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Natural Infections of Leptomonad Flagellates in Panamanian *Phlebotomus* Sandflies[1]

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In the search for vectors of leishmaniasis in Panama, over 5000 wild-caught *Phlebotomus* females were dissected and examined for leptomonad flagellates. Natural infections were found in 416 out of a total of 4861 females of six of the seven common man-biting species (*gomezi, panamensis, sanguinarius, shannoni, trapidoi,* and *ylephiletor*). A single female *longipalpis* was also infected. None of 365 females of 13 other species which never or only occasionally bite man was infected. A total of 262 males, including 242 of common man-biting species, was negative.

Leptomonad infections were found in sandflies from each of five endemic areas and in two others where human cases have not been reported. The over-all infection rate of the common man-biters was 8.5%, with rates varying from 1.9% for *panamensis* to 15.4% for *trapidoi*. During the rainy season, June through December, the over-all rate (10.6%) was much higher than during the dry season (4.1%).

The source of these infections in unknown. However, the vertical distribution of species in simultaneous ground-level and tree-platform catches, together with differential infection rates, suggest that the reservoirs may be found among arboreal animals.

Leptomonads were always present in the hindgut of infected females. They also occurred occasionally in the posterior part of the midgut (stomach), rarely in the anterior part (cardia), and only twice were they found in the foregut (esophagus and pharynx, respectively). In over 20% of the infections leptomonads occurred in the Malpighian tubules.

In culture the morphology of leptomonads from the natural infections is consistent with that of *Leishmania*. Lesions produced in hamsters by two of the sandfly strains are indistinguishable from those produced by Panamanian human strains. In the sandfly gut the growth pattern and morphology of the leptomonads in natural infections are similar to those of sandflies fed on hamster lesions produced by both Panamanian human strains and a wild-caught sandfly strain.

For many years it has been generally considered that *Phlebotomus* sandflies are probably the vectors of American leishmaniasis as well as of the leishmaniases of the Old World, where transmission has been experimentally demonstrated in several cases. With this hypothesis several South American investigators, working in areas where *Leishmania braziliensis* is endemic, have searched for natural infections of leptomonad flagellates in sandflies, with results reviewed in some detail by both Pessôa and Barretto (1948) and Pifano (1960).

In Venezuela, Pifano (1940) found leptomonads in 5 of 72 *Phlebotomus panamensis*, some taken while feeding on the border of a human lesion. He also found infections in

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2 *Phlebotomus migonei* and 1 *Phlebotomus longipalpis* out of an unspecified total of these species.

Pessôa and associates (Pessôa and Pestana, 1940; Pessôa and Coutinho, 1940, 1941; Coutinho, 1940) examined a large number of sandflies from several endemic areas in the state of São Paulo, Brazil. They found 26 infections out of a total of 11,393 sandflies of 3 species: *whitmani*, 10/4940; *migonei*, 8/3742; *pessoai*, 8/2711; the over-all infection rate was 0.22%. The only other species examined was *limai*, of which 20 were negative. In these four Brazilian studies, examination was made entirely by dissections in one case (528 specimens); in serial sections in two cases (total, 1612 specimens); in the fourth instance, with 9273 sandflies examined, both methods were used, but the total for each was not specified. Serial sections revealed one each of *migonei* and *pessoai* with flagellates in the pharynx. In no case do the two reviews give any information about the location or growth pattern of the flagellates in the sandfly gut, except for the two pharyngeal infections. Even here, in the one instance for which the original publication is available to us, Coutinho (1940) carefully described and figured the flagellates in the pharynx but did not mention whether any occurred in the rest of the gut.

Forattini and Dos Santos (1952), in the course of sandfly studies in the state of São Paulo, found a natural infection in one female *intermedius*. In further examinations by means of Giemsa-stained smears of the triturated bodies, these workers (Forattini, 1954) brought the total of sandflies examined to 678 *intermedius* and 104 *whitmani*, without finding any additional infections.

All these investigators felt they were probably dealing with *L. braziliensis*.

We have been concerned with the transmission problem of American cutaneous leishmaniasis in Panama. We recognize that we are dealing with one of the geographic strains of this disease and that the nomenclature of the causative parasites requires revision. For the present we shall use the name *L. braziliensis, s. lat.*

During 1961 more than 5000 wild-caught female sandflies were dissected and the gut examined for leptomonads. A preliminary report of this work has been published (Johnson *et al.*, 1962).

Several places where the human disease is known to occur have been sampled from two to many times. Two of the areas are on the wet Caribbean slope of the continental divide (Changuena River and Almirante); one is on the drier Pacific slope, west of the Canal Zone (Santa Rita); and two are in the continental divide region (La Zumbadora and Quebrada Bonita (Fig. 1). Our efforts were concentrated at Quebrada Bonita, which has been well studied by our group since 1956. This area, on the Transisthmian Highway, was at one time heavily wooded and was actively being cleared for agricultural purposes. A large proportion of the population has had leishmaniasis. Even though the remaining wooded tract where our collections were made in 1961 has been reduced to little more than two acres, there was a new case of leishmaniasis in a child living nearby as recently as September, 1961.

We also have collected and dissected sandflies from two areas where human leishmaniasis has not been reported. Both are on the Pacific side of the Isthmus, one in the Canal Zone, and the other at about 2000 feet on Cerro Campana. The latter collection was made early in January, 1962.

Materials and Methods

Collecting and transport of specimens. Most of the females dissected were individuals of the 7 most common man-biting species of *Phlebotomus* in Panama. They were collected, unfed, at dusk or predawn, when attracted to the collectors or to a horse. This assured that physiologically hungry females formed a large part of the samples. Some samples were taken from tree platforms in the forest canopy

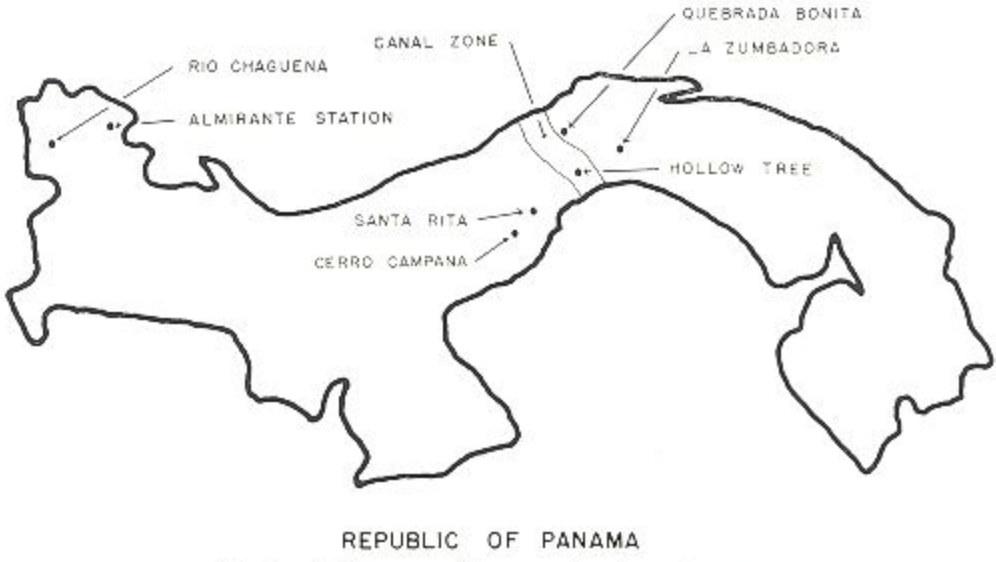


FIG. 1. Outline map of Panama, showing study areas.

at heights ranging from 6 to 50 feet. At Quebrada Bonita, in order to sample other than physiologically hungry females of the man-biting species, as well as those species not attracted to man, daytime collections were made from tree trunks, tree buttresses, and animal burrows. At Cerro Campana a Shannon trap collection was made of sandflies attracted to light.

Flies were caught singly or in groups of about 15 in small, moistened, plaster-lined vials. Depending on the distance involved and the conditions of transport to the laboratory, the vials were either maintained at ambient temperatures or placed over ice. Details of the holding vials, aspirators, and methods of transport are given in Hertig and Johnson (1961).

Method of dissection. In the laboratory the catch was dissected as soon as convenient, usually within 24 hours after capture except that in special cases females were held in the laboratory up to 120 hours. The vials containing flies were placed in a refrigerator at 4.5°C to immobilize the flies. Flies in groups of 4 or 5, were shaken vigorously in a vial of sterile saline to remove as much organic material, dust, and scales as possible. Flies were then removed singly from the vial to a drop of sterile saline on a flamed slide, and the wings and legs were removed. After removal of legs and wings each fly was transferred to a small drop of sterile saline on a flamed cover slip for the actual dissection. After cutting off the head, the thorax was held with one needle, the second needle was pressed lightly on the penultimate segment of the abdomen, and the tail end plus the entire mid- and hindgut and Malpighian tubules slowly pulled out the rear. No actual cutting was done in this step. The preparation was arranged so that the gut from the proventriculus to the rectal ampulla was clearly visible; the cover slip was inverted over the concave surface of a depression slide, the edge sealed with saline, and the gut examined microscopically By using the shallow hanging drop there was a minimum of distortion of the gut and a maximum of visibility of all the parts. Leptomonad-infected guts could be easily removed from the drop to a culture tube.

After examination a drop of phenol was introduced into the hanging drop of saline to clear the tail end, which contains the spermathecae, for species determination.

Results

Infection rate in the sandfly population. Females of 6 of the 7 most common man-biting species were found infected with flagellates indistinguishable from leptomonad forms of the genus *Leishmania*. Positive females of 1 or more of the 6 species were taken from all the endemic localities sampled as well as from two areas where human leishmaniasis has not been reported. We also found leptomonad flagellates in a female *longipalpis* collected at Cerro Campana, the only specimen of this species dissected during the survey. One *trapidoi* female from Quebrada Bonita and one *ylephiletor* female from Cerro Campana were infected with crithidiform flagellates. We have dissected 365 specimens of 13 other species which never or only occasionally are taken biting man, and none was infected with leptomonads. Samples of most of the 13 species were small except for *trinidadensis* and *vespertilionis*. The species and number of females dissected were as follows: *camposi*, 7; *cruciatus*, 15; *hansoni*, 6; *ovallesi*, 7; *runoides*, 4; *rorotaensis*, 1; *serranus*, 1; *spinosus*, 5; *trinidadensis*, 190; *triramulus*, 3; *vesiciferus*, 2; *vespertilionis*, 122; and *vexillarius*, 2. We have found crithidiae of trypanosomes in both *vespertilionis* and *trinidadensis*. These infections will be discussed in a separate note. The progeny of some of the crithidia-infected *vespertilionis* were reared to adults and proved to be this species by examination of males. However, *vespertilionis* and the closely related *isovespertilionis* cannot be distinguished in the female, with the result that the latter species may have been included in our series.

We also dissected 262 males of 6 species, including 242 males of 3 common man-biting species which were taken in collections containing infected females. Species and numbers of males were: *camposi*, 7; *gomezi*, 136; *sanguinarius*, 96; *trapidoi*, 10; *trinidadensis*, 1; and *vespertilionis*, 12. Males were consistently negative for any type of flagellate.

The over-all infection rate in females of the man-biting species was 8.5% (Table I). The five most common species, *gomezi*, *panamensis*, *sanguinarius*, *trapidoi*, and *ylephiletor*, differed widely in infection rates, with a low of 1.9% for *panamensis* and a high of 15.4% for *trapidoi*. *Phlebotomus pessoana* was not common in our collections, and although none of 24 females was infected, larger samples would be necessary to ascertain the infection rate of this species.

Seasonal differences in infection rates are marked (Table I). During the dry season (January–April), there was an over-all infection rate of 4.1%, whereas the rate during the rainy season (June–December) was 10.6%. No collections were made during the month of May. In the rainy season all species

TABLE I

Leptomonad Infections in Wild-Caught, Man-Biting Species of Panamanian Sandflies

(All sources; calendar year 1961)

Species of *Phlebotomus*	Entire year			Dry season (Jan.–April)			Rainy season (June–Dec.)		
	No. diss.	No. pos.	% pos.	No. diss.	No. pos.	% pos.	No. diss.	No. pos.	% pos.
gomezi	545	27	5.0	430	20	4.7	115	8	7.0
panamensis	579	11	1.9	29	1	3.5	550	10	1.8
sanguinarius	1404	66	4.7	711	19	2.7	693	47	6.8
trapidoi	1591	245	15.4	327	19	5.8	1264	226	17.9
ylephiletor	668	63	9.4	59	3	5.1	609	60	9.9
shannoni	74	4	5.4	18	3	16.6	56	1	1.8
pessoana	24	—	—	—	—	—	24	—	—
Total	4885	416	8.5	1574	65	4.1	3311	352	10.6

TABLE II

Leptomonad Infections in Wild-Caught, Man-Biting Species of Panamanian Sandflies

(Quebrada Bonita, from man and horse, 1961)

Species of *Phlebotomus*	Entire year			Dry season (Jan.–April)			Rainy season (June–Dec.)		
	No. diss.	No. pos.	% pos.	No. diss.	No. pos.	% pos.	No. diss.	No. pos.	% pos.
gomezi	382	23	6.0	284	16	5.6	98	7	7.1
panamensis	344	6	1.7	24	1	4.2	320	5	1.6
sanguinarius	1190	62	5.2	567	18	3.2	623	44	7.1
trapidoi	1304	211	16.2	222	16	6.8	1082	195	18.0
ylephiletor	200	31	15.5	29	2	6.9	171	29	17.0
shannoni	25	3	12.0	15	3	20.0	10	—	—
Total	3445	336	9.5	1141	56	4.9	2304	280	12.2

except *shannoni* and *panamensis* showed an abrupt increase in infection rates, particularly *trapidoi*, which rose from 5.8 to 17.9%.

1. Quebrada Bonita, collections from man and horse. This study area lies to the north or Caribbean side of the continental divide. Rainfall is heavy during the months May to December, but a definite dry season occurs from January through April. At Quebrada Bonita, 55 collections averaging 60 females each were made mainly in the evening at weekly intervals during the months of January to April and June to December. The proportion of infected females increased almost threefold during the rainy season, from 4.9 to 12.2% (Table II). *Phlebotomus trapidoi* and *ylephiletor* had the highest infection rates during the rainy season, and *P. shannoni* was rare, but during the dry season, 3 of 15 were found infected. During the dry season, *panamensis* is an uncommon species, and we lack sufficient data for a true comparison of its dry and rainy season infection rates.

Because leishmaniasis had been contracted by Gorgas Memorial Laboratory personnel making biting-insect collections in the forest canopy (at Almirante), during the rainy season paired collections of roughly equal numbers were made in the evening from a 36-foot tree platform and at ground level below the platform. All species except *panamensis* occurred regularly both at ground level and in the canopy (Table III). All but 2 of the 304 *panamensis* were taken from ground level. Of the other species, *trapidoi, ylephiletor,* and *gomezi* were more frequently captured at the 36-foot level, and *sanguinarius* at ground level. *Phlebotomus trapidoi* and *ylephiletor,* as well as being more common at 36 feet, had somewhat higher infection rates there, particularly *ylephiletor,* while *gomezi*, which was also most common

TABLE III

Evening Collections, Comparison of Infection Rates from Ground Level and 36-Foot Platform

(Quebrada Bonita, June–Dec., 29 paired collections)

Species of *Phlebotomus*	Ground level			36-foot platform		
	No. diss.	No. pos.	% pos.	No. diss.	No. pos.	% pos.
gomezi	27	3	11.1	44	2	4.6
panamensis	302	5	1.7	2	—	—
sanguinarius	326	24	7.5	147	9	6.1
trapidoi	346	52	15.0	486	91	18.7
ylephiletor	43	4	9.3	114	20	17.5
Total	1044	88	11.4	793	122	15.4

TABLE IV

Evening and Predawn Collections

Comparison of Ground Level and 36-Foot Platform Infection Rates

(Quebrada Bonita, Oct.-Dec., 8 paired collections)

Species of *Phlebotomus*	Ground level						36-foot platform					
	Evening			Predawn			Evening			Predawn		
	No. diss.	No. pos.	% pos.	No. diss.	No. pos.	% pos.	No. diss.	No. pos.	% pos.	No. diss.	No. pos.	% pos.
gomezi	3			2			14	1	7.1	15	2	13.3
panamensis	13			3								
sanguinarius	171	11	6.4	101	6	5.9	64	8	12.5	27	4	14.8
trapidoi	37	7	18.9	72	18	25.0	94	19	20.2	115	16	13.9
ylephiletor	3			5	2	40.0	8	2	25.0	5	1	20.0
Total	227	18	7.9	183	26	14.2	180	30	16.7	162	23	14.2

at the 36-foot level (based on a small sample), had a distinctly higher infection rate at ground level.

We were interested to see if the proportion of infected females and growth pattern of the leptomonads might differ according to what time of night catches were made. During October through December, 8 of the regular evening catches made after sundown at about 7 P.M. were followed by 8 catches made before daybreak at about 5 A.M. These paired catches were made both at ground level and at 36 feet. The pattern of infection was similar in predawn and evening collections.

Phlebotomus trapidoi, which had a slightly higher infection rate at the 36-foot level in the evening catches, reversed itself. The predawn ground-level rate (25.5%) was almost twice that of the platform (13.9%) (Table IV). We have omitted from the data presented in Table IV one morning collection taken 12 October which lacked a paired evening catch. This collection is worthy of mention because 16 of 39 *trapidoi* (41.0%) were found infected, as well as 2 of 3 *ylephiletor* (66.6%), including both ground and platform catches.

2. *Quebrada Bonita, daytime collections from adult resting places.* Most of these collections, made during the rainy season at weekly intervals, were from tree trunks or within tree buttresses. A few were made from animal burrows and from leaves on the forest floor. The most frequently encountered species was *ylephiletor*: 425 females were collected and 31 were found infected with leptomonads (7.3%). Infected individuals of *sanguinarius, trapidoi,* and *shannoni* were also found, although these species were collected only in small numbers. The two specimens of *gomezi* and one of *panamensis* were negative. Nine species which never or only occasionally bite man were also taken from resting places and found consistently negative for leptomonads. Of these species only *trinidadensis* was common, 189 specimens of the total 227 being this species.

There were no readily discernible differences between the pattern of leptomonad infection found in resting females and physiologically hungry females of the man-biting species.

3. *Santa Rita, collections from man and horse.* Human leishmaniasis is common in and around Santa Rita, although the wooded areas are mainly second growth, generally much drier than at Quebrada Bonita, and thus less satisfactory for *Phlebotomus*. In 12 ground-level collections made from February through September only 10 of 442 females were infected with leptomonads (Table V). During the dry season, *gomezi* was the only common species and the only one found infected. Its infection rate of 2.8%

TABLE V

Leptomonad Infections in Wild-Caught, Man-Biting Species of Panamanian Sandflies

(Santa Rita, from man and horse, 1961)

Species of *Phlebotomus*	Entire year			Dry Season (Feb.–April)			Rainy season (June–Sept.)		
	No. diss.	No. pos.	% pos.	No. diss.	No. pos.	% pos.	No. diss.	No. pos.	% pos.
gomezi	159	4	2.5	145	4	2.8	14	—	—
panamensis	228	5	2.2	1	—	—	227	5	2.2
sanguinarius	29	—	—	17	—	—	12	—	—
trapidoi	6	1	16.7	2	—	—	4	1	25.0
ylephiletor	1	—	—	1	—	—	—	—	—
shannoni	1	—	—	1	—	—	—	—	—
pessoana	18	—	—	—	—	—	18	—	—
Total	442	10	2.3	166	4	2.4	276	6	2.2

is roughly half that of *gomezi* from the Quebrada Bonita collections. The common species during the rainy season was *panamensis*, with an infection rate of 2.2%, which is similar to that found in *panamensis* from Quebrada Bonita. Only 6 specimens of *trapidoi* were taken, 4 during the rainy season, with 1 of them infected. Circumstances prevented making collections at Santa Rita during the latter part of the rainy season.

4. Almirante station, Bocas del Toro Province, collections from man and horse. The Caribbean coast of Panama is covered with a tropical rain forest that lacks a true dry season, although there is less rain during January to April. We had 3 paired collections from Almirante, at ground level and at 40 feet in the forest canopy: one in March during the "dry" season, and the other two in August and November, during the heavy rains. The leptomonad infection rate, as at Quebrada Bonita, increased sharply during the "rainy" season (Table VI). *Phlebotomus trapidoi* and *ylephiletor* were the only two species found infected. During the "rainy" season *trapidoi* was not only the dominant species but more than 20% of this species were infected with leptomonads. In the November collection, *trapidoi* was the only species collected from the 40-foot tree platform, with 14 of 51, or 27.5%, infected. During 1961 three Almirante collectors developed cutaneous leishmaniasis. Two of the cases were acquired during the "rainy" season, one in December, 1960, and the other in July, 1961. The third case was acquired during the "dry" season.

5. La Zumbadora, collections from man

TABLE VI

Leptomonad Infections in Wild-Caught, Man-Biting Species of Panamanian Sandflies

(Almirante Station, from man, 1961)

Species of *Phlebotomus*	Entire year			Dry season (24 Mar.)			Rainy season (4 Aug., 29 Nov.)		
	No. diss.	No. pos.	% pos.	No. diss.	No. pos.	% pos.	No. diss.	No. pos.	% pos.
gomezi	—	—	—	—	—	—	—	—	—
panamensis	5	—	—	4	—	—	1	—	—
sanguinarius	13	—	—	10	—	—	3	—	—
trapidoi	169	29	17.2	48	3	6.3	121	26	21.5
ylephiletor	15	1	6.7	6	1	16.7	9	—	—
shannoni	1	—	—	—	—	—	1	—	—
Total	203	30	14.8	68	4	5.9	135	26	19.3

and horse. La Zumbadora, lying at about 2000 feet within the mountains of the continental divide, has sharply defined rainy and dry seasons with heavy rains during May to December as at Quebrada Bonita. From 1958 through 1960, 94 females of the 7 common man-biting species were dissected and all were negative for any type of flagellate. These females were taken in 9 small collections from ground level at various times during both rainy and dry seasons. We have had six 1961 collections from La Zumbadora, all made in February and March during the dry season, and all from ground level. Of the 199 females collected, 117 were *sanguinarius* and only 1 was positive, an infection rate for this species of less than 1%. Of the 55 *trapidoi* dissected none was positive. In the 3 years prior to our 1961 dissections five members of Gorgas Memorial Laboratory field teams acquired leishmaniasis at La Zumbadora. Cases in the local population were frequent and still occur.

6. Changuena River, Bocas del Toro Province, collections from man. In September, 1961, a short-term field station was established on the Changuena River in uninhabited virgin jungle. Like Almirante, this area is in the tropical rain forest. In two collections of 101 individuals taken from a 50-foot tree platform, 2 of 49 *sanguinarius* and 2 of 41 *trapidoi* were found infected. Other species taken included 6 *pessoana*, 4 *ylephiletor*, and—unexpectedly—1 *panamensis*. None of these was infected. The over-all infection rate was 4.0%. Human leishmaniasis has been reported from the Changuena River area in hunters and other transients.

7. Canal Zone, collections from hollow tree. On the Pacific side of the Isthmus in the Canal Zone are large stands of virgin or old second growth forest. There are no human habitations in or near these forests, which are undisturbed by man and frequented in the main only by occasional hunters. Human cases of leishmaniasis have not been reported from these areas. We have made a few collections of *Phlebotomus* from a large hollow tree near the edge of one of the forested areas. All but one of the flies collected were of non-man-biting species. The single exception was a female *gomezi* which was infected with leptomonad flagellates similar in appearance and position to those found in *gomezi* from areas of endemic leishmaniasis.

8. Cerro Campana, collections from man and Shannon trap. Cerro Campana lies about 35 miles southwest of the Canal Zone near the Pacific Coast. Human leishmaniasis has not been reported from Cerro Campana but is present at Santa Rita, which lies less than 20 airline miles away at the foot of the same mountain chain. Our collections in early January, 1962, were taken at about 2000 feet and included females attracted to human bait and to light in a Shannon trap.

The dominant species captured both from man and Shannon trap was *ylephiletor* (213 of 248 females dissected). Seven of 87 *ylephiletor* collected from man (8.0%) were infected with leptomonads similar in appearance and position to those found in sandflies from Quebrada Bonita and other endemic areas. The Shannon trap catch included 126 *ylephiletor* with 4 infected (3.2%), and a further specimen of this species infected with crithidiae. The only other common man-biting species taken by either collecting method was *sanguinarius*. None of 30 specimens dissected was infected. Of particular interest was a single female *longipalpis* taken from the Shannon trap and found infected with leptomonads. Typically a dry-land species, *P. longipalpis* is associated with neotropical kala azar in Guatemala, El Salvador, and several South American countries.

Site and type of infection in the sandfly gut. A diagrammatic drawing of the sandfly gut is given in Fig. 2. The buccal cavity, mouthparts, rectum, and anus are not shown. Below the sclerotized pharynx is the short esophagus with its membranous, distensible diverticulum. The esophageal diverticulum serves as a reservoir for fluids. The pharynx and

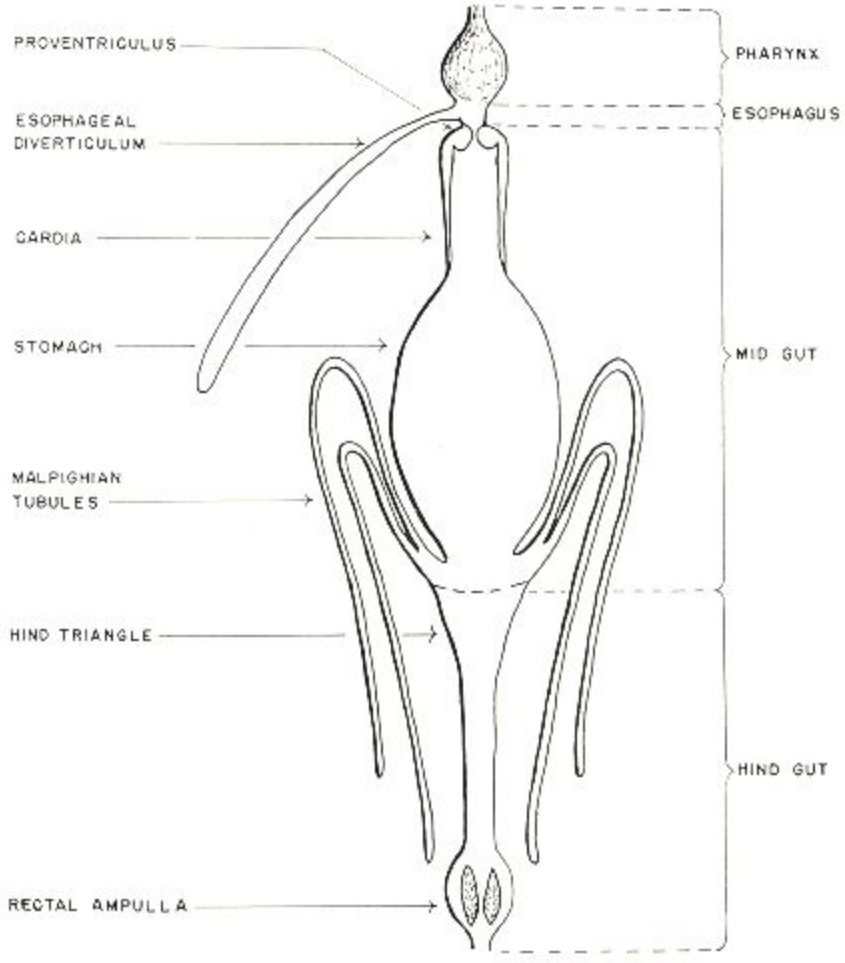
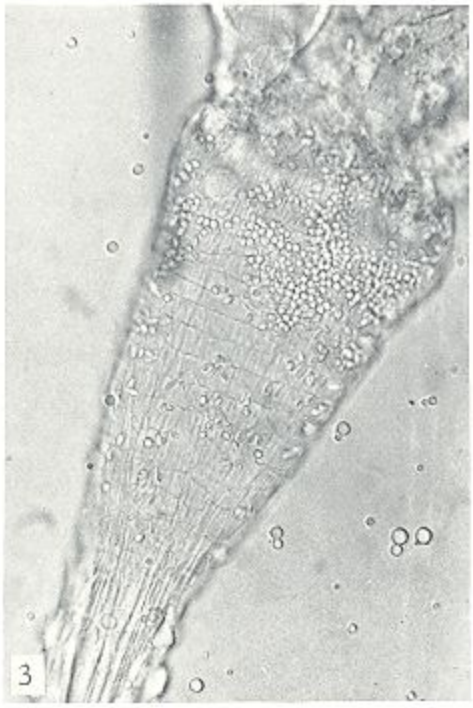
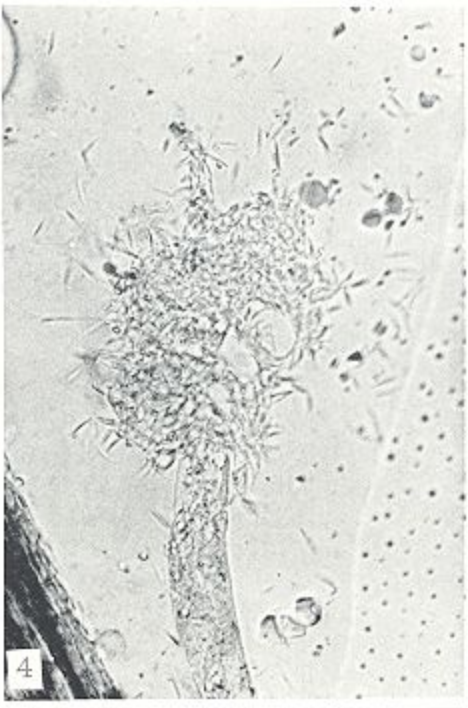


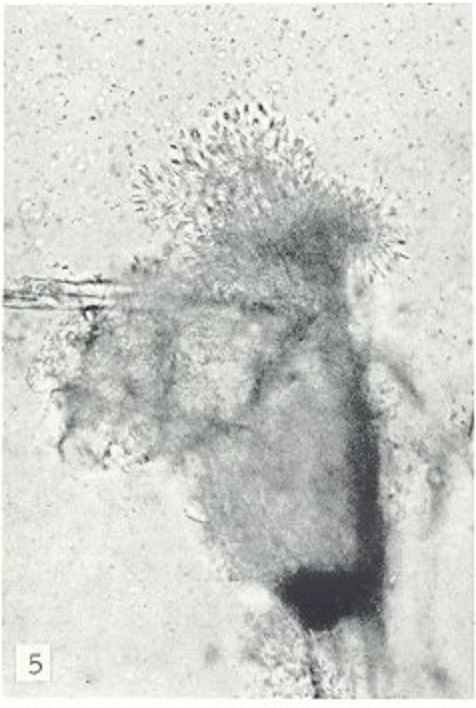
FIG. 2. Diagram of the gut of *Phlebotomus*. Mouthparts, buccal cavity, rectum, and anus omitted.

esophagus are parts of the foregut. Posterior to the esophagus is the muscular proventriculus, which is the valvular opening into the midgut. The nondistensible, anterior part of the midgut is called the cardia, and the posterior elastic portion, which may be greatly distended by a blood meal, is the stomach. On either side at the posterior extremity of the midgut arise the Malpighian tubules, beginning as two common tubes which quickly branch into two tubules each. *In situ* the distal ends of the Malpighian tubules are closely appressed to the median portion of the hindgut. The anterior-most part of the hindgut is roughly triangular in optical section and we call it the "hind triangle." Below the thin-walled hind triangle, the hindgut forms a narrow muscular tube which broadens into the rectal ampulla, with its two rectal glands. Beyond the rectal ampulla follow the tubular rectum and anus.

In wild-caught Panamanian sandflies, leptomonad flagellates were always found in the hindgut with the majority of infections confined to this area. Often, the hind triangle was the only area infected (Fig. 3). In the hindgut, leptomonads usually occurred in rosettes, attached at the anterior (flagellar) end to the gut wall, with most of the flagellates round to oval in shape, and small. We hope that sections of infected guts, now being prepared, will show whether at-







FIGS. 3–6. Natural infections of *Phlebotomus* with leptomonads; fresh dissections; (350 ×).

FIG. 3. *Phlebotomus trapidoi*; flagellates attached to epithelium of the hind triangle of the hindgut; oval forms predominate; in the large patch most of the organisms are perpendicular to the epithelium and are seen foreshortened.

tachment to the gut wall is only by the flagellum where it arises from the cell or by the cell itself. At times there were a few spindle-shaped to long, thin, very active leptomonads, particularly at the outer edges of the rosettes. These larger and more active flagellates were attached by any part of the flagellum. Eventually some of such precariously attached leptomonads would break loose from the gut wall and swim about in the lumen. In more than 20% of the infected flies, spindle-shaped, long, thin flagellates were found in the Malpighian tubules (Fig. 4).

Probably the long, active organisms are the ones which migrate within the gut, usually in an anterior direction, with some flagellates lost in the Malpighian tubules and others going forward into the midgut. Midgut infections usually consist of spindle-shaped, long, thin flagellates, although in heavy infections shorter organisms may also be present. Usually the infection does not progress beyond the stomach. We have held females in the laboratory for varying periods up to 120 hours after collection and the trend seems to be toward a progression of the infection to the midgut. About 14 hours after collection, 3 of 22 females, or less than 15%, had flagellates in the stomach; at 72 hours, 2 of 7 females, almost 30%, had such infections; and at 96 and 120 hours, 10 of 24 females, or more than 40%, had flagellates in the stomach.

In less than 5% of the infections (0.2% of the total females dissected) organisms were found in the cardia. Cardia infections may consist either of a loose mass of mainly spindle-shaped and long, thin organisms or of actual rosette formation at the proventriculus.

Pharyngeal and esophageal infections are rare. We found only one pharyngeal infection, in a *trapidoi* from Quebrada Bonita (Fig. 5). This infection represents 0.08% of the total *trapidoi* dissected from that area. A second *trapidoi* from the Changuena River station had an esophageal infection. Considered together, these foregut infections equal only 0.1% of the total *trapidoi* dissected from all sources.

After a primary infective meal, a second noninfective blood meal probably does not alter the course of infection. Groups of wild-caught females which had been allowed to feed on human or horse at the time of collection, and then were kept up to 120 hours in the laboratory, did not evidence a lower infection rate at the end of 5 days than did their unfed counterparts, and the forward growth of flagellates was similar in both groups.

There were no real differences in type of infection according to the species of sandfly except that infected *gomezi* often had a heavier infection in the hind triangle than did the others.

The type of infection we have found in the wild-caught sandflies is similar to but not so intense as that found in laboratory-reared flies experimentally infected with human strains of Panamanian *Leishmania* by allowing them to feed on infected animals. That is, in both experimentally and naturally infected flies, the hindgut is invariably infected. The proportion of midgut infections is much greater in experimentally infected flies, but this is possibly because the experimentally produced animal lesion is extremely rich in organisms, and sandflies thus receive a massive dose of parasites.

The infection rate according to method of

FIG. 4. *Phlebotomus sanguinarius*; flagellates at ruptured apex of Malpighian tubule; spindle-shaped and long, thin forms predominate.

FIG. 5. *Phlebotomus trapidoi*; heavily infected pharynx; flagellates, still attached to tissue, protruding from pharynx at junction with esophagus.

FIG. 6. *Phlebotomus gomezi*; midgut of infected fly showing large, round, refractile, intracytoplasmic inclusions.

collection. At a given time the possibility of human or other animal infections occurring depends upon the proportion of infective flies which are actively seeking a blood meal. For this reason biting collections probably give the best index of potential transmission. At Cerro Campana 8.0% of *ylephiletor* in a biting collection were infected with leptomonads. At the same time and place and only a few yards away, *ylephiletor* collected when attracted to light had an infection rate of only 3.2%. A similar difference occurred in Quebrada Bonita biting collections of *ylephiletor* (15.5% infection rate) as opposed to daytime resting-place catches (7.3% infection rate.)

Discussion

Identification of the leptomonads. The leptomonad strains we have found in wild-caught, man-biting sandflies have not as yet been completely identified. However, we have strong presumptive evidence that one or more human-infecting strains of *Leishmania* are included. Since only females are infected there is no need to consider insect leptomonads (never reported in sandflies) which either infect insects in the larval stage or are acquired by nymphs and adults of both sexes. Our leptomonad strains are morphologically consistent with leptomonads of the genus *Leishmania*. The strongest evidence that human-infecting strains are involved will be mentioned only briefly since it is the subject of a separate paper by one of us (McConnell, 1963), which directly follows the present report. Suffice it to say that two strains from wild-caught *trapidoi* produced lesions in the Syrian hamster indistinguishable from those caused by Panamanian human strains. Furthermore, the infection pattern in sandflies fed on such lesions not only matched that of the natural infections but in addition was the same as in sandflies fed on hamsters inoculated with the human strain.

A comparison may be made of the growth pattern of leptomonad infections derived from four sources. Two of these are of unknown origin, namely, (1) the natural infections, and (2) those of sandflies fed on hamster lesions produced by sandfly strains. The other two are of known human origin, i.e., (3) infections of sandflies fed on hamsters inoculated with human strains of *L. braziliensis*, and (4) of those fed artificially on cultures of human strains (Hertig and McConnell, 1963). In the artificially fed sandflies early infection of the hind-triangle usually, but not invariably, takes place as in the others. The artificial infections differ chiefly in the early establishment of growth in the cardia, particularly at or near the proventricular valve, and often with a massive accumulation of flagellates within 4 or 5 days.

This difference, as between the artificial infections and those derived from the human strain hamsters, cannot be attributed to different strains or species. A better explanation would be the number and stage of the parasites ingested by the sandflies. In the natural and hamster derived infections there were frequently only a few flagellates, at times less than a dozen, which gave the appearance of incipient infections by a few surviving parasites which would have been ingested in the aflagellate stage at least 4 or 5 days previously. On the other hand, the artificial culture-blood meals provided an initial supply of large numbers of motile flagellates.

The vector-reservoir problem. Two of the infected species, *trapidoi* and *ylephiletor*, are members of the *intermedius-whitmani* group of *Phlebotomus*, a species complex associated by South American investigators with cutaneous leishmaniasis in Brazil and Paraguay.

Assuming human-infecting strains of *Leishmania* are involved in our series, from the standpoint of infection rate, *trapidoi* leads the list of suspected vectors. This species has been found in all endemic areas. In Almirante, where human infection takes place at a brisk rate, *trapidoi* is the dominant species in biting collections from humans, and an extremely high proportion of females is

infected. If anterior station transmission takes place, the foregut infections we found in *trapidoi* and no other species may be of significance. The virtual absence of *trapidoi* from Cerro Campana, where human cases of leishmaniasis have not been reported, also suggests that *trapidoi* may be necessary for transmission to humans. At Cerro Campana *ylephiletor* is the dominant sandfly species with a leptomonad infection rate of 8.0% in the one biting collection which we dissected. In 41 collections including all seasons of the year made at Cerro Campana during November, 1958, through July, 1960, there were 3392 *ylephiletor* females and only 42 *trapidoi* females of a total 3915 females of the man-biting species collected.

We do not yet know the entire host range of any of our common man-biting sandflies. Laboratory evidence suggests that *gomezi* and *sanguinarius* have a broad host spectrum. Our impression is that both species prefer humans to spiny rats. Four genera of opossums (*Caluromys, Didelphis, Marmosa*, and *Philander*), climbing rats (*Tylomys*), squirrels (*Sciurus*), and kinkajous (*Potos*) are very attractive to *sanguinarius*. All these animals except *Philander* are readily accepted by *gomezi*. *Philander* is fed upon by *gomezi* but in limited numbers. Baby chicks are acceptable to both species and, although reluctant, *gomezi* and *sanguinarius* will feed in limited numbers on Syrian hamsters. Baby white mice are rejected. Horses are attractive to all the man-biting species.

In a feeding trial of *sanguinarius* on a female woolly opossum (*Caluromys*) with attached young, more than half of 46 flies were observed to feed on the babies. Of the 46 fed females, 29 developed needle crystals in the blood meal, indicating improper digestion of the meal, and most of these 29 died within 2 days after feeding. Nine days later another batch of 29 *sanguinarius* was fed on the same opossum with the babies shielded from bites. None of these females developed needle crystals in the blood meal. We plan to continue investigations along this line with live-trapped animals.

Unfortunately, *trapidoi*, the most likely suspect, is our most difficult species to rear and feed in the laboratory, so nothing can be said of its host preferences on the basis of laboratory experience.

Our collections at Quebrada Bonita indicate that vertebrate reservoirs of Panamanian leishmaniasis may be arboreal animals. *Phlebotomus panamensis*, which has the lowest infection rate of any of the common man-biting species, is also seldom captured above ground level. This is true even though the tree platform may be only a few yards from ground level on a steep hillside. On the other hand, more *trapidoi* are found biting in the canopy than at ground level, and this species has the highest infection rate.

Posterior or anterior station transmission? In Old World sandflies experimentally infected with organisms of oriental sore or kala azar, investigators have found heavy infections in the midgut, particularly the cardia, to be the rule. Forward extension of flagellates into the pharynx is common, and occasionally parasites are found in the mouthparts. Hindgut infections are rare and usually associated with midgut infections. Partly because of the anterior infections found in experimentally infected Old World sandflies it has been assumed that the human leishmaniases are transmitted by bite. However, a Russian worker found many heavy hindgut infections in *Phlebotomus minutus* collected from rodent burrows and assumed posterior station transmission took place in Old World cutaneous leishmaniasis (Shoshina, 1953). Regarding Shoshina's finding of hindgut infections in *P. minutus*, Adler and Theodor (1957) believed she was working with a lizard *Leishmania*. Since in Panamanian man-biting sandflies leptomonad infections of the hindgut are the rule, it is possible that Shoshina was, indeed, dealing with a *Leishmania* which will infect humans. Her assumption that hindgut infections indicate

posterior transmission (to whatever vertebrate host) is not necessarily the case. We have observed in our dissections that leptomonad flagellates are not released from the anus of dissected-out guts into the saline in any great number even in heavy hindgut infections. Only once, from one of the man-biting species, a female *trapidoi*, did we observe a few flagellates in the saline, and in this case the parasites appeared to be crithidial in nature. As evidence that flagellates in unbroken, dissected-out, sandfly guts may pass through the anus into the saline, specimens of *vespertilionis* and *trinidadensis* infected in the hindgut with trypanosomes and crithidiae usually do shed flagellates in large numbers into the surrounding saline. Also the general growth pattern of leptomonads in Panamanian sandflies seems to be forward once the infection has been established in the hindgut, and free leptomonads are not common in the posterior part of the hindgut.

In favor of the possibility of posterior station transmission it must be added that by an unknown mechanism, living, undamaged sandflies can propel with considerable force a drop of liquid which has come from the anus and which is held by the cerci. We do not know at what point in the gut this event is initiated. If the process begins anterior to or at the rectal ampulla (we have seldom seen free leptomonads posterior to the rectal ampulla), it is probable that available free flagellates would be forced out, willy-nilly, with the fecal drop.

The feeding state of wild-caught females. An important factor in the transmission potential of a sandfly species is the number of times a female may be expected to feed. Unlike the Old World species, *papatasi*, which is associated with transmission of oriental sore in certain areas, our species usually lay the eggs produced by one blood meal before taking another, so that Panamanian man-biting sandflies have fewer chances to acquire or transmit an infection of *Leishmania* during their lifetime than is true of *papatasi*.

We do not know whether major differences occur in Panamanian sandfly species in the adult life-expectancy or the number of times a female will take a blood meal. At the present time it cannot always be told which nonblooded wild flies caught while coming to animal bait have fed previously. At times the proportion of previously fed flies must be very high. We assume leptomonad-infected females have fed at least once, and in one instance more than 40% of a single catch of *trapidoi* were found infected, indicating that at least that proportion of the catch was attempting to take a second or perhaps a third or fourth blood meal.

Follicular relics in the ovaries indicate that a batch of eggs has been laid; with the exception of *gomezi*, which sometimes reproduces autogenously, such relics indicate an earlier blood meal. Unfortunately, easily visible follicular relics are not always present in females which have laid eggs. In the laboratory 3 or 4 days after egg laying all obvious evidence usually has disappeared from the ovaries. Since time is of essence in our dissecting schedule we have not applied refined methods of ovarial examination which might reliably indicate whether prior oviposition has occurred.

The presence of granules in the lumen of the accessory glands of the ovaries is said to give a fair indication that a female sandfly has oviposited previous to examination. Lewis and Minter (1960) gave a review of the Old World literature on this subject. The same authors suggested that in most nongravid individuals of East African species the presence of granules in the accessory glands indicates prior oviposition. Since their observations were made on wild-caught flies, we assume that their statement is based on corroborative evidence offered by the presence of follicular relics in the flies which had granules in the accessory glands. In labora-

tory-reared Panamanian sandflies granules may be present or absent with no regard to the parous or nulliparous condition of the female. That the same is true of wild-caught females seems evident since accessory gland granules may be abundant, scarce, or absent in leptomonad-infected females. Thus an examination of the condition of the accessory glands does not aid in determining whether females of a Panamanian species of *Phlebotomus* have or have not fed previously.

Taking the presence of leptomonads as evidence of a previous blood meal, certain conditions of the gut and its associated structures in infected flies may give indications of the physiological state. Often the secretory cells of the Malpighian tubules of infected females are mainly hyaline with granular material present only along the basement membrane, and the lumen is small, clear, and lacking granular material. We assume that the eggs are laid about 4 or 5 days after a previous meal, as in the laboratory; that the wild female almost immediately seeks a new blood meal; and that Malpighian tubules in the condition described are probably found in flies 4 or 5 days after a blood meal. We have observed that almost immediately after a full blood meal wild-caught females discharge the accumulation of a granular material from the Malpighian tubules. Probably a build-up of waste material in the secretory cells follows the discharging state, resulting first in large partly hyaline cells, followed by formation of granular waste within the entire cell, the eventual extrusion of such granules to the enlarging lumen, and, completing the cycle, final discharge of the granules at the time of a new blood meal. This process is not matched in laboratory-reared females. In laboratory-reared females, hyaline excretory cells are not common in individuals of any age or feeding state. Forty-five laboratory-reared *sanguinarius* which had fed 5–12 days previous to dissection lacked any partly hyaline cells in the Malpighian tubules. Four females in the same group had some of these cells partly hyaline, and no females had a majority of the secretory cells partly hyaline. Laboratory-reared females usually die after the first oviposition, and one might speculate that a physiological deficiency in waste disposal could be a contributing cause.

In a small number of cases the cytoplasm of the midgut wall was filled with large, round, refractile globules or granules (Fig. 6). These individuals were mostly *gomezi* with an occasional *trapidoi* or *ylephiletor*. Furthermore, nearly all such specimens were infected with flagellates. (This led us to take this phenomenon as a sign of probable infection, which was usually confirmed on further examination.) It was later found that similar globules appear in the midgut wall of laboratory-reared *gomezi*, but not *sanguinarius*, about 3 days after feeding in the laboratory on various animals (marsupials, rodents, carnivores) and usually disappear 6 or 7 days after feeding. These cytoplasmic inclusions in wild-caught sandflies thus probably indicate a recent blood meal, but we have no explanation for the disproportionately high infection rate in females exhibiting this phenomenon.

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