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EXPLANATION OF PLATES

Plate 1

Skin appearance in forest mammals naturally infected with dermatotropic species of *Leishmania*.

A. Two-toed sloth, *Choloepus hoffmanni*, infected with *L. braziliensis*. The parasite was consistently isolated in culture from sound skin mainly from the nose, jaw and ears.

B. Tropical porcupine, *Coendou rothschildi*. This rodent showed a high infection rate due to *L. hertigi*. The parasite was found throughout the skin without any macroscopic skin alteration.

C. Incrusted lesions at the border of both ears (arrows) in an arboreal spiny rat, *Diplomys labilis*. A dermatotropic species of *Leishmania*, not identified as yet, was isolated in culture from the right ear on the 6th and 30th days after its capture; simultaneous cultures from the other ear yielded negative results. The lesions from both ears almost disappeared during the time (1 month) the animal was maintained alive in the laboratory.

D. Small, rounded and depigmented areas on the lower aspect of right ear in an olingo, *Bassaricyon gabbii*, from which *L. braziliensis* was cultured.

E. Active lesion due to *L. mexicana* at the base of the tail of a rice rat, *Oryzomys capito*. Initially the lesion was prominent, whitish in colour, denuded of hair and covered by scaly-like crusts.

F. Typical scar of leishmanial etiology on the tail of the rice rat. The scars are conspicuously depigmented, depressed (arrow) and usually located near the base of the tail.

G. Ulcerated skin lesion at the base of the tail of a brown murine opossum, *Marmosa robinsoni*, from where *L. mexicana* was observed in skin smears. The parasite was isolated in culture twice during the period of 24 days the animal was maintained alive in the laboratory. The lesion was rounded in outline and 7 mm in diameter, and resembled human ulcerated lesions produced by *L. braziliensis* in Panama.

MATERIALS AND METHODS

Sixteen species of blood-engorged mosquitoes were collected in Panama from 10 October 1969 through 6 June 1971. Of these, 7 were collected in sufficient numbers to give some baseline information as to their host preferences. These species were as follows: *C. aikenii*, *Culex* (*M.*) *egcymon* Dyar, *Culex* (*M.*) *tecmarsis* Dyar, *Culex* (*M.*) *dunni* Dyar, *Culex* (*M.*) *elevator* Dyar & Knab, *Culex* (*M.*) *epanastasis* Dyar (*crybda* Dyar) and *Culex* (*Aedinus*) *amazonensis* (Lutz). The main areas of collection were Juan Mina, on the Atlantic watershed of the Canal Zone and the Tocumen swamps, located about 24 km east of Panama City, Republic of Panama. The area of Juan Mina has been used by the Gorgas Memorial Laboratory for more than 30 years as a field station, while the Tocumen swamps, also, have been utilized for many years as a study site for entomologists of the same institution.

Juan Mina is located on the left bank of the Chagres River near the Transisthmian Highway that joins the cities of Panama and Colon. The area contains an old abandoned citrus orchard, which during the study was being used as a pasture for grazing about a dozen cattle.

The Chagres River in this area is a wide, deep, meandering river with dense growths of aquatic plants along the sunlit edges. The numerous inlets, channels and oxbow lakes are deeply shaded along the shallow edges by palm and swamp trees with deep buttresses. Beyond the shore line of the river, the contour of the land rises abruptly, forming hills several hundred meters in height. These hills are mostly covered with evergreen, broad-leaved forest intermediate between tropical rain and deciduous forest.

Several mosquitoes breed in masses of aquatic vegetation. Those found breeding in this vegetation during this study were *C. aikenii*, *C. dunni* and *C. egcymon*. *Culex elevator* was usually found in shaded, slowly running waters, along the edges of streams.

Along the marshy edges are numerous rodent burrows. In the flooded galleries of some of these burrows immature stages of *C. epanastasis* were found.

In the Juan Mina area, 2 collecting stations provided the great majority of the engorged specimens. Both are located on the right bank of the river and are about 1.5 km apart.

Included among the common wild mammalian fauna found in this area were bats, monkeys, opossums, sloths, armadillos, squirrels, the Hispid Cotton Rat (*Sigmodon hispidus*) and the Tomes' Spiny Rat (*Proechimys semispinosus*). The domestic mammals were represented principally by dogs and bovines, as well as man.

Birds found included representatives from 19 different orders. Water birds were especially numerous. The most common avian groups included Ciconiiformes (egrets and herons), Gruiformes (rails, gallinules), Charadriiformes (jacanas), Columbiformes (doves) and several species of Passeriformes. A few domestic birds (chickens, ducks) were present in the area.

Engorged mosquitoes were collected at Juan Mina from 8:00 AM to 12 noon every day by disturbing natural resting places such as tree buttresses, base of palm fronds, animal burrows and emergent vegetation. Mosquitoes disturbed were collected by means of battery-run vacuum cleaners or midge nets. Engorged mosquitoes were identified, placed individually into gelatin capsules, and frozen at −20 °C within 30 hr of collection. When a sufficient number of mosquitoes was accumulated, they were packed in dry ice and sent in insulated shipping boxes to the University of California by air express.

Supplemental collections were made at Tocumen and Almirante (Grayson & Galindo 1968). The Tocumen swamps form part of the extensive coastal freshwater swamps, which extend for some 48 km from the ruins of Old Panama east to the mouth of the Bayano river. These swamps are formed by a maze of oxbows, sloughs and channels of stream deltas which precipitate with force onto the lowlands from the mountains of the Cerro Azul range, spilling over from their natural courses during the rainy season, which extends from May to December. The landscape of these flatlands is made up of extensive grasslands used as pasture for cattle, intermingled with gallery forests along the main streams and cut off by mangrove swamps as the salinity of the water increases near the seacoast. The swamps are choked with a variety of aquatic plants such as *Pistia*, *Eichhornia*, *Hydrilla*, *Salvinia*, *Nymphea*, etc., which either form extensive pure associations or mixed communities with little or no dominance. A large variety of mosquitoes breed in the swamps, including many species of *Melanoconion* and allied subgenera. Of the species reported herein, the most common ones in the area were *C. aikenii*, *C. amazonensis* and *C. tecmarsis*.

Bovines were present in the Tocumen swamps in large numbers on some collecting days, and at other times were completely absent. Other mammals were scarce with the exception of rodents, both

cricetids and echimyids, marsupials, lagomorphs and the Three-toed Sloth (Edentata). Birdlife was abundant, dominated by columbiforms, anis and aquatic groups, such as herons, egrets and jacanas. Of the Passeriformes, there was abundance of flycatchers, and seed-eating fringillids. Iguanas, ameiva lizards, turtles and caimans were the commonest reptiles, while toads and frogs were also very common.

The testing procedure was the same as that reported earlier (Tempelis & Galindo 1970). This varied from our usual procedures in that all blood meals were suspended in 0.75 ml phosphate-buffered saline. Initial tests were done with 3 antisera: a broadly reacting antiserum for mammals, a broadly reacting antiserum for birds and a specific antilizard serum. Those positive for either the mammal or bird antiserum were further tested with more specific antisera. These antisera were chosen on the basis of species abundance in the collecting areas.

Blood meals that failed to react in the initial test procedure were tested further with the benzidene reagent for the detection of blood. If blood was detected, further testing was done with antisera to Lagomorpha, Marsupilia and various poikilothermic animals.

RESULTS

Approximately 3000 blood meals from 7 species of *Culex* mosquitoes collected in Panama were identified. The results are summarized in TABLE 1. *Culex aikenii* showed considerable versatility in feeding pattern. It fed equally well on either mammals or birds. Among the mammals, significant numbers fed on echimyid rodents (20.2%) which were represented principally in the collecting areas by the spiny rat. Water birds, especially the Ciconiiformes (herons and egrets), were the primary hosts in the avian group. Significant numbers of mammal (10.5%) and avian hosts (12.8%) remained unidentified. Cold-blooded animals, principally lizards, also served as hosts.

Three species, *C. egcymon*, *C. tecmarsis* and *C. elevator*, fed predominantly on lizards, while the

TABLE 1. Feeding patterns of 1 *Culex* (*Aedinus*) and 6 *Culex* (*Melanoconion*) species collected in the Republic of Panama, 1969–1971.

	Mosquito species													
	Culex (*M.*) *aikenii*		*Culex* (*M.*) *egcymon*		*Culex* (*M.*) *tecmarsis*		*Culex* (*M.*) *dunni*		*Culex* (*M.*) *elevator*		*Culex* (*M.*) *epanastasis*		*Culex* (*A.*) *amazonensis*	
Total no. of mosquitoes tested	2378		633		390		133		200		105		211	
	No.	%*	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Hosts														
Mammalia														
Bovine	168	9.6	1	0.2			3	3.9			2	2.3	12	7.7
Sloths	23	1.3									4	4.7		
Man	26	1.5									1	1.2	2	1.2
Cricetidae rodent	89	5.1			1	0.4					2	2.3	102	65.0
Echimyidae rodent	352	20.2	71	14.0			2	2.7			1	1.2	5	3.2
Bat	7	0.4					1	1.3					5	3.2
Opossum	10	0.6									1	1.2	1	0.6
Other	29	1.6									3	3.5		
Unknown	184	10.5			1	0.4	1	1.3			8	9.3	11	7.0
Subtotal	888	50.8	72	14.2	2	0.8	7	9.2			22	25.7	138	87.9
Aves														
Ciconiiformes	359	20.6	3	0.6	9	3.3	6	7.9	2	1.5	12	13.9	2	1.3
Passeriformes	16	0.9	5	1.0	6	2.2	6	7.9			8	9.3		
Gruiformes	108	6.2			1	0.4	1	1.3						
Other	3	0.2									1	1.2	1	0.6
Unknown	224	12.8	7	1.4	4	1.5	4	5.3	3	2.3	16	18.6	1	0.6
Subtotal	710	40.7	15	3.0	20	7.4	17	22.4	5	3.8	37	43.0	4	2.5
Reptilia														
Lizard	105	6.0	410	80.7	239	88.8	48	63.2	117	90.0	21	24.4	10	6.4
Turtle	29	1.7												
Other											2	2.3	2	1.3
Amphibia														
Toad	5	0.3	9	1.7	1	0.4	3	3.9			2	2.3	3	1.9
Frog	9	0.5	2	0.4	7	2.6	1	1.3	7	5.4	2	2.3		
Other	1	0.1							1	0.8				
Total	1747	100.0	508	100.0	269	100.0	76	100.0	130	100.0	86	100.0	157	100.0

*Percentage based on total identified feedings.

majority of *C. dunni* fed on lizards (63.2%). *Culex dunni* also fed in fairly large numbers on birds (22.4%) and mammals (9.2%). Only *C. elevator* of these 4 species did not feed on any mammals.

Culex epanastasis had a catholic feeding pattern. It fed readily on both warm and cold-blooded hosts with a preference for birds.

Only *C. amazonensis* showed an almost complete preference for mammals, with 87.9% of the blood meals from this group. Most, 65.0%, fed on cricetid rodents, probably hamsters, which were used as sentinel animals, or the cotton rat which was common in the Tocumen swamps.

DISCUSSION

A recent report on the feeding habits of several *Deinocerites* mosquitoes indicated a broad host range among 3 species of these principally Neotropical mosquitoes (Tempelis & Galindo 1970). The broad host range included homoiothermic as well as poikilothermic vertebrates. This feeding versatility had not previously been reported for any species of mosquitoes. In the present report, several species of *Culex* (*Melanoconion*) and 1 *Culex* (*Aedinus*) demonstrate a similar broad host range.

Of the 6 species of *Melanoconion* and 1 *Aedinus* whose host ranges have been studied, *C. aikenii* is the most important, as it has been shown to be the main endemic vector of VEE virus in the Chagres River drainage of the Republic of Panama (Galindo & Grayson 1971). In the present study, *C. aikenii* demonstrated an almost equal feeding choice for mammals and birds. Within these 2 major vertebrate classes some host preferences were indicated.

Among the mammals studied, 20.2% of the identified blood meals indicated *C. aikenii* feeding on echimyid rodents, and among the birds, 20.6% of the mosquitoes fed on members of the order Ciconiiformes. The major echimyid rodent found in the area of Juan Mina was the spiny rat. Rodents have been shown to be primary hosts in the maintenance of endemic VEE virus in nature (Grayson & Galindo 1968, Jonkers et al. 1968, Chamberlain et al. 1969). Several rodent species have been implicated, including the spiny rat. In the study reported by Grayson & Galindo (1968) on the epidemiology of VEE virus in the Almirante, Panama area, it was shown that the spiny rat followed by the cotton rat had the highest incidence of hemagglutination-inhibiting antibody of all the vertebrate species tested. Both rodents were found in the collecting areas of the present study; however, the spiny rat was predominant. In the collecting area where most of the feedings on cricetid rodents were demonstrated, hamsters were used as sentinel animals; some of these blood meals assigned to cricetids may refer to cotton rats.

The significance of the high rate of *C. aikenii* feeding on birds, especially the Ciconiiformes, and its relationship to the epidemiology of endemic VEE is not known. Some investigators have suggested that compared with mammals, birds do not seem to play a major role in the ecology of this disease, but it is of interest to note that VEE virus has been isolated from the Green Heron, *Butorides virescens*, in Panama (Galindo et al. 1966). Further, hemagglutinating and neutralizing antibodies have been found in other Ciconiiformes in Mexico (Dickerman et al. 1972). Recent experiments demonstrate that the Striated Heron, *Butorides striatus*, is capable of circulating significant titers of VEE virus for several days following subcutaneous inoculation of virus (Grayson 1972) and that such titers are high enough to infect *C. aikenii* feeding on the viremic birds. The mosquitoes, in turn, replicate the virus and transmit the infection when allowed to feed on clean herons and hamsters after an incubation period of less than 2 weeks (P. Galindo, M. A. Grayson & A. J. Adames, unpubl.). In view of these facts and if herons, like rodents, serve as hosts for *C. aikenii* as frequently as reported here, these birds also may play a role in the ecology of the endemic cycle of VEE. A reason for the apparently obscure role of birds in the epidemiology of VEE virus may be related to the inability to secure good samplings of these vertebrates which would provide the necessary serologic and virologic data (Dickerman 1972). The feeding pattern of *C. aikenii* presented here, plus the data summarized above, indicates the necessity to reevaluate the role of certain bird species, particularly herons and egrets, in the natural transmission and possible dissemination of VEE virus. The migratory habits of some species like the Green Heron and Little Blue Heron, *Florida caerulea*, and the mass movements and population displacement of the Cattle Egret, *Bubulcus ibis*, recently introduced into the Western Hemisphere from Africa, lend themselves potentially to the wide distribution of VEE virus.

Almost 25% of the *C. aikenii* blood meals were not identified to family or order. There were numerous birds and mammals in the collecting areas for which we had no specific antisera, due to the difficulty in obtaining sufficient quantities of sera from them. These included various carnivores, Charadriiformes (jacanas), and Cuculiformes (anis), all 3 common groups in the area.

It is apparent from these data and those previously

reported on the feeding habits of *Deinocerites* that several mosquito species in Panama feed preferentially on cold-blooded animals. Three species of *Melanoconion* mosquitoes, *C. egcymon*, *C. tecmarsis* and *C. elevator*, fed almost exclusively on reptiles, while 1 other, *C. dunni*, apparently feeds on reptiles the majority of the time; the latter species also fed readily on birds and mammals.

These data further indicate that at least some groups of Neotropical mosquitoes have more extensive host ranges than those found among Nearctic groups.

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