entered into the world on June 30, 1956, in Wilmington, Delaware. He attended Wilmington Friends School and graduated from there in 1974. He began his college career as an undergraduate at the University of Delaware in that same year. He received his B.S. with a major in entomology and applied ecology in 1977. In the fall of that year, he travelled to Gainesville, Florida, to enroll at the University of Florida and was subsequently awarded a research assistantship in medical-veterinary entomology. After receiving his M.S. degree in December 1979, Richard continued on at the university in a Ph.D. program the following January. In the process of performing research for the doctoral dissertation, he spent over 14 months in the Dominican Republic.

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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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