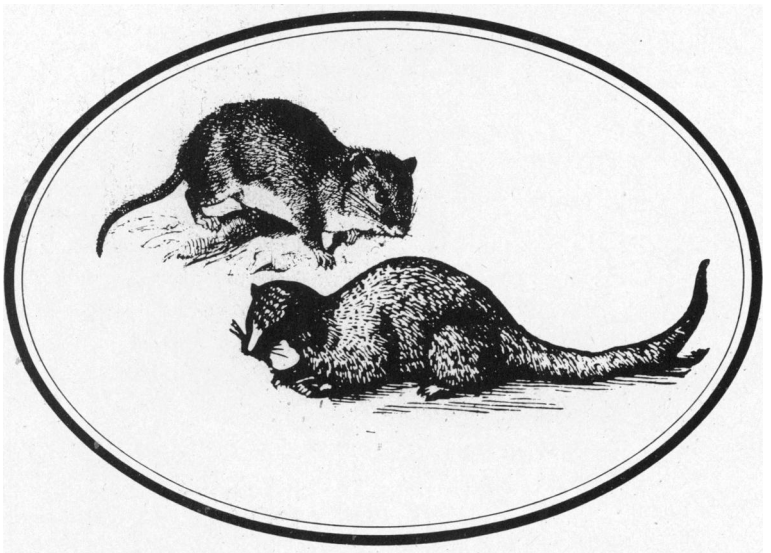
Prevalence of Rodent and Mongoose Leptospirosis on the Island of Oahu



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LEPTOSPIROSIS IS NOT COMMON in the continental United States (*1*), but in the State of Hawaii it occurs fairly frequently. In the period 1966–70, for example, 47 human cases of the infection were reported in the State, one of which resulted in death (*2*). The disease in man, an accidental host, may vary from an icterogenic type with severe kidney and liver involvement to a mild catarrh-like illness of short duration (*3*). Rodents, and possibly mongooses, appear to be associated with human leptospiral infections, and therefore a number of surveys of these animals have been undertaken. We conducted such a survey on the Island of Oahu from December 1970 to June 1973. There had also been earlier studies in the Hawaiian Islands.

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Previous Studies

In 1943, Alicata and Breaks (*4*) took serological samples of rats, mongooses, and humans in Honolulu, on Oahu, to determine the presence of leptospirosis. Of the 344 human serums they tested, 12 were positive for *Leptospira icterohaemorrhagiae,* and 1 was positive for *Leptospira canicola.* Thus, 3.78 percent of the 344 persons tested were infected. Of 97 rats they trapped along freshwater streams, 8.2 percent were infected;

of 12 mongooses they trapped along the Nuuanu stream on Oahu, 4 showed leptospires. In further work by Alicata (*5*) in 1944 among 860 plantation workers on the Island of Hawaii, 105 workers, or 12.2 percent, had positive reactions. A 1964 report by Minette (*6*) on leptospirosis in rodents and mongooses on the same island provides data comparable to those of Alicata.

Study Area

Oahu is one of the eight main islands of the Hawaiian archipelago (*7*). The island comprises 589 square miles of the 6,406 that constitute the eight main islands. According to the State Department of Planning and Economic Development's 1973 census, 881,000 persons lived in the State of Hawaii. Honolulu County accounted for 678,124 of these persons, and the city of Honolulu—as of 1970—accounted for 324,871.

Much of the island is rugged and mountainous; peaks rise to an average elevation of approximately 4,000 feet above sea level. There are plateaus between the mountains in the central region and also along the coasts. Because of the northwesterly trade winds, the climate is mild, and the temperature changes little over the seasons. In Honolulu, on the leeward side of Oahu, the mean temperature for August, the warmest month, is 78°F.; for January, the coldest month, it is 71°F.

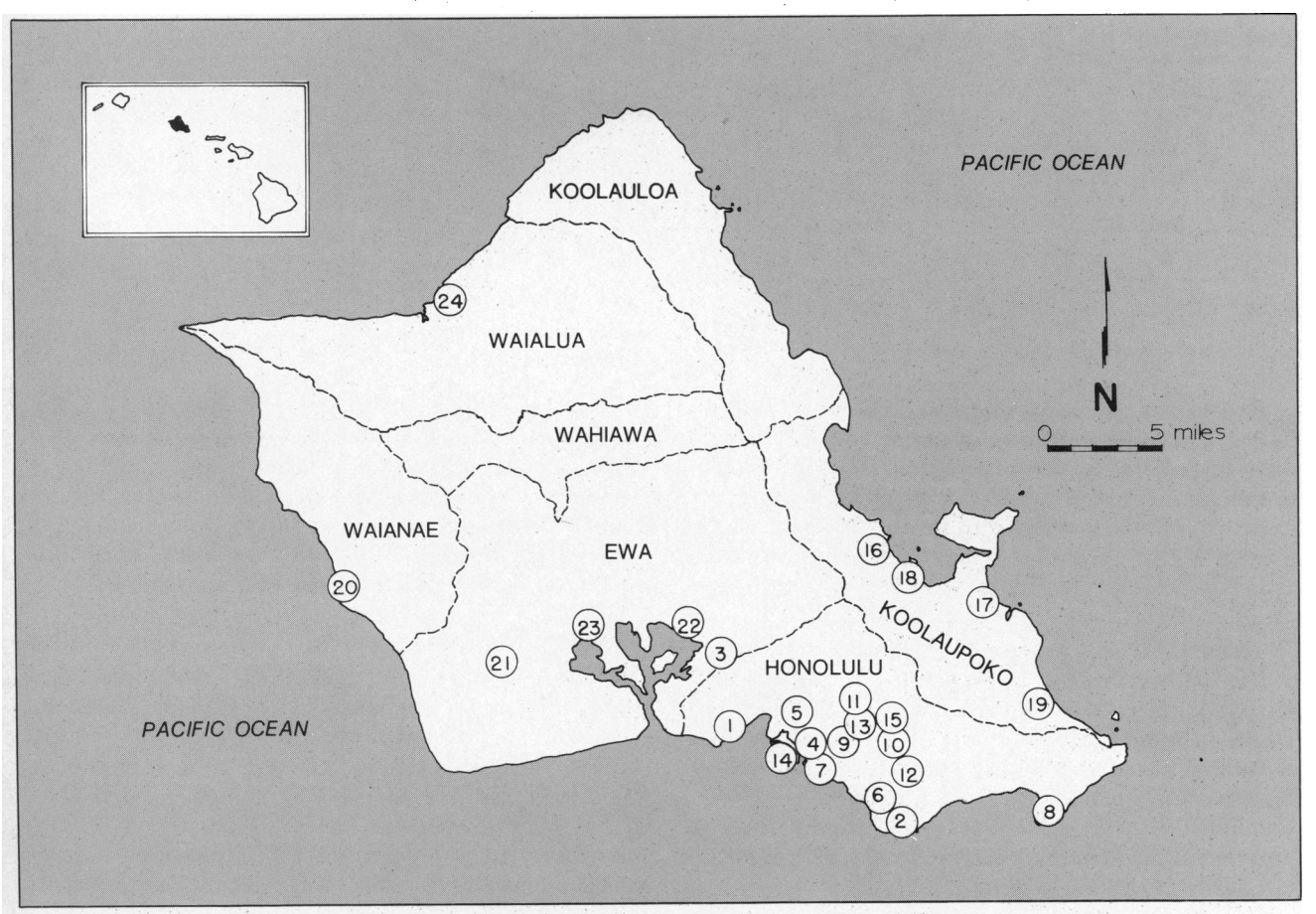
Rainfall varies with the topography. Mountainous and valley areas average heavy rainfalls of approximately 100 inches annually, in contrast to the lowlands (near sea level) which average only 20 to 30 inches.

Methods

The first step in our survey was to trap the animals for study. In urban areas, we conducted our trapping along streams, in vacant lots, and in residential properties. In rural areas, the trapping sites at the Kaneohe location in the Koolaupoku District were in the mountains, while the one at Waimalu was in a valley. Traps were set in and around pig farms, dairies, dumping sites, and in vacant lots near human dwellings.

The numbers on the map indicate our rodent and mongoose trapping locations. The number of rodents and mongooses trapped alive at each location is shown in table 1. Table 2 categorizes the various species trapped. As the map shows, trapping was concentrated in the urban Honolulu district. The other trapping sites were rural; at the inland sites of the Honolulu dis-

Map of Oahu showing districts and trapping locations



trict, they were near mountains and valleys, the areas with the most fresh water from either streams or rainfall. These wet areas were: Halawa (valley), Makiki (mountain), Manoa (valley), Nuuanu (valley), Palolo (valley), and Tantalus (mountain).

To capture the animals alive, we used small animal cages for traps—one trap (*A*) for the rodents and a different kind (*B*) for the mongooses. We anesthetized the animals, bled them from the heart, and removed both kidneys aseptically for culturing. The blood serums were used in the macroscopic slide agglutination test to detect and identify leptospiral antibodies.

The leptospires were cultured by first grinding the kidneys in sterile mortars with pestles. The ground tissue was mixed with sterile phosphate-buffered saline (*8*), pH 7.5, to give a 10 percent tissue suspension and was then cultured in a Difco-supplemented Ellinghausen, McCullough, Johnson, Harris (EMJH) medium (*9,10*), containing 0.20 percent agar. The antibacterial agent 5-fluorouracil (*11*) was incorporated into the supplemented basal medium at 100 microgram (ug) per milliter of concentration to prevent contaminative growth.

Four screw-capped tubes, 16 by 125 mm, each containing 5 ml of medium, were inoculated with 1 drop from each of the 4 dilutions (1:10, 1:100, 1:1,000, 1:10,000) of the kidney suspension from each animal. Both kidneys were used to make the suspension. Approximately 4 to 6 weeks are normally required to obtain visible growth of leptospires, which usually appear as a ring within 1 cm of the medium's surface in the tube. The cultural growth was examined for leptospires by darkfield microscopy.

In serotyping isolated leptospires, the isolated kidney leptospiral cultures were transferred weekly, twice in an EMJH medium containing agar and, also, at least twice in an EMJH medium containing no agar. These weekly transfers help prevent the formation of "breeding nests" (*8*), or groupings of leptospires, and insure a uniform leptospiral culture. The cultures were serotyped by the microscopic agglutination method (*8*).

Table 1. Prevalence of rodent and mongoose leptospirosis at various locations on Oahu

District and location of trapping sites[1]	Rodents			Mongooses		
	Number examined	Number positive	Percent positive	Number examined	Number positive	Percent positive
Honolulu:						
Airport (1)	23	1	4.3	0	...	...
Diamond Head (2)	29	0	0	26	2	7.7
Halawa (3)	0	...	...	33	4	12.1
Harbor (4)	79	3	3.8	0	...	...
Kalihi (5)	44	7	15.9	0	...	...
Kapahulu (6)	17	0	0	0	...	...
Kewalo (7)	11	0	0	0	...	...
Koko Head (8)	10	1	10.0	0	...	...
Makiki (9)	16	7	43.8	0	...	...
Manoa (10)	16	6	37.5	0	...	...
Nuuanu (11)	167	41	24.6	14	2	14.3
Palolo (12)	196	73	37.2	15	3	20.0
Pauoa (13)	21	12	57.1	0	...	...
San Island (14)	22	3	13.6	0	...	...
Tantalus (15)	23	11	47.8	0	...	...
Koolaupoko:						
Kahaluu (16)	18	2	11.1	0	...	...
Kailua (17)	27	6	22.2	28	9	32.1
Kaneohe (18)	77	27	35.1	36	11	30.6
Waimanalo (19)	0	...	...	26	10	38.5
Waianae:						
Maili (20)	0	...	...	22	2	9.1
Ewa:						
Makakilo (21)	24	4	16.7	0	...	...
Waimalu (22)	0	...	...	19	2	10.5
Waipahu (23)	25	2	8.0	0	...	...
Waialua:						
Haleiwa (24)	0	...	...	63	20	31.7
Total	845	206	24.4	282	65	23.0

[1] Numbers in parentheses refer to trapping areas shown on map.

Table 2. Prevalence of leptospirosis among rodent species and the mongoose according to locations on Oahu

District and location of trapping sites [1]	Total examined	Rattus rattus [2]			Rattus norvegicus [2]			Rattus exulans [2]			Mus musculus [2]			Herpestes auropunctatus [2]		
		Number examined	Number positive	Percent positive	Number examined	Number positive	Percent positive	Number examined	Number positive	Percent positive	Number examined	Number positive	Percent positive	Number examined	Number positive	Percent positive
Honolulu:																
Airport (1)	23	22	1	4.5	0	.....		0	.....		1	0	0	0	.....	
Diamond Head (2)	55	28	0	0	0	.....		1	0	0	0	.....		26	2	7.7
Halawa (3)	33	0	.....		0	.....		0	.....		0	.....		33	4	12.1
Harbor (4)	79	69	3	4.3	9	0	0	0	.....		1	0	0	0	.....	
Kalihi (5)	44	32	5	15.6	11	2	18.2	1	0	0	0	.....		0	.....	
Kapahulu (6)	17	13	0	0	3	0	0	0	.....		1	0	0	0	.....	
Kewalo (7)	11	11	0	0	0	.....		0	.....		0	.....		0	.....	
Koko Head (8)	10	10	1	0	0	.....		0	.....		0	.....		0	.....	
Makiki (9)	16	12	4	33.3	1	1	...	3	2	...	0	.....		0	.....	
Manoa (10)	16	15	5	33.3	0	.....		1	1	...	0	.....		0	.....	
Nuuanu (11)	181	136	36	26.5	7	1	...	24	4	16.7	0	.....		14	2	14.3
Palolo (12)	211	152	49	32.2	21	14	66.6	23	10	43.5	0	.....		15	3	20.0
Pauoa (13)	21	16	10	62.5	2	2	...	3	0	0	0	.....		0	.....	
Sand Island (14)	22	22	3	13.6	0	.....		0	.....		0	.....		0	.....	
Tantalus (15)	23	22	11	50.0	1	0	0	0	.....		0	.....		0	.....	
Koolaupoko:																
Kahaluu (16)	18	15	1	6.7	1	0	0	2	1	...	0	.....		0	.....	
Kailua (17)	55	23	5	21.7	3	1	...	1	0	0	0	.....		28	9	32.1
Kaneohe (18)	113	68	25	36.8	5	1	...	2	0	0	2	1	...	36	11	30.6
Waimanalo (19)	26	0	.....		0	.....		0	.....		0	.....		26	10	38.5
Waianae:																
Maili (20)	22	0	.....		0	0	...	0	.....		0	.....		22	2	9.1
Ewa:																
Makakilo (21)	24	3	0	0	0	.....		0	.....		21	4	19.0	0	.....	
Waimalu (22)	19	0	.....		0	.....		0	.....		0	.....		19	2	10.5
Waipahu (23)	25	23	2	8.7	2	0	0	0	.....		0	.....		0	.....	
Waialua:																
Haleiwa (24)	63	0	.....		0	.....		0	.....		0	.....		63	20	31.7
Total	1,127	692	161	23.3	66	22	33.3	61	18	29.5	26	5	19.0	282	65	23.0

[1] Numbers in parentheses refer to trapping areas shown on map.

[2] *Rattus rattus*—roof rat. *Rattus norvegicus*—Norway rat, *Rattus exulans*—Hawaiian rat, *Mus musculus*—mouse, *Herpestes* auro-*punctatus*—mongoose.

NOTE: (. . .) No value; insufficient examinations.

We used the 'box" titration procedure, in which two-fold dilutions (1 to 25 and 1 to 800) of known antiserums were prepared in horizontal rows, and then suitable isolated live leptospiral cultures were added in respective vertical rows. Antiserums from 12 leptospiral serotypes, as recommended by Galton and associates (*8*), were used, namely *ballum, canicola, icterohaemorrhagiae, bataviae, grippotyphosa, pyrogenes, autumnalis, pomona, sejroe, australis, hyos,* and *mini-georgia.*

The tubes were shaken and incubated at 30°C. for 3 hours and examined for agglutination by dark-field microscopy. To confirm our results, selected isolated leptospiral cultures were sent to the Leptospirosis Reference Laboratory, Center for Disease Control, Atlanta, Ga., where agglutination-absorption studies were performed.

The slide agglutination test was used to determine whether the rodent or mongoose had been exposed to pathogenic leptospires. The procedures (*8*) consisted of screening the serum with four pooled formalinized antigen preparations. The pools of *Leptospira* serotypes were: pool 1—*ballum, canicola,* and *icterohaemorrhagiae;* pool 2—*bataviae, grippotyphosa,* and *pyrogenes;* pool 3—*autumnalis, pomona,* and *sejroe;* pool 4—*australis, hyos,* and *mini-georgia.*

The serum to be tested was mixed with one drop of its respective pooled antigen (0.055 ml) on a ruled glass plate. This mixture was hand-rotated five or six times and then placed on a mechanical rotator for 4 minutes at 125 rpm. The results were then read over an illuminated background. A serum and pool that showed a positive reaction was further tested with the individual antigens of the pool for identification. A

titer was obtained by serum dilution and titration against selected antigens. If cross-reactions with two or more serotypes occurred, the antigen giving the highest titer was presumed to be the infecting serotype. We confirmed positive reactions by the previously described microscopic agglutination test, using known live antigens of the serotype that had reacted in the slide agglutination test.

Results

As table 1 shows, 845 rodent kidney cultures were studied from December 1970 to June 1973, and 206 (24.4 percent) were positive. Of 282 mongoose kidney cultures examined, 65 were positive, a prevalence rate of 23 percent. The highest prevalence of rodent infections was in the wet areas of Oahu, in Kaneohe, Makiki, Manoa, Palolo, Pauoa, and Tantalus. The mean annual rainfall in inches for 1970, 1971, and 1972 for these areas was Kaneohe 72.41, Makiki 40.00, Manoa 95.83, Palolo 111.61, Pauoa 155.06, and Tantalus 107.56 (*12*). Infected mongooses were most often found in Haleiwa, Kailua, Kaneohe, and Waimanalo.

Table 2 shows the number of animals positive for leptospirosis among various rodent species and the mongooses by their locations. The results for Palolo are of interest. Since at this site, all animals except the mouse (*Mus musculus*) are represented, the rate of infection for each species could be compared. The following infection rates were observed: *Rattus norvegicus* 66.6 percent, *Rattus exulans* 43.5 percent, *Rattus rattus* 32.2 percent, and *Herpestes auropunctatus* 20 percent. To include the mouse in our comparison, we used data from the Makakilo site in the Ewa district. These data showed a 19 percent infection rate. Therefore, it was obvious that the Norway rat posed the greatest threat as a carrier of leptospirosis, followed by the Hawaiian rat.

The cultures of leptospires isolated from rodent and mongoose kidneys were identified by the microscopic agglutination test. This test revealed (table 3) that *L. icterohaemorrhagiae* accounted for 72.2 percent of the leptospiral infections in *R. norvegicus*. More than 70 percent of the leptospiral infections in the kidneys of *R. rattus, R. exulans,* and *M. musculus* were caused by *L. ballum;* 85 percent of the leptospiral infections in mongooses were caused by *L. sejroe*. Six *L. sejroe* isolates were tested and confirmed by the Center for Disease Control in Atlanta.

Results of the slide macroscopic agglutination test for identifying leptospiral serum antibodies and the

Table 3. Identification of leptospira serotypes isolated from rodents and mongooses of Oahu

Animal species	Number of cultures examined	Leptospira ballum		Leptospira canicola		Leptospira icterhaemorrhagaie		Leptospira sejroe	
		Number	Percent	Number	Percent	Number	Percent	Number	Percent
Rattus rattus	142	100	70.4	0	....	39	27.5	3	2.1
Rattus norvegicus	18	5	27.8	0	....	13	72.2	0	....
Rattus exulans	11	9	81.8	0	....	2	18.2	0	....
Mus musculus	5	5	100.0	0	....	0	....	0	....
Herpestes auropunctatus	60	0	.....	1	1.67	8	13.3	51	85.0

NOTE: (. . .) No value; insufficient examinations.

Table 4. Serological results of rodents and mongooses by the macroscopic slide agglutination test and Ellinghausen, McCullough, Johnson, Harris (EMJH) cultural results

Animal species	Number of serum samples	Screening test results		Antibody identification for leptospiral serotype										Culture test results		
				ballum		canicola		icterohaem-orrhagiae		sejroe		Unidentified				
		Number positive	Percent positive	Number	Percent	Number	Percent	Number	Percent	Number	Percent	Number	Percent	Total number	Number positive	Percent positive
Rattus rattus	551	84	15.2	49	8.9	0	...	26	4.7	9	1.6	0	....	551	106	19.2
Rattus norvegicus	50	12	24.0	3	6.0	0	...	8	16.0	1	2.0	0	...	50	12	24.0
Rattus exulans	44	6	13.6	4	9.1	0	...	2	4.5	0	...	0	...	44	10	22.7
Mus musculus	2	1	...	1	...	0	...	0	...	0	...	0	...	2	1	...
Herpestes auropunctatus	241	53	22.0	4	1.7	2	0.8	17	7.1	29	12.0	1	0.4	241	59	24.5
Total	888	156	17.6	.....										888	188	21.2

NOTE: (. . .) No value; insufficient examinations.

corresponding number of positive kidney culture tests are shown in table 4. Titers of 1 to 64 and greater were considered positive. The predominant antibody in *R. norvegicus* was *L. icterhaemorrhagiae* (16 percent), while in *R. rattus* and *R. exulans, L. ballum* predominated, with percentages of 8.9 and 9.1. Twelve percent of the mongooses examined showed antibody reactions to *L. sejroe*. Table 4 also shows that for the identical animals examined, there were 188 positive culture tests and 156 serologic positives.

In the macroscopic slide agglutination test, in which formalinized antigens are used to identify serum antibodies, nonspecific clumping of some prepared antigen lots sometimes occurs and may give rise to false-positive interpretations (*13*). This clumping may be eliminated by vigorously shaking the antigen before use. It is imperative that suitable positive and negative controls be included in the tests.

Discussion

A high prevalence of leptospiral infections among rodents was found in the wet areas of Oahu, that is, in areas with considerable amounts of fresh water. Such areas are presumably conducive to an increased rat population (per unit area) as well as to the survival and transmission of the leptospiral infection. A higher rat population favors more contact between rats, and moisture is required for prolonged survival of the organism and for its transport. Because the ecological niches of rodents and mongooses are different and data are lacking on the wet and dry areas for the mongoose, we cannot discuss the prevalence of leptospirosis in the mongoose.

A comparison of our data with those from the 1943 study of Alicata and Breaks (*4*) shows that the rate of infection among *R. norvegicus* has been increasing while its numbers have been decreasing. In 1943, the rats in Honolulu trapped in cages had been mostly *R. rattus* (198) and *R. norvegicus* (233) in a ratio of 1 to 1.2. Our study shows (table 2) that for approximately the same number of rats trapped in cages in similar locations (Harbor, Kalihi, Nuuanu, and Palolo), 389 were *R. rattus* amd 48 were *R. norvegicus,* in a ratio of 1 to 0.12. These figures show a tenfold proportional decrease in the numbers of Norway rats to roof rats.

In 1943, only 11 (10.5 percent) of 105 Norway rats in Honolulu had been found to be infected with leptospirosis. In our study, 17 of 98 (35.4 percent) of the Norway rats were infected. This result amounts to an increase of more than 300 percent in leptospiral infections. The ecological niche of the Norway rat evidently favors the occurrence of leptospirosis. Table 3 shows that for *R. norvegicus,* 72.2 percent of the leptospiral infections were caused by *L. icterohaemorrhagiae* and 27.8 percent by *L. ballum.* (*L. icterohaemorrhagiae* may cause severe illness in man, but human infections due to *L. ballum* are relatively few and mild.) In all other rodent species on Oahu—*R. rattus, R. exulans,* and *M. musculus*—the predominant leptospire was *L. ballum.* Thus, on Oahu, the Norway rat is the greatest potential health hazard to man. Table 3 also shows that of 60 leptospiral cultures isolated from mongoose kidneys, 51 (85 percent) were identified as *L. sejroe* and 8 (13.3 percent) as *L. icterohaemorrhagiae.* One culture (1.7 percent) was of *L. canicola.* On Oahu, the mongoose is the preeminent carrier of *L. sejroe,* whereas on the island of Hawaii (*6*), *L. icterohaemorrhagiae* was found to be the predominant organism (76.5 percent) in 17 leptospiral isolates from mongooses. *L. sejroe* had first been isolated in 1937 from a sick fisherman on the island of Sejro, Denmark (*14*). In Denmark, *L. sejroe* has been associated with the field mouse, *Mus musculus spicilegus.* The first reported isolation of *L. sejroe* in the Western Hemisphere was on the island of Hawaii in 1964 (*6*).

When the same animals (*R. rattus, R. exulans,* and *H. auropunctatus*) were examined by the two methods we used to detect infection, a greater number of results were positive by kidney culture than by the slide agglutination test. The reason is that after an infection sets in, the leptospires colonize in the convoluted renal tubules (*15*) and become inaccessible to circulating antibodies. Eventually, the antibody concentration diminishes, but the organism persists. The leptospires are discharged in the urine, and in this carrier state the animal becomes a potential source of infection.

The high prevalence of rodent leptospirosis in certain areas of Oahu presents a potential human health hazard. Further studies of environmental survival of the leptospiral organism without the mammalian body and transmission mechanisms seem indicated. These studies should also concentrate on the role of the mongoose in public health. In those districts in our study with a high number of leptospirosis infections, control measures—such as rodent extermination, institution of better sanitary measures, and dissemination of health education—may be indicated.

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Equipment References

A. Hotei-Ya animal trap. Hotei-Ya, Honolulu, Hawaii.
B. Havahart No. 2 trap. Alcock Manufacturing Co., Ossining, N.Y.

SYNOPSIS

HIGA, HARRY H., and FUJINAKA, IVAN T. (Hawaii Department of Health): *Prevalence of rodent and mongoose leptospirosis on the Island of Oahu. Public Health Reports, Vol. 91, March-April 1976, pp. 171–177.*

Sporadic occurrences of human leptospirosis in recent years throughout the State of Hawaii have resulted in at least one death. Because of the apparent association of rodents and possibly mongooses with human leptospiral infections, a survey for leptospirosis was conducted among rodents as well as mongooses on Oahu. No such work had been recorded since a survey of rodents and mongooses for leptospirosis 31 years ago. In the current work, the prevalence of rodent and mongoose leptospirosis in the districts of Oahu was determined by the kidney-culture method. A serologic study of the rodents and mongooses subjected to kidney culturing was also conducted by use of the microscopic slide agglutination test. There were 1.2 times as many kidney culture results that were positive as serologic results.

High prevalences of rodent leptospirosis were found where there was considerable rainfall or fresh surface water such as from streams. The overall leptospirosis prevalence for rodents was 23.4 percent, and for mongooses it was 23.0 percent. The Norway rat (*Rattus norvegicus*) had the highest infection rate, 33.3 percent, and the predominant (72.2 percent) organism in these infections was *Leptospira icterohaemorrhagiae,* which causes Weil's disease in man. Observations of rodent leptospirosis recorded 31 years ago were compared with results of the current study. The mongoose (*Herpestes auropunctatus*) is the preeminent carrier of *Leptospira sejroe,* a serotype that generally causes a mild form of leptospirosis in man.