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Annals of Tropical Medicine and Parasitology, 1972. *Vol.* 66, *No.* 1

Enzootic cutaneous leishmaniasis in eastern Panama
II: entomological investigations

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Received 19 *August* 1970

Leishmanial promastigotes (leptomonads) have been found in only five wild-caught species of *Lutzomyia* in the New World, *L. panamensis*, *trapidoi*, *gomezi*, *ylephiletrix* and *flaviscutellata* (Christensen *et al.*, 1969). Although the finding of leishmanial promastigotes in sandflies under natural conditions is not proof that the species is a natural vector, it does satisfy an important prerequisite. Five Neotropical species, *L. flaviscutellata* (= *olmeca*), *cruciata*, *longipalpis*, *renei* and *pessoana* (Biagi *et al.*, 1965; Williams, 1966; Coelho and Falcao, 1962; Strangways-Dixon and Lainson, 1966) have been shown to transmit cutaneous leishmaniasis experimentally by biting. The parasite of cutaneous leishmaniasis has been shown to develop in 23 species of *Lutzomyia* (Pessoa and Coutinho, 1941; Hertig and McConnell, 1963; Strangways-Dixon and Lainson, 1966; Coelho *et al.*, 1967), and most probably will be shown to develop in other species when studied. Certainly not all of these species are essential to the existence of the disease in nature, and knowledge of the ecological relationships between infected mammals and the various sandfly species is essential to an understanding of the epidemiology of cutaneous leishmaniasis.

The present study was closely integrated with parasitological and mammalian ecological investigations (Herrer *et al.*, 1971; Telford *et al.*, 1971) of cutaneous leishmaniasis in Sasardi, San Blas Territory, Panama. Two lines of approach were used in the entomological work, field studies on the bionomics of sandfly populations and laboratory efforts to isolate the leishmanial agent from potential vectors.

Collections of sandflies at Camp Sasardi were made in September and December 1968, and in March and June 1969, providing information on sandfly activities in all seasons.

MATERIALS AND METHODS

CDC miniature light-trap collections provided most of our data on the species composition of the sandfly population, and some information on seasonal variations. Light-trap collections were made at 3 ft and 35 ft above the ground. Some traps were operated continuously for a 12-hour period from 18·00 to 06·00 hours, others were tended at midnight to separate evening and morning collections.

Differences in trapping effort during the periods sampled necessitated a method of standardizing all light-trap collections. Although the number of flies collected per hour provides the best indicator of density fluctuations, this measurement is not entirely satisfactory

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when it represents the total number of flies collected divided by the number of hours of collection, as it then cannot reflect hourly fluctuations.

Castor oil traps baited with small animals provided some evidence of host-preferences of sandflies in the collecting area. Our trap (Fig. 1) is a modification of that designed by Disney (1966). In addition, a Shannon trap was set up and tended at dawn and dusk.

Habitats sampled included tree buttresses and tree holes, forest-floor litter and low vegetation, and animal burrows. Collections from forest-floor litter were accomplished by draping a white sheet over the head of a collector and allowing one end to trail on the ground. The collector then disturbed the litter with a shuffling action of his feet, and collected the flushed-out flies as they alighted on the sheet. Both mouth-operated and battery-operated aspirators were used to collect flies from tree holes and tree buttresses. Flies were collected from animal burrows by blowing cigarette smoke deep into the burrow through a length of polyethylene tubing, and collecting the flies in a cloth cage placed over the opening of the burrow.

Five hundred and seventy-five sandflies comprising 13 species, collected by the various techniques, were dissected and examined for leishmanial promastigotes. The dissection technique described by Hertig and McConnell (1963) was used in our study. Some of the dissections were made at the Sasardi camp site and others at the Gorgas Memorial Laboratory in Panama City.

One thousand, three hundred and sixty-eight *L. olmeca*, collected in castor oil traps one to six days previously, were washed in a weak solution of detergent, triturated in saline, and inoculated into the nasal skin of 11 golden hamsters. The flies were divided into seven lots ranging from 104 to 284 flies per lot. All the hamster inoculations were performed in the Gorgas Memorial Laboratory.

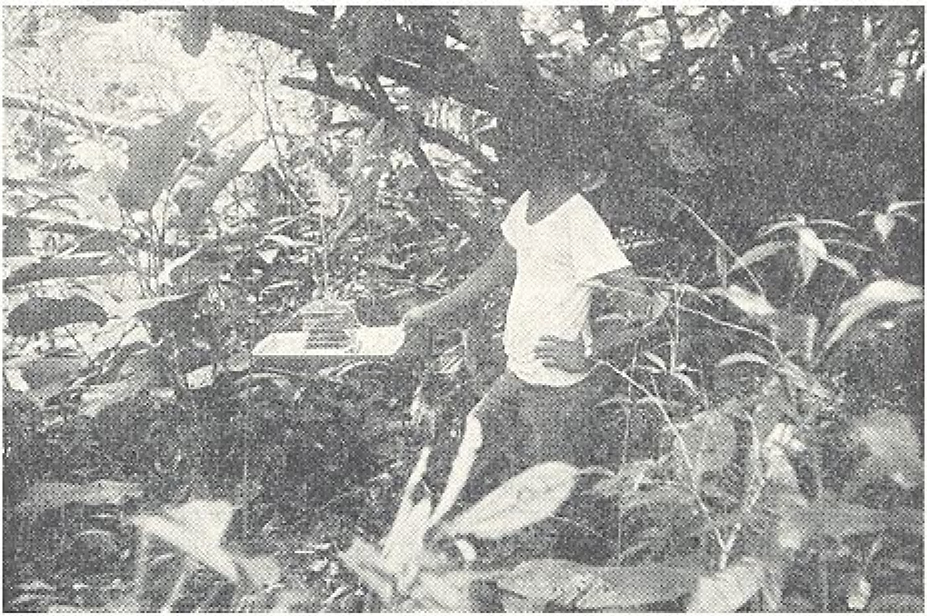

FIG. 1. Animal-baited castor oil trap used to collect the species of sandflies attracted to mammalian hosts of cutaneous leishmaniasis.

RESULTS

Bionomics

Out of a total of 8099 specimens belonging to 24 species of sandflies collected at Sasardi, 1444 specimens comprising 19 species were collected by light-traps (Tables I–IV). The average trap-night (one CDC light-trap operated for 12 hours) netted 20·9 sandflies. Table II shows the density fluctuations of the eight most abundant species in relation to the total light-trap catch. The results of light-trap collections made at 3 ft and 35 ft above the ground are summarized in Table III, which shows the vertical stratification of the commonest species. The collections at 3 ft represent 49 individual collections (588 trap-hours) which netted a total of 878 sandflies or 17·9 per collection. Collections at 35 ft represent 20 collections (240 trap-hours) which netted 553 sandflies or 27·7 per collection. A comparison was also made of the abundance of the most common sandfly species between 18·00 and 24·00 hours and between 24·00 and 06·00 hours (Table IV). The evening trapping represents 40 collections (240 trap-hours) which netted a total of 551 sandflies or 13·8 per collection, while the morning trapping (with the same trapping effort) netted 503 flies or 12·6

TABLE I

Sandfly species arranged alphabetically collected from traps and resting sites*

Lutzomyia species	Traps		Resting sites			Total all sources
	CDC light	Animal baited	Animal burrow	Tree buttress	Forest floor	
aclydifera	49	—	—	1	—	50
barrettoi	—	—	1	—	—	1
camposi	26	—	—	—	—	26
cayennensis ...	3	—	—	3	—	6
carpenteri ...	388	—	1146	89	—	1623
dasymera	—	—	—	10	—	10
dysponeta	521	1	65	27	—	614
gomezi	40	2	—	8	11	61
hansoni	—	—	—	3	—	3
isovespertilionis ...	4	—	—	42	—	46
marajoensis ...	1	—	—	—	—	1
micropyga	1	—	—	33	—	34
nordestina	1	—	—	—	—	1
odax	1	—	—	—	—	1
olmeca	158	1748	—	1	246	2153
ovallesi	1	—	—	—	—	1
panamensis ...	145	10	—	—	1	156
punctigeniculata ...	1	—	—	2	—	3
shannoni	6	—	—	312	—	318
trapidoi	11	—	—	8	—	19
trinidadensis ...	83	4	—	2811	—	2898
undulata	—	—	—	2	—	2
ylephiletrix ...	—	—	—	2	—	2
Brumptomyia sp. ...	4	—	5	—	—	9
Totals ...	1444	1765	1217	3354	258	8038

* Table does not include 61 specimens collected in a Shannon trap

TABLE II

The eight most common sandflies in light-trap collections

Lutzomyia species	September 1968 72 trap–hours				December 1968 228 trap–hours				March 1969 240 trap–hours				June 1969 288 trap–hours				Total 828 trap–hours
	Number ♂	Number ♀	Average/h	(%)	Number ♂	Number ♀	Average/h	(%)	Number ♂	Number ♀	Average/h	(%)	Number ♂	Number ♀	Average/h	(%)	Average/h
dysponeta	2	3	0·07	(6·9)	48	19	0·29	(23·9)	125	98	0·93	(37·7)	123	103	0·74	(45·1)	0·63
carpenteri	10	16	0·36	(36·1)	89	56	0·64	(51·8)	86	82	0·70	(28·4)	33	16	0·17	(9·8)	0·47
olmeca	6	4	0·14	(13·9)	8	20	0·12	(10·0)	17	85	0·43	(17·3)	4	14	0·06	(3·6)	0·19
panamensis	1	24	0·35	(34·7)	—	4	0·02	(1·4)	—	4	0·02	(0·7)	1	111	0·39	(22·3)	0·18
trinidadensis	—	—	—	—	—	5	0·02	(1·8)	12	27	0·16	(6·6)	16	23	0·14	(7·8)	0·10
aclydifera	—	3	0·04	(4·2)	13	10	0·10	(8·2)	7	8	0·06	(2·5)	4	4	0·03	(1·6)	0·06
gomezi	—	1	0·01	(1·4)	1	4	0·02	(1·8)	1	8	0·04	(1·5)	4	21	0·09	(5·0)	0·05
camposi	—	—	—	—	—	—	—	—	5	13	0·08	(3·0)	4	4	0·03	(1·6)	0·03
all others	—	2	0·03	(2·8)	2	1	0·01	(1·1)	6	7	0·05	(2·3)	3	13	0·06	(3·2)	0·04
Totals	19	54	1·00	(100·0)	161	119	1·22	(100·0)	259	332	2·47	(100·0)	192	309	1·76	(100·0)	1·75

flies per collection. The histograms (Fig. 2) compare the most common sandflies collected at light during the evening and morning hours at 3 ft and 35 ft. The activity of males and females is measured as the number of flies collected per hour.

Table V shows the five most common species found in tree buttresses, and their density fluctuations during the months sampled. Sixteen species were represented in the total of 3354 sandflies collected from tree buttress habitats. The 11 species included in Table V as "all others" comprised only 2% of the total (Table I). Forest-floor litter and animal burrows appear to be much more restrictive as habitats, as shown in Tables VI and VII.

L. dysponeta and *carpenteri* were the commonest species taken based on the combined data from all collections. *L. dysponeta* is apparently the most abundant species, and its particularly high density during the months of March and June (Table II) indicates that it

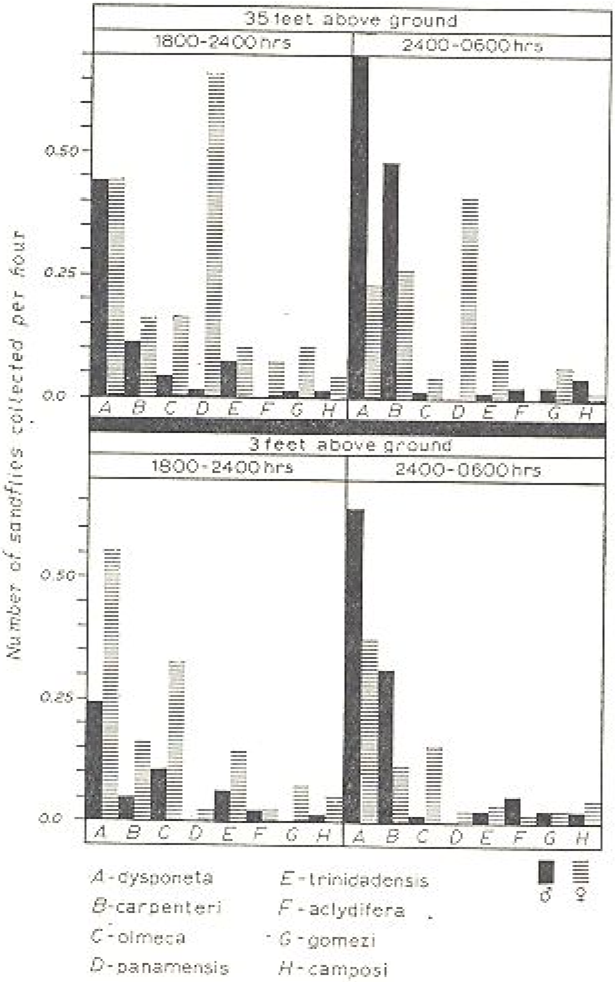

FIG. 2. Histogram comparing the abundance of the eight commonest sandflies collected at the light traps during the evening and morning hours, at 3 ft and 35 ft above ground level.

TABLE III

The eight most common sandflies in light traps at different heights above ground

Lutzomyia species	3′ above ground 49 collections (588 h)				35′ above ground 20 collections (240 h)			
	Number ♂	Number ♀	Average/h	(%)	Number ♂	Number ♀	Average/h	(%)
dysponeta ...	173	150	0·55	(36·8)	125	73	0·83	(35·8)
carpenteri ...	151	119	0·46	(30·7)	67	51	0·49	(21·3)
olmeca	29	90	0·20	(13·6)	6	21	0·11	(4·9)
panamensis ...	1	18	0·03	(2·2)	1	125	0·52	(22·8)
trinidadensis ...	19	35	0·09	(6·1)	9	20	0·12	(5·3)
aclydifera ...	22	18	0·07	(4·6)	2	7	0·04	(1·6)
gomezi ...	3	17	0·04	(2·3)	3	17	0·08	(3·6)
camposi ...	4	12	0·03	(1·8)	5	5	0·04	(1·8)
all others ...	7	10	0·03	(1·9)	3	13	0·07	(2·9)
Totals ...	409	469	1·50	(100·0)	221	332	2·30	(100·0)

is most active during the late dry season. Table III shows it to be the most abundant species collected at 3 ft as well as 35 ft above the ground. Table IV shows that the flight activity of this species is somewhat greater during the hours from midnight to 06·00 hours in terms of total numbers; however, the females are most active before midnight and the males after midnight. The peak activity period for females (Fig. 2) occurs before midnight at 3 ft above the ground, and for males during the morning hours, with little difference in the height above the ground. Natural resting sites of this species include tree holes, tree buttresses and animals burrows (Table I). In none of these resting sites, however, has *L. dysponeta* been found to be more abundant than *carpenteri*, the second most common species taken in light-traps.

L. carpenteri, unlike *L. dysponeta*, was found to be far more abundant in light-traps during the months of December and March (Table II), indicating that early dry season

TABLE IV

The eight most common sandflies in light traps before and after midnight

Lutzomyia species	40 collections (240 h) from 1800 to 2400 h				40 collections (240 h) from 2400 to 0600 h			
	Number ♂	Number ♀	Average/h	(%)	Number ♂	Number ♀	Average/h	(%)
dysponeta ...	80	120	0·83	(36·3)	160	74	0·98	(46·5)
carpenteri ...	18	38	0·23	(10·2)	93	42	0·56	(26·8)
olmeca	17	59	0·32	(13·8)	2	24	0·11	(5·2)
panamensis ...	1	68	0·29	(12·5)	0	47	0·20	(9·3)
trinidadensis ...	27	67	0·39	(17·1)	4	13	0·07	(3·4)
aclydifera ...	2	9	0·05	(2·0)	9	1	0·04	(2·0)
gomezi ...	1	20	0·09	(3·8)	4	9	0·05	(2·6)
camposi ...	2	11	0·05	(2·3)	7	6	0·05	(2·6)
all others ...	5	6	0·05	(2·0)	3	5	0·03	(1·6)
Totals ...	153	398	2·30	(100·0)	282	221	2·09	(100·0)

TABLE V

Five most common sandflies in tree buttress collections

Lutzomyia species	September 1968			December 1968			March 1969			June 1969			Total		
	♂	♀	(%)	♂	♀	(%)	♂	♀	(%)	♂	♀	(%)	♂	♀	(%)
trinidadensis	88	34	(56·5)	159	92	(49·7)	45	19	(44·4)	1417	957	(95·4)	1709	1102	(83·8)
shannoni	12	5	(7·9)	132	32	(32·5)	55	7	(43·0)	51	18	(2·8)	250	62	(9·3)
carpenteri	35	1	(16·7)	53	2	(10·9)	—	—	(—)	—	—	(—)	88	1	(2·6)
isovespertilionis ...	9	15	(11·1)	11	—	(2·2)	6	—	(4·2)	1	—	(0·0)	27	15	(1·3)
micropyga	3	4	(3·2)	2	3	(1·0)	3	3	(4·2)	9	6	(0·6)	17	16	(1·0)
all others	9	1	(4·6)	16	3	(3·7)	2	4	(4·2)	21	10	(1·2)	48	19	(2·0)
Totals	156	60	(100·0)	373	132	(100·0)	111	33	(100·0)	1499	991	(100·0)	2139	1215	(100·0)

TABLE VI

Sandfly collections from animal burrows

Species	♂	♀	% of total
L. carpenteri	983	163	94·2
L. dysponeta	58	7	5·3
Brumptomyia sp. ...	1	4	0·4
L. barrettoi	—	1	0·1
Totals	1042	175	100·0

factors are more favorable to this species. Surprisingly, this species was not encountered in tree buttresses during the months of March and June. Table III shows that its abundance at 35 ft was slightly greater than at 3 ft above the ground (individuals collected per hour). The number of *L. carpenteri* collected per hour in the morning hours was more than twice that collected in the period before midnight (Table IV). Figure 2 clearly shows that the greatest period of activity for both sexes occurs during the morning hours, with a preference for a relatively high elevation above the ground level. Females dominate the evening hours while males outnumber females in the morning hours. *L. carpenteri* was the most prevalent species collected from animal burrows (Table VI), and the third most common species encountered in tree buttresses (Table V).

L. olmeca, the third most abundant sandfly in the study area, has been reported in the literature from Mexico and British Honduras as *flaviscutellata*, a species restricted to South America (G. B. Fairchild, *personal communication*). On Dr. Fairchild's recommendation, we refer to it here as *L. olmeca* pending future clarification. This species was collected in greatest numbers during March, the height of the dry season (Table II). Peak activity occurred near ground level (Table III), but this species was found to range up to 35 ft at least, and is more often taken in the hours before midnight (Table IV). *L. olmeca* was the dominant species among forest-floor litter (Table VII). It is impossible to be precise about the habitat of this species because, for reasons inapparent to us, relatively high densities were found within small areas of forest-floor litter, while it was completely absent in adjacent areas with comparable litter conditions. Castor oil traps baited with *Oryzomys capito*, *Proechimys semispinosus* and *Metachirus nudicaudatus* attracted *L. olmeca* almost exclusively. This species accounted for 1748 (99·0%) out of 1765 sandflies collected by this technique. Four other species, *L. panamensis*, *trinidadensis*, *dysponeta* and *gomezi*, together represented 1% of the collection. Little difference was noted between the attractiveness of

TABLE VII

Sandfly collections from forest-floor litter

Species	♂	♀	% of total
L. olmeca	200	46	95·5
L. gomezi	5	6	4·3
L. panamensis	—	1	0·2
Totals	205	53	100·0

the three bait-animals used. Approximately 18% of the *L. olmeca* removed from traps of each bait-animal were engorged with fresh blood, indicating that all three animals were acceptable hosts for this fly.

L. panamensis, the fourth most abundant sandfly species in the study area is definitely a wet season species with highest densities during June and September (Table II). Flight activity at 35 ft is about 18-fold greater (flies/h) than at the 3 ft level (Table III). Highest densities occurred during the evening hours; significant numbers however, were taken in morning collections as well (Table IV). Females dominated all light trap collections, with only two males collected by this method. *L. panamensis* was the second most frequent sandfly collected by the castor oil trap method, although it represented less than 1% of the accumulated catch.

L. trinidadensis, the next most abundant species, appeared to be most active in March and June (Table III). It was the dominant sandfly in tree buttresses (Table V), and was particularly abundant in this habitat during June, a month in which it comprised more than 95% of the buttress collections. This species appeared to be somewhat arboreal, displaying slightly more activity at the 35 ft level (Table III) during the evening hours (Table IV).

L. shannoni, although not one of the common species, was the second most frequently encountered sandfly in tree buttresses. Its density in this habitat approached that of *L. trinidadensis* at the beginning of dry weather in December, and almost equalled the latter in March, the peak of the dry season.

Other sandfly species were not collected in sufficient numbers to justify comment on their activities.

Search for Leishmanial Infections

Only four flagellate infections were encountered in dissections of 575 individual sandflies comprising 13 different species, *L. olmeca* (449), *L. carpenteri* (39), *L. trinidadensis* (34), *L. dysponeta* (23), *L. shannoni* (11), *L. panamensis* (5), *L. micropyga* (4), *L. gomezi* (3), *L. camposi* (2), *L. aclydifera* (2), *L. punctigeniculta* (1), *L. odax* (1), and *L. isovespertilionis* (1). These infections (three in *L. trinidadensis* and one in *L. micropyga*) showed either epimastigote or trypomastigote forms of the flagellates, and were restricted to the alimentary tract posterior to the midgut.

None of the hamsters inoculated with triturated *L. olmeca* developed leishmaniasis. Although some of the hamsters showed noticeably swollen noses several months after inoculation, leishmanial organisms have not been detected in skin smears or cultures from nasal biopsies at the time of this report.

DISCUSSION AND CONCLUSIONS

The epidemiological similarities between the rodent strain of *Leishmania* in Sasardi and *Leishmania mexicana* have been considered in the preceding report (Herrer *et al.*, 1971). Other workers have contributed a good deal of information about vector-host relationships of *L. mexicana*. *L. flaviscutellata* (= *olmeca*) was shown to transmit *L. mexicana* in Mexico (Biagi et al., 1965). Disney (1968) incriminated the same species as the vector of *L. mexicana* in British Honduras and he noted that *L. cruciata*, *L. permira*, and possibly *L. panamensis* may be vectors of secondary importance. Williams (1970*a*) reported that *L. olmeca* is the principal vector of *Leishmania* among rodents in British Honduras, but that the mode of transmission to man remains obscure. Shaw and Lainson (1968) suggested that although *L. flaviscutellata* maintains leishmaniasis among the rodents another species, which bites both man and rats, transmits the disease to man in Brazil. Extremely thorough work by Williams (1970*b*) showed that *L. olmeca* is probably the principal vector of the disease both among rodents and man in British Honduras. Lainson and Shaw (1968), working in Brazil, reported that 99·2% of 2774 sandflies captured with rodent-baited traps were *L. flaviscutellata*. Flagellates isolated from six *L. flaviscutellata* proved to be *Leishmania*. The authors noted the similarities between this parasite and *L. mexicana*.

The high *Leishmania* infection rate among rice rats in Sasardi (Herrer *et al*, 1971), and the apparent absence of the disease among Choco and Cuna Indians in the region, indicated a non-anthropophilic sandfly vector closely associated with rodents. *L. carpenteri* and *L. dysponeta* dominated the animal burrow habitat and our light trap collections. But as only a single *L. dysponeta* was collected in rodent-baited castor oil traps, and not even one *L. carpenteri*, these two species are not considered to be involved in the epidemiology of *Leishmania* in Sasardi. The overwhelming predominance of *L. olmeca* among sandflies attracted by *Oryzomys capito* and *Proechimys semispinosus*, the primary reservoir hosts of *Leishmania* in Sasardi, implicated this species of sandfly as the most likely vector. *L. panamensis* showed a definite arboreal flight behaviour, and is considered less likely to encounter terrestrial reservoir hosts in significant numbers. If the flight activity of this species had been primarily terrestrial, as it is in other parts of Panama (Johnson *et al.*, 1963), its role as a potential vector would have been more seriously considered. Its frequency in rodent and marsupial-baited traps was only 0·6% of the entire catch. The absence of human leishmaniasis in the area further diminished the importance of *L. panamensis* as a vector since this species is one of the most common man-biters in Panama.

A previous survey, which included our study area, investigated the medical and ecological factors of importance in the area of a proposed sea level canal. Under the auspices of the Interoceanic Canal Commission feasibility studies were done on Route 17 which transects the Republic of Panama from Sasardi on the Caribbean coast to Santa Fe on the Pacific. A total of 7850 sandflies were collected from October 1966 to November 1967. Man-biting collections accounted for a total of 2697 sandflies, 2231 of which were *L. panamensis* (82·7%). *L. olmeca*, not considered an important anthropophilic species in Panama, represented 3·2% of the man-biting collection. These findings corroborate our conclusions concerning the relative importance of these species as potential vectors of the zoonosis in Sasardi.

Because of the remoteness of the study area, the triturated flies used for hamster isolation trials had been collected one to six days before inoculation. Although the flies were retained in the castor oil collecting medium and placed on ice shortly after collection, the delay between capture and inoculation may have accounted for our failure to isolate the agent. Studies are currently in progress to determine if castor oil harms leishmanial promastigotes within the alimentary tract of experimentally-infected sandflies. Triturates of infected flies are being inoculated into hamsters after various periods of incubation in ice-chilled castor oil. Field conditions encountered in Sasardi, in which sandflies were held before trituration and inoculation into hamsters, are being duplicated so far as possible.

The results, to be reported in a later paper in this series, will have direct application to the present work and may result in modification of future techniques.

To briefly review the evidence for considering *L. olmeca* as the principal vector of the Sasardi zoonosis: this species (1) accounted for 99·0% of the sandflies attracted to reservoir rodent species in castor oil traps; (2) is primarily active near ground level where it may encounter infected rodents; (3) is the third most abundant sandfly species in the area; and (4) has been implicated as a vector in Mexico and British Honduras.

SUMMARY

1. In Sasardi, Darien Province, Republic of Panama 8099 specimens of *Lutzomyia* sandflies belonging to 24 species were collected. Studies were conducted in September and December 1968, and March and June 1969, thus providing information on sandfly activities at different seasons.

2. Bionomic data are given for the eight most common species, *L. dysponeta*, *L. carpenteri*, *L. olmeca*, *L. panamensis*, *L. trinidadensis*, *L. aclydifera*, *L. gomezi* and *L. camposi*. These species accounted for 94% of the total collection. *L. dysponeta* was the most common sandfly species encountered, but although it was found in several different habitats, it was not the dominant species in any. *L. olmeca* was the most common sandfly found in the forest-floor litter. It also comprised 99% of the species collected from castor oil traps baited with the principal rodent reservoir hosts of *Leishmania* in the area. *L. panamensis*, the most anthropophilic species of the group, was much more abundant at 35 ft than near ground level, contrary to findings in other regions of Panama. *L. carpenteri* was the dominant species found in animal burrows, and *L. trinidadensis* was the most common sandfly in the tree buttress habitat.

3. Although other epidemiological evidence incriminated *L. olmeca* as the principal vector of the infection, techniques which included sandfly dissections and inoculation of hamsters with pooled, triturated sandflies, failed to isolate a leishmanial agent.

ACKNOWLEDGMENTS. The work reported here was supported in part by a research grant (AI-1251) from the NIAID, NIH, U.S. Public Health Service.

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