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Animal Leptospirosis in Malaya *

1. Methods, Zoogeographical Background, and Broad Analysis of Results

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In recent years leptospirosis has been shown to be an important cause of human febrile illness in Malaya. Studies were therefore undertaken to determine its animal reservoirs and the factors influencing spread of infection from them to man and domestic animals. This paper presents the board picture obtained. A wide range of animal species were trapped in forest localities, ricefield areas, areas of scrub and cultivation and in several towns and villages. The maintenance hosts of leptospirosis in Malaya appear to be mainly or entirely rats, although evidence of infection has been found throughout the animal kingdom. Some rat species have characteristics which suggest that they are better maintenance hosts than others. Evidence was found of practically every serogroup of leptospires infecting animals in Malaya. Altogether 104 strains were isolated and identified, and 155 animals were found to have serological evidence of infection. Of 1763 rodents examined, 194 had evidence of infection, and 41 of 1083 other animals. A serum survey of domestic animals showed the highest incidence of antibodies to be in goats and the lowest in oxen.

The first substantial work on leptospirosis in Malaya was by Fletcher (1928), who isolated strains from both man and rats. Extensive work has been done in Indonesia and has been reviewed by Walch-Sorgdrager (1939) and by Collier (1948). Since the Second World War, interest in leptospirosis in Malaya has been increased by the high incidence in British troops engaged in military operations; studies of human infections were made by Wissemann et al. (1955), Alexander et al. (1955, 1957) and McCrumb et al. (1957). Wissemann et al. also made a limited study of wild animal leptospirosis.

The present study was designed to provide a background of information about leptospirosis in wild animals which might facilitate understanding of the epidemiology of human and domestic animal infections in Malaya.

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Animal collections were made, as part of a larger survey, from sample localities believed to be typical of various animal communities, as well as from areas where leptospirosis was known to occur. Sampling was continued, if possible, until at least thirty of each of the principal species in each locality had been examined.

COLLECTING AREAS

Many of these were described by Audy & Harrison (1954), while the principal vegetation types, and animal communities are summarized by Harrison (1957). The localities are shown in the maps (Fig. 1 and 2).

The names of animals used are as in Chasen (1940), as modified by Ellerman & Morrison-Scott (1955). A convenient summary is given by Harrison (1957b).

Forest areas

The four main study areas were in dipterocarp forest lying between about 200 and 2000 feet (60-600 m) above sea level. Most of the vegetation was hill forest, with some lowland forest and secondary

FIG. 1

MAIN LOCALITIES IN MALAYA MENTIONED IN THE TEXT

forest fringes. The main differences were as follows:

Ulu Langat Forest Reserve. This was the highest, most remote and least disturbed of the areas studied.

Ulu Gombak Forest Reserve. The area covered comprised those parts of the forest easily accessible from the Kuala-Lumpur–Pahang road, which traverses it. For this survey the area was trapped by the laboratory staff, and not by aborigines, as mentioned in Audy & Harrison (1954). This area was rather more diffuse than the others and comprised a greater range of vegetation, from iundsturbed primary forest to secondary forest and scrub.

Bukit Lagong Forest Reserve. Trapping was mainly in the fringe of a large well-established hillside clearing, which contained a settlement of aborigines and their fruit and vegetable gardens.

Jugra Hill. This is an isolated, steep-sided, forested hill surrounded by rubber and scrub, except on one side where it runs into mangrove forest.

Other areas. Small numbers of animals were collected from other places on various occasions—a number of them by Colonel R. Traub.

Areas of scrub and cultivation

Ricefields. (1) Bukit Meriam—an area of ricefields in the coastal fringe of the Kedah rice-plain. The water in the ricefields is slightly brackish as the seashore is only a mile or so distant.[1] (2) Ulu Langat—a small group of ricefields near scrub and forest in the valley at the head of which lies the Ulu Langat Forest Reserve. (3) An area of ricefields near Malacca.

Scrub and grassland. (1) Pahang road, 7th mile—an area of scrub and secondary forest on the outskirts of Kuala Lumpur. (2) Bentong—a scrubby area surrounding and including the camp area where an outbreak of leptospirosis occurred among the Royal West Kent Regiment. (3) Kuala Lumpur—animals were trapped in a number of wasteland places in and around the city.

Towns and villages

(1) Kuala Lumpur—trapping mainly by municipal ratcatchers in and around houses. (2) Klang—trapping in a selected group of shop-houses near the river to obtain samples of *Rattus rattus diardi* and *R. norvegicus* from the same place. (3) Port Swettenham—trapping around warehouses with the same intention. (4) Jeram—a small village north of Klang from which a variety of house-infesting animals were obtained.

METHODS

Animal body service

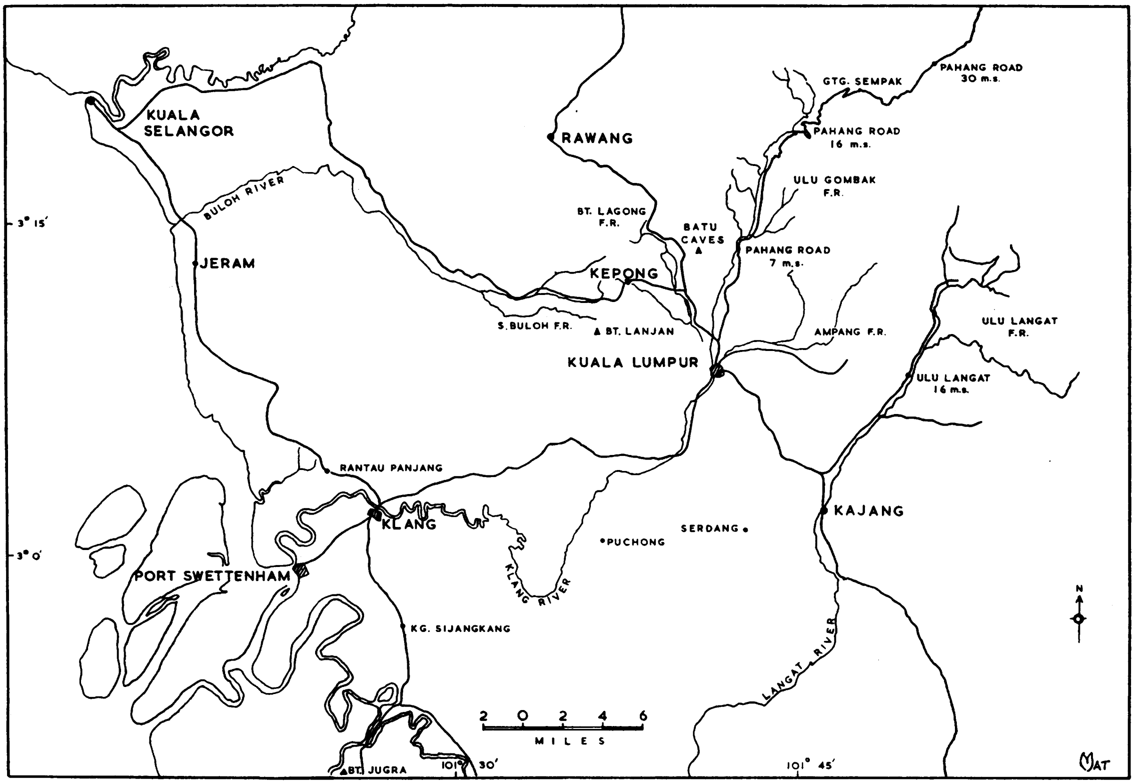
Trapped animals were given as full an examination as possible. In addition to the bleeding and culture described below, they were also weighed, measured and examined for parasites, etc. as described by Audy & Harrison (1954).

Bleeding and culture

Where possible animals were anaesthetized and bled from the heart. Smaller animals were bled by cutting the axillary vessels or (especially with birds) with a syringe and needle from the jugular vein.

[1] See also the article by Smith & Turner on page 35 of this issue.

FIG. 2
MAIN COLLECTING AREAS FOR ANIMALS STUDIED



The abdomen was then opened with sterile precautions and the kidneys removed into a sterile Petri dish. A piece of renal cortex was punched out of one kidney with a sterile Pasteur pipette and expelled into a tube of 10 ml of Korthof's medium. A small amount of cortical material was emulsified in a drop of saline with the tip of a Pasteur pipette and then examined microscopically under the dark ground for motile leptospires. The second kidney was fixed in 10% formalin. A general examination was then made of abdominal and thoracic organs for gross abnormalities.

Cultures

The cultures in Korthof's medium (Alston & Broom, 1958) were kept at room temperature (27°-31°C) in the dark and examined by dark-ground microscopy at intervals for 28 days. When leptospires were seen, they were subcultured into another tube of Korthof's medium and into a hard-glass ampoule of the medium. The ampoule was sealed and sent by air to London.

Sera

The sera containing 0.4% sodium azide were sent to London in sealed glass ampoules by air. A fair number were lost by breakage but those which arrived unbroken were in good condition.

Kidney sections

Some kidneys were examined after silver impregnation by Levaditi's method and histological section. This technique was later abandoned owing to staff shortage.

Identification of cultures

The identification of strains of leptospires depends on determining their serological characteristics by a microscopic agglutination test. As a wide variety of serological types (serotypes) was found to be present in Malaya, it was necessary to test the newly isolated strains against 32 serotype-specific antisera.[1] These tests were performed in London.

Experience had shown that the work involved could be considerably reduced by making a three-stage "screening test". The method employed depends on the fact that the agglutination of a strain by its homologous antiserum is not inhibited by the presence of heterologous antisera. We therefore made six mixtures each containing five or six antisera, and the first stage of the test consisted in allowing a single dilution (1/300) of each mixture to interact with the culture. In the second stage, the strain was tested against a single dilution of each of the antisera in the mixture or mixtures with which it reacted. Lastly, the culture was tested against a full range of dilutions of the individual antisera which agglutinated it. From the results of these tests it was possible to decide which serogroup the strains belonged to, but for identification of the serotype it was necessary to carry out agglutinin-absorption tests.

The last stage of identification is not complete for all our strains and, in addition, some represent new serotypes whose characteristics have not yet been published. In Table 4, therefore, only the serogroups to which the strains belong have been recorded. As a matter of convenience we have extended some serogroups beyond the limits used by Wolff & Broom (1954). Thus, *L. celledoni* and *L. sarmin* have been included in the Javanica, *L. schüffneri* and *L. benjamin* in the Canicola, and *L. sentot* and *L. djasiman* in the Autumnalis serogroups.

Agglutination tests with animal sera

With the same aim of economizing time and labour, we used a comparable screening method for testing sera for the presence of leptospiral antibodies. This testing, also, was done in London. Ten mixtures were prepared, each consisting of formolized cultures of three serotypes. We found experimentally that it was inadvisable to include more than three serotypes in the mixtures, otherwise weakly positive reactions might escape detection since only the homologous leptospires are agglutinated by any antibodies in the serum. *L. australis B* and *L. sejroe* were not included in the antigen mixtures, but the series comprised all the other serotypes mentioned earlier in the footnote.

A 1/100 dilution of each serum was first tested against the ten mixed suspensions. The same dilution was then tested against the individual serotypes in any mixtures which had given positive reactions, and finally the actual titre of the serum was determined in the usual way.

RESULTS

Table 1 shows the incidence of leptospirosis found by various techniques in rats and mice, and Table 2 presents the same information about other species. Leptospires were isolated mainly from rats (92%), the other species being the palm civet, a domestic cat, and two bats. Serological evidence of infection was found in a much wider range of species.

The results below are presented in terms of the proportions of positive animals detected by all methods.

House-infesting animals

R. norvegicus, with an incidence of 34%, accounted for 80% of house-infesting rats with evidence of leptospirosis. *R. norvegicus*, however, is of very limited distribution in Malaya, being confined to seaports, and is probably a comparatively recent introduction. The over-all level of leptospirosis in house-rats in Malaya is thus better expressed by the incidence in the Malayan house-rat (*R. r. diardi*) which amounted to 3%; in four localities from which samples of 41-74 animals were examined, the incidence was 4%, 2%, 2% and nil. No evidence of infection was found in the house-mouse (*Mus musculus*), but very few sera were examined as this species was caught almost exclusively on "sticky boards" and the animals were rarely suitable for bleeding. Although classified as a rat of scrub and cultivation, *R. exulans* commonly enters houses; it had an incidence of 7% (2/27) in Kuala Lumpur (mostly in grassland). Other house-infesting animals are the shrew (*Suncus murinus*) with an infection rate of 5% (2/44) and the palm civet (*Paradoxurus hermaphroditus*), which commonly enters roofs, with an incidence of 19% (8/42). The civet is, of course, a much less numerous animal than the shrew and small rodents.

Animals of scrub and cultivation

Among the rats, *R. jalorensis* had the lowest incidence (3%); it is found in scrub, in gardens, and in rubber and oil-palm estates and becomes a

[1] The antisera used had been prepared against the following serotypes: *andaman, australis A, australis B, autumnalis, ballum, bangkinang, bataviae, benjamin, canicola, celledoni, djasiman, grippotyphosa, hardjo, hebdomadis, hyos, icterohaemorrhagiae, javanica, kremastos, mankarso, medanensis, mini, naam, poi, pomona, pyrogenes, sarmin, saxkoebing, schüffneri, sejroe, semarang, sentot* and *wolffii*.

TABLE 1. INCIDENCE OF LEPTOSPIROSIS IN RATS AND MICE

Species	Total examined	Proportion positive by				Total positive		Serogroups cultured	Antibody to additional serotypes [a]
		Culture	Serology	Kidney section	Dark-ground of kidney	By species	By group		
HOUSE RATS AND MICE									
R. r. diardi	260	2/259 (1 %)	4/233 (2 %)	2/98	0/260	7/260 (3 %)		Javanica, Pyrogenes	*schüffneri*, *poi*, *andaman A*
R. norvegicus	82	20/82 (24 %)	22/77 (29 %)	5/53	1/82	28/82 (34 %)	35/393 (9 %)	Icterohaemorrhagiae, Javanica, Bataviae	*hebdomadis*, *sarmin* (m) *saxkoebing* (m)
Mus musculus	51	0/50	0/51	0/5	0/51				
RATS OF SCRUB AND CULTIVATION									
R. jalorensis	263	6/263 (2 %)	4/220 (2 %)	0/52	0/263	9/263 (3 %)		Icterohaemorrhagiae, Pyrogenes, Autumnalis	*sentot*, *australis A*
R. argentiventer	140	42/140 (29 %)	32/126 (25 %)	1/8	2/140	53/140 (38 %)	83/701 (12 %)	Icterohaemorrhagiae, Javanica	*sentot*, *schüffneri*, *hebdomadis*, *benjamin* (m)
R. exulans	298	14/298 (5 %)	6/111 (5 %)	4/59	8/298	21/298 (7 %)		Icterohaemorrhagiae, Javanica, Canicola, Hebdomadis	*saxkoebing*, *bataviae*
FOREST GROUND RATS									
R. bowersi	67	10/67 (15 %)	2/63 (3 %)	2/14	0/67	12/67 (18 %)		Autumnalis, *hyos*	*grippotyphosa*
R. mülleri	140	11/140 (7 %)	11/139 (7 %)	0/27	0/140	19/140 (14 %)		Icterohaemorrhagiae, Pyrogenes, Bataviae, *australis A*, *hyos*, *grippotyphosa*	*poi*, *schüffneri*, *medanensis* *javanica* (m), *ballum* (m), *hebdomadis* (m)
R. sabanus	148	2/146 (1 %)	29/143 (20 %)	0/18	2/148	31/148 (21 %)	75/522 (14 %)	*australis A*, Bataviae	*naam*, *poi*, *canicola*, *sentot*, *autumnalis*, *grippotyphosa*, *medanensis*, *saxkoebing*
R. rajah	90	4/90 (4 %)	5/89 (6 %)	1/31	0/90	10/90 (11 %)		Javanica, Hebdomadis, *hyos*	*canicola*, *grippotyphosa*, *bataviae*, *naam* (m), *medanensis* (m)
R. whiteheadi	63	0/63	3/37 (8 %)	0/8	0/63	3/63 (5 %)			*hebdomadis*, *wolffi* *canicola* (m)
Rhizomys sumatrensis	10	0/10	0/8		0/10				
Other species (*Rattus*)	4	0/4	0/3	0/1	0/4				
FOREST TREE RATS									
R. canus	33	0/33	1/24	0/10	0/33	1/33			*schüffneri*
R. cremoriventer	68	0/68	0/22	0/4	0/68	0/68	1/147 (1 %)		
Chiropodomys gliroides	46	0/46	0/8	0/19	0/46	0/46			

[a] m = antibody found only in serum with multiple antibody.

TABLE 2. INCIDENCE OF LEPTOSPIROSIS IN ANIMALS OTHER THAN RATS AND MICE

Group	Species	Total examined	Positive by		Total positive		Serotypes (by serology) [a]
			Culture	Serology	By species	By group	
Primates	*Macaca irus*	16	0/5	0/16	0/16	0/35	
	Other monkeys (4 spp.)	4	0/1	0/4	0/4		
	Nycticebus coucang	15	0/11	0/15	0/15		
Tree shrews	*Tupaia glis*	78	0/78	3/72	3/78 (4 %)	3/101 (3 %)	*pyrogenes*, *javanica*, *pomona* (m), *sentot* (m)
	T. minor	23	0/23	0/15	0/23		
Squirrels	*Callosciurus notatus*	105	0/103	0/99	0/105	1/237 (0.4 %)	
	C. caniceps	73	0/71	1/71	1/73 (1 %)		*djasiman*
	C. nigrovittatus	36	0/36	1/36	1/36 (3 %)		*hebdomadis*
	5 other tree spp.	14	0/14	0/11	0/14		
	2 ground spp.	9	0/9	0/9	0/9		
Shrew	*Suncus murinus*	44	0/42	2/26	2/44 (5 %)	2/44 (5 %)	*saxkoebing*, *autumnalis*
Carnivores	*Paradoxurus hermaphroditus*	42	7/39 [b]	4/40	8/42 (19 %)	9/57 (16 %)	*pomona*, *sentot*, Icterohaemorrhagiae group
	Civets: 3 other spp.	11	0/9	1/11	1/11 (9 %)		*semarang*
	Felis bengalensis	4	0/4	0/4	0/4		
Bats	*Myotis* spp.	123	2/123 [c]	0/1	2/123 (2 %)	4/295 (1.3 %)	*wolffii*, *medanensis*
	Scotophilus spp.	62	0/41	0/35	0/62		
	Eonycteris spelaea	51	0/51	2/37	2/51 (4 %)		
	12 other genera	59	0/59	0/8	0/59		
Other mammals	*Tragulus* spp.	5	0/4	2/5	2/5 (40 %)	5/25 (20 %)	*medanensis*, *icterohaemorrhagiae* (m), *sentot* (m), *djasiman* (m), *pomona* (m)
	Atherurus macrourus	4	0/3	1/4	1/4 (25 %)		*saxkoebing*
	Cynocephalus variegatus	7	0/7	2/7	2/7 (29 %)		*djasiman*, *bangkinang*
	4 other spp.	9	0/9	0/9	0/9		
Birds	*Pycnonotus* spp. (B. Lagong)	77	0/72	1/7	1/77 (1 %)	1/173 (0.6 %)	*javanica*
	Other birds	96	0/96	0/9	0/96		
Reptiles	*Varanus* spp.	18	0/18	1/11	1/18 (6 %)	16/116 (14 %)	*bataviae*
	Gonocephalus borneensis	41	0/41		0/41		
	Misc. lizards	20	0/20		0/20		
	Acrochordus javanicus	17		13/17	13/17 (76 %)		Ictero. gp (2+11 m), Javanica gp (3m), *canicola* (m), *pomona* (6m)
	Python spp.	5		1/5	1/5 (20 %)		Autumnalis gp (2m), Hebdomadis gp (3m), *semarang* (2m), *andaman A* (m)
	Elaphe oxycephala	8		1/8	1/8 (12 %)		*pomona*
	Homalopsis buccata	7		0/7	0/7		*hebdomadis*

[a] m = antibody found only in serum with multiple antibody. [b] Cultures: 4 *L. pomona*, ***L. sentot***, Hebdomadis group. [c] Cultures died; unidentified.

pest in the latter; the range of incidence was 1%, 1%, 4% and 10% in four areas from which samples of 27-82 animals were obtained. The differences between the 1% incidences and the 10% incidence are significant at the 5% level. *R. exulans*, common in scrub and grassland and invading the forest fringe, had incidences of nil, 7%, 12% and 17% in samples of 27-110 animals from four areas. The nil incidence in the Bukit Lagong Forest Reserve differed significantly at the 5% level from the two highest incidences. The highest incidence in any species was in *R. argentiventer*, which lives in grassland and ricefields. The over-all incidence in this species was 38%, and in three ricefield areas with samples of 59, 52 and 17 animals the incidence was 29%, 50% and 53% respectively. The lowest incidence (in the Ulu Langat ricefields) differed significantly from the other two places at the 5% level.

Two species of tree squirrel, *Callosciurus caniceps* and *C. notatus*, are common in gardens, in estates and around coconut palms. Only one animal (*C. caniceps*) of these species had evidence of infection in samples of 73 and 105 animals respectively.

Forest animals

There were six principal species of mainly terrestrial rats with an over-all incidence of 14%; and three species of tree rats, in which only 1/147 animals was found to have evidence of infection. The over-all incidence in the four species of giant rats was 17%; *R. mülleri* lives in the wetter parts of the forest, notably in valley bottoms; *R. bowersi* is of similar habit but predominates at a higher altitude than *R. mülleri*; the overlap is marked, however, and from the Ulu Langat Forest Reserve they were trapped in almost equal numbers. *R. bowersi* had an incidence of 0/19 in one forest area and of 12/45 (27%) in another (significant difference). *R. mülleri* had incidences of 7%, 12% and 24% in three forest areas from which samples of 42, 33 and 42 animals, respectively, were examined. The lowest incidence, in the Ulu Langat Forest Reserve, is significantly lower than the other two at the 5% level. *R. sabanus* is the common giant rat of the drier parts of the forest, such as hillsides, whereas the first two species would be in the valley bottoms. *R. sabanus* had incidences of 18%, 19% and 23% in samples of 40, 32 and 62 animals, respectively, from three forest areas. These incidences do not differ significantly.

The over-all incidence (8%) in spiny rats (*R. rajah, R. whiteheadi*) was about half that in the giant rats. *R. rajah* had incidences of 9%, 12% and 13% in samples of 23, 34 and 31 animals from three forest areas. The incidences do not differ significantly. *R. whiteheadi* was sampled adequately from only one area of forest and had an incidence of 5% in 39 animals.

There were very small samples of a few other terrestrial forest mammals; evidence of infection was found only in 2/5 mouse-deer (*Tragulus* spp.), and 1/4 porcupines (*Atherurus macrourus*).

Only one tree-rat (*R. canus*) was found to have evidence of infection and by serology only. Both *R. canus* and *R. cremoriventer* live primarily in trees but do come to ground. *Chiropodomys* is believed never to come to ground.

As with tree-rats the incidence of infection in other arboreal or partly arboreal species was generally low: 3/101 tree-shrews (*Tupaia* spp.), and 1/36 *Callosciurus nigrovittatus* had antibodies. Many of the other two species of *Callosciurus* also came from the forest fringe but the incidence was low throughout the genus. One forest civet (*Arctogalidia trivirgata*) and 2/7 flying lemurs (*Cynocephalus variegatus*) also had antibodies.

No evidence of infection was found in 36 primates. Leptospires were isolated from 2/123 *Myotis* bats; these were very small species living mainly in the stems of large bamboos. Antibodies were also found in 2/51 cave bats (*Eonycteris spelaea*). Cultures were made of kidneys from about 200 birds and no leptospires were grown; few sera were examined as they were required for another investigation but one bulbul (*Pycnonotus b. bruneus*) was found to have antibody.

The finding of antibodies in reptiles was of great interest although as yet of unknown significance. One of *Varanus salvator*, an aquatic and largely fish-eating species of monitor lizard, had antibody, but none of the other lizards of *Varanus* species did. A high incidence of antibodies (13/17) was found in the aquatic fish-eating snake, *Acrochordus javanicus*. Antibodies were also found, but in much lower incidence, in two species of snake which eat rodents: 1/8 *Elaphe oxycaphala* and 1/5 pythons. Experimental work is in progress to evaluate the significance of these findings.

Domestic animals

Table 3 shows the incidence of leptospirosis found in domestic animals. Apart from two dogs and 40 cats which were cultured and bled in the laboratory, all the examinations were of sera collected

TABLE 3

INCIDENCE OF LEPTOSPIROSIS IN DOMESTIC ANIMALS

Animal	No. examined [a]	No. positive	Positive to a single serotype	Positive to several serotypes [b]
Ox	52	2	ictero., pomona	
Goat	61	17	ictero., 2 naam, 3 sarmin, canicola grippotyphosa, 3 medanensis, 3 saxkoebing	(naam+ictero.) (medanensis+sarmin) (autumnalis+australis A)
Buffalo	34	5	2 hebdomadis, semarang	(hardjo+canicola) (sarmin+saxkoebing)
Pig	47	5	2 ictero., canicola saxkoebing	(ictero.+canicola)
Dog	51 (2)	9	3 canicola, medanensis, hardjo	(ictero. + pyrogenes + javanica +poi) 2 (hebdomadis+ictero.) (canicola + hebdomadis + ictero.)
Cat	68 (40)	7	2 canicola, ictero, pomona [c]	(canicola+hebdomadis.+ictero.) (javanica+grippotyphosa+hebdomadis) (hebdomadis+australis A+ictero.+naam)

[a] Figures in parentheses indicate the number examined by culture as well as by serology.

[b] Serotypes underlined gave higher titre.

[c] Isolation of strain from kidney.

at abattoirs or by veterinarians in various parts of the country. Only one culture was successful: *L. pomona* from a cat.

The highest incidence in ungulates was in goats (28%) and the lowest in oxen (4%). Antibodies were found in 15% of buffaloes and 11% of pigs. The incidence in dogs was 18% and in cats 10%. All the positive cats were among the 40 killed in the laboratory (from the city of Kuala Lumpur); the 28 sera from elsewhere all yielded negative results.

Distribution of leptospiral serogroups

Culture. A total of 104 strains of leptospires was isolated and identified: a further 16 strains were isolated but for various reasons did not survive to be identified in London.

The strains identified and their hosts are tabulated in Table 4. Strains of the Icterohaemorrhagiae serogroup were isolated mainly (15/23) from the

TABLE 4

NUMBERS AND HOSTS OF STRAINS ISOLATED

Serotype	Number of strains	Animal hosts
L. icterohaemorrhagiae	16	*R. norvegicus* (15), *R. mülleri* (1)
Icterohaemorrhagiae group	7	*R. argentiventer* (2), *R. exulans* (2), *R. mülleri* (2), *R. jalorensis* (1)
L. javanica	18	*R. argentiventer* (16), *R. norvegicus* (1), *R. r. diardi* (1)
Javanica group	24	*R. argentiventer* (21), *R. rajah* (2), *R. exulans* (1)
L. celledoni	1	*R. argentiventer*
L. schüffneri	1	*R. exulans*
L. canicola	2	*R. exulans*
L. benjamin	1	*R. exulans*
Benjamin group	1	*R. exulans*
Pyrogenes group	3	*R. r. diardi*, *R. jalorensis*, *R. mülleri*
L. sentot	1	*P. hermaphroditus*
Autumnalis group	2	*R. jalorensis*
L. djasiman	2	*R. bowersi*
L. australis A	4	*R. mülleri* (3), *R. sabanus* (1)
L. pomona	5	*P. hermaphroditus* (4), domestic cat (1)
L. grippotyphosa	1	*R. mülleri*
Hebdomadis group	3	*R. exulans*, *R. rajah*, *P. hermaphroditus*
L. bataviae	2	*R. mülleri*, *R. sabanus*
Bataviae group	2	*R. norvegicus*
L. hyos	8	*R. bowersi* (6), *R. rajah* (1), *R. mülleri* (1)
Died and unidentified	16	*R. exulans* (5), *R. jalorensis* (2), *R. norvegicus* (2), *R. argentiventer* (2), *R. bowersi* (1), *R. mülleri* (1), *P. hermaphroditus* (1), *Myotis* spp. (2)
Total	120	

house-rat of seaports, *R. norvegicus*, but also from rats of scrub and cultivation (5/23) and from a forest-rat (3/23 from *R. mülleri*).

Strains of the Javanica serogroup were also isolated from the three main habitats; but the majority (37/42) were from *R. argentiventer*, which was trapped mainly in ricefields. Strains of the Pyrogenes and Hebdomadis serogroups came also from all three habitats. The Canicola serogroup came only from *R. exulans*, and 4/5 of them were from an army camp near Bentong. *L. djasiman*, *L. australis A*, *L. grippotyphosa* and *L. hyos* came only from forest-rats; both strains of *L. djasiman* and all six of *L. hyos* came from the Ulu Langat Forest Reserve, while all three strains of *L. australis A* were from the Ulu Gombak Forest Reserve. Strains of the Bataviae serogroup came from house- and forest-rats. *L. pomona* was isolated only in towns and villages from the palm civet and a domestic cat. *L. sentot* came only from a palm civet and the single strains of the Autumnalis serogroup and of *L. celledoni* from rats of scrub and cultivation.

Serology. The serotypes to which antibodies were detected in rats are shown in Table 1. There were relatively few sera with antibody to more than one serotype and these are indicated. No cross-reactions are known (Wolff & Broom, 1954) between the pairs found in rats except in *R. argentiventer* where *L. benjamin* were associated with *L. schüffneri* antibodies; in one case the titres were equal against the two strains and in the other the titre against *L. schüffneri* was the greater. One serum of *R. whiteheadi* had antibodies to both *L. canicola* and *L. hebdomadis* and a very small degree of cross-reaction was found between these strains by Wolff & Broom (1954). In general, however, rat sera with antibodies to multiple serotypes probably represent multiple infection.

In species other than rats (Table 2) the sera were again mainly positive against only one serotype. One tree-shrew had a higher titre against *L. poi* than *L. sentot*; a low level of cross-reaction was found between these serotypes by Wolff & Broom (1954). The palm civet with a higher titre against *L. icterohaemorrhagiae* than *L. naam* probably represents an infection by the former. The finding of multiple antibodies in the mouse-deer parallels what is commonly found in domestic ungulates (see below). All the positive sera of the snake *Acrochordus* had antibody to more than one serotype except two, and to more than one serogroup except three, all of which were Icterohaemorrhagiae serogroup. Eight of the enlarged serogroups were encountered more than once in *Acrochordus* sera.

In domestic ungulates (Table 3) the commonest serogroups were Hebdomadis (in 12 sera, 3 of them multiple reactors), Icterohaemorrhagiae (in 8 sera, 1 of them multiple), Javanica (5 sera, 2 multiple) and Canicola (4 sera, 2 multiple). Pomona, Grippotyphosa and Semarang serogroups appeared only once, and only multiple reactors had antibody to Autumnalis and Australis A serogroups.

In dogs and cats, the Icterohaemorrhagiae (7 sera, 6 multiple), Canicola (7 sera, 2 multiple) and Hebdomadis (8 sera, 6 multiple) serogroups were most frequent; Pomona appeared in one serum only, and only multiple reactors had antibody to the Javanica, Pyrogenes, Australis A or Grippotyphosa serogroups.

Relation between culture and serology. Culture was much more successful with, for example, *R. norvegicus* than *R. sabanus*: with the former the culture rate was 26%, the serology rate 29% and the combined rate 35%; only a very low rate of infection (1%) was found in *R. sabanus* by culture but the serology rate was high (21%) giving a total rate of 22%.

The differences between rat species may, however, be largely due to differences in the predominant serogroup of leptospires infecting them. The serology of the rats from which leptospires were isolated is shown in Table 5. Of 21 rats from which strains of the Icterohaemorrhagiae serogroup were cultured, 16 (76%) also had circulating antibody. But only half the 38 rats from which Javanica serogroup strains were isolated had antibody, and none of the seven rats infected with *L. hyos* whose sera were examined had antibody. Conversely, 22% of rats with Icterohaemorrhagiae serogroup antibody, and 32% of those with Javanica serogroup antibody gave negative cultures, while 88% of rats with Bataviae serogroup antibody and 75% of those with Australis A serogroup antibody had negative cultures. All rats with antibody against the Canicola, Autumnalis, Grippotyphosa and Hebdomadis serogroups had negative cultures. There is, apparently, a spectrum of leptospiral serotypes or serogroups with the Icterohaemorrhagiae serogroup at one end and *L. hyos* at the other, which have a diminishing antigenic power for rats although all apparently behave similarly in ability to infect and also multiply in the same site. This has been studied experimentally and will be discussed in greater detail in a later paper.

TABLE 5

SEROLOGY OF ANIMALS FROM WHICH LEPTOSPIRES WERE ISOLATED

Serogroup	No. of strains cultured	No. of sera tested	Positive sera (%)	Animal species [a]
Ictero-haemor-rhagiae	23	21	15 (79%)	*R. norvegicus*, 2 *R. exulans* (1 NS), *R. jalorensis* (+), 3 *R. mülleri* (2+), 2 *R. argentiventer* (1+)
Javanica	43	38	20 (53%)	*R. argentiventer*, *R. norvegicus* (+), *R. exulans* (NS) *R. r. diardi*, 2 *R. rajah*
Canicola	5	2	0	*R. exulans*
Pyrogenes	3	3	0	*R. r. diardi*, *R. jalorensis*, *R. mülleri*
Autumnalis	5	5	1	2 *R. jalorensis*, 2 *R. bowersi*, *P. hermaphroditus* (+)
L. australis A	4	4	1	3 *R. mülleri*, *R. sabanus* (+)
L. pomona	5	4	2	4 *P. hermaphroditus* (+, 1NS), domestic cat
L. grippo-typhosa	1	1	0	*R. mülleri*
Hebdomadis	3	2	0	*R. exulans* (NS), *R. rajah*, *P. hermaphroditus*
Bataviae	4	3	2	2 *R. norvegicus* (+), *R. sabanus* (+), *R. mülleri* (NS)
L. hyos	8	7	0	6 *R. bowersi* (1 NS) *R. mülleri*, *R. rajah*

[a] + = positive serum; NS = no serum.

Host-list of serogroups

The animals with evidence of infection by each serogroup are listed below with the number of animals from which cultures (C) were made, the number with antibodies to the serogroup only (S) and the number with antibodies to the serogroup in addition to antibody to at least one other serogroup (m).

Icterohaemorrhagiae serogroup

R. norvegicus (15C+4S+1m), *R. jalorensis* (1C) *R. argentiventer* (2C), *R. exulans* (2C), *R. mülleri* (3C), *R. sabanus* (1S), *R. rajah* (1m), *P. hermaphroditus* (1S), *Tragulus* (1m), *Acrochordus* (2S+11m), Ox (1S), Goat (4S), Pig (2S+1m), Dog (4m), Cat (1S+2m).

Javanica serogroup

R. r. diardi (1C+2C), *R. norvegicus* (1C+1S+1m), *R. argentiventer* (38C+3S), *R. exulans* (1C), *R. mülleri* (1S+2m), *R. sabanus* (3S+1m), *R. rajah* (2C), *T. glis* (1S), *Pycnonotus* (1S), *Acrochordus* (3m), Goat (3S+1m), Buffalo (1m), Dog (1m), Cat (1m).

Canicola serogroup

R. r. diardi (1S), *R. argentiventer* (5S), *R. exulans* (5C+1S), *R. mülleri* (1S), *R. sabanus* (1S), *R. rajah* (1S), *R. whiteheadi* (1m), *R. canus* (1S), *Acrochordus* (1m), Goat (1S), Buffalo (1m), Pig (1S+1m), Dog (3S+1m), Cat (2S+1m).

L. ballum

R. mülleri (1m).

Pyrogenes serogroup

R. r. diardi (1C), *R. jalorensis* (1C), *R. mülleri* (1C+1m), *T. glis* (1S), Dog (1m).

Autumnalis serogroup

R. jalorensis (2C+2S), *R. argentiventer* (1S), *R. bowersi* (2C), *R. sabanus* (3S), *T. glis* (1m), *C. caniceps* (1S), *S. murinus* (1S), *P. hermaphroditus* (1C+1S), *Tragulus* (1m), *Cynocephalus* (2S), *Acrochordus* (2m), Goat (1m).

L. australis A

R. jalorensis (1S), *R. mülleri* (3C), *R. sabanus* (1C), Goat, (1m), Cat (1m).

L. pomona

T. glis (1m), *P. hermaphroditus* (4C), *Tragulus* (1m), *Acrochordus* (6m), *Python* (1S), Ox (1S), Cat (1m).

L. grippotyphosa

R. bowersi (1S), *R. mülleri* (1C), *R. sabanus* (4S), *R. rajah* (1S), Goat (1S), Cat (1m).

Hebdomadis serogroup

R. norvegicus (1S+1m), *R. argentiventer* (1S), *R. exulans* (1C+1S), *R. mülleri* (2S+1m), *R. sabanus* (6S+1m), *R. rajah* (1C+1S+1m), *R. whiteheadi* (2S+

1m), *C. nigrovittatus* (1S), *S. murinus* (1S), *P. hermaphroditus* (1C), *Eonycteris* (2S), *Tragulus* (1S), *Atherurus* (1S), *Acrochordus* (3m), *Elaphe* (1S), Goat (6S+1m), Buffalo (2S+2m), Pig. (1S), Dog (2S+3m), Cat (3m).

Bataviae serogroup

R. norvegicus (2C), *R. exulans* (1S), *R. mülleri* (1C+1S+1m), *R. sabanus* (1C+10S), *R. rajah* (1S), *Varanus* (1S).

L. semarang

Arctogalidia (1S), *Acrochordus* (2m), Buffalo (1S).

L. andaman A

R. r. diardi (1S), *Acrochordus* (1m).

L. hyos

R. bowersi (6C), *R. mülleri* (1C), *R. rajah* (1C).

Comparison of survey methods

Culture and serology. Considering only rats examined by both techniques (Table 1), culture alone revealed an incidence of 7% compared with 9% by serology alone, and 13% when the two methods were combined.

Kidney sections. These were done for a limited period only, and the proportions of different rat species examined differs from those considered above under the heading " Culture and serology ". Only 7 rats were shown to be infected which would otherwise have been missed. But the incidence detected in some species (e.g., *R. r. diardi, R. exulans*) was not lower than that found by culture and serology, and in some other species the numbers examined were small. The over-all rate found by section was 4%.

Dark-ground examination of kidney. Only 12 positive examinations were made, 8 of them in *R. exulans*. In one *R. exulans* this was the only evidence of infection found. The over-all rate detected was only 1%.

Combined totals. The proportions of rats with evidence of infection calculated on the basis of all animals examined, however incompletely, do not differ significantly from the proportions calculated from animals which were completely examined (Table 1). The largest difference was in *R. whiteheadi*, where 8% were positive of fully examined animals compared with 5% when all animals were included. The gross incidences found by inclusion of all animals may therefore be used as satisfactory estimates.

DISCUSSION

The results have been presented only in general terms and will be discussed in the same vein. A more detailed analysis of specific localities and differences between them, and of factors such as sex and weight in the rats, will be discussed in later papers.

There are two main aspects of leptospirosis in wild animals to be considered here: (1) factors which control the continued survival of leptospires in an area, and (2) the risk of infection of man and domestic animals from the reservoirs.

Factors which control the survival of leptospires in an area

The requirements for survival appear to be the occurrence (1) of a host which (*a*) can excrete leptospires, (*b*) has suitable habits, and (*c*) has a sufficient population to ensure transmission; and (2) of favourable environmental conditions.

A suitable host is presumably one which is easily infected but relatively unaffected by the infection, and in which the leptospires can multiply in the renal tubules for long periods and be excreted in urine which is not too acid. Different species probably vary in their efficiency as carriers, and a useful measure may be found in the ratio between culture rate and serology rate. If these are equal, then infections can be presumed to be persistent; whereas if the serology rate greatly exceeds the culture rate, then infections are probably fleeting, and leptospires are not excreted for long. By this criterion, *R. norvegicus*, *R. argentiventer*, *R. exulans* and *R. mülleri* are highly efficient. It is known that *R. norvegicus* once infected continues to excrete more or less for the rest of its life (Walch-Sorgdrager, 1939). Such species are " maintenance hosts " (Audy, 1958) for the leptospires they carry. On the other hand, species like *R. sabanus*, where the serology rate is twenty times the culture rate, and *R. whiteheadi*, where there is similar disproportion, are probably poor reservoirs as infection apparently does not persist long in their kidneys. The difference between the two classes is further supported by the fact that 10 of the 15 rats with positive kidney sections and 10 of the 13 rats in whose kidneys leptospires were seen by dark-ground microscopy were of the first class. Of course, in the case of *R. bowersi*, where the infecting serotype (*L. hyos*) does not stimulate antibody formation, this criterion cannot be applied.

The population of a reservoir species must be dense enough for the range of several animals to overlap and for transmission to occur (Karaseva, 1956). Common drinking places might suffice for this. Dense local populations probably create " islands " of infection, as was suggested by the finding of *L. hyos* only in one small area of forest.

Favourable environmental conditions include the presence of water, soil and surface water of pH above 6.0, and perhaps the physical nature of the soil.[1] Adverse factors are salinity of the water and cold climate. Such factors increase in importance with less dense maintaining populations.

The influence of water is readily seen. Most of the species with the highest infection rates are especially associated with water. *R. norvegicus* lives near river banks and frequents drains and sewers; it had an infection rate of 32% at Klang, while *R. r. diardi*, living in the same area but in dry conditions in the roof and walls, had an infection rate of only 2%, *R. argentiventer* abounds in ricefields, which are flooded for much of the year. *R. bowersi* and *R. mülleri* live in the forested bottoms of valleys close to streams. Of the other animals, *Varanus salvator* and *Achrocordus javanicus* are both aquatic.

Factors influencing the degree of risk to man and domestic animals

Infections of man and domestic animals, and in fact of all animals which are not effective reservoirs, are incidental to the life history of the leptospires and do not normally contribute to their continued existence in an area. Such animals with their wider range of movement may, however, act from time to time as agents for the dissemination of infection from one area to another and from one reservoir population to another, the reservoir species being generally of very short range. Man is always a casual victim (or incidental host—Audy, 1958), but domestic animals sometimes become maintenance hosts; the origin in either case is, however, focal leptospirosis in some wild maintenance species.

Other things being equal, the greatest risk to man and domestic animals will be where there is contact through water with a reservoir population of high efficiency and of high population density. This is, of course, the situation in the occupational leptospiroses, for example, in the canefields of Queensland or the ricefields of Italy where the fields are infested with rodents. In Malaya, dense populations of a single species of rat are found only where deforestation has taken place. In a study of about 16 000 animals trapped in Malaya in roughly equal numbers from the three main habitats, Audy & Harrison (1954) found 49 species from forest, 15 species from scrub and cultivation, and only 5 species from towns; the numbers of animals per species were, of course, in inverse proportion, and this was demonstrated more critically by trap-mark-release experiments in an area of 25 acres of forest, in parallel with 25 acres of grassland and scrub. In grassland and scrub, 9.0 rats per acre compared with 5.8 per acre in forest were trapped in the same period (Audy, 1954).

The risk in towns and villages

Frank clinical leptospirosis is apparently uncommon in Malaya, but studies by the United States Army Medical Research Unit at the Institute for Medical Research, Kuala Lumpur, have shown that 15% of " pyrexias of unknown origin " in adult Malayan civilians and about 2% of such fevers in children in the Kuala Lumpur General Hospital are due to leptospirosis (Institute for Medical Research, Kuala Lumpur, 1958). The incidence of pyrexias of unknown origin in Malaya is very high so that these represent a large annual number of infections. Within towns the main source of infection is probably rats, although cats and dogs may account for some infections. The low incidence (3%) in the house-rat, *R. r. diardi*, corresponds to the findings summarized by Collier (1948) from Indonesia. Where rats are numerous, however, a 1%-2% excretion rate is probably high enough under the close conditions of contact within dwellings and places of work, especially as rats are known to contaminate food and utensils with urine. The risk may be higher in seaports where *R. norvegicus* (excretion rate about 25%) is common, but on the other hand its habits probably bring it less into contact with man than the house-rat. No history of frequent Weil's disease could be elicited from the Chinese residents of the shop-houses where rats were trapped in Klang. Mild fevers would not have been recognized as leptospirosis, but severe forms were obviously uncommon, although *L. icterohaemorrhagiae* was the predominant serotype present. (An attempt to obtain blood samples from these people failed.) Other sources of infection in houses, especially in villages and on the fringes of towns, are *R. exulans* (excretion rate about 5%) and the palm civet, with an excretion rate approaching 20%.

[1] In this connexion, see the article by Smith & Turner on page 35 of this issue.

The risk in scrub and cultivation

Here, the risk is presumably largely occupational but, apart from the armed forces, there is no recognizable occupational leptospirosis in Malaya. In the ricefields there is contact in wet conditions with very large populations of *R. argentiventer*, which may have very high excretion rates; however, only mild fevers, some of which may be of leptospiral origin, occur commonly. Sera were obtained from people of the village at Bukit Meriam where the excretion rate in *R. argentiventer* was over 30%; and of 25 animals where the infecting serogroup was determined, the Javanica group was identified in 22. Sera from 51 persons aged 12-70 years were examined; 15 (29%) had antibody. There was no increase in incidence with age; in fact, it fell from 42% of 12 sera positive below 20 years of age, through 29% of 14 aged 29-39 years, to 24% of 35 aged over 39 years. Unexpectedly, there was no predominance of the Javanica serogroup in the antibodies found. Three sera had antibody only to the Icterohaemorrhagiae serogroup and 2 other sera had antibody to it and to at least one other serogroup; in the same way there were 3 and 3 sera respectively with Javanica antibody, 2 with Canicola antibody only, 1 and 2 with Pomona antibody, and 1 and 1 with Autumnalis antibody. While, however, only 1 of 7 positive sera from persons aged 11-20 years had Javanica antibody, 6 of 8 over 20 years did. These figures, of course, suggest that only a small proportion, if any, of the infections were acquired in the ricefields. Of 48 sera from another rice-growing community in a narrow valley in Negri Sembilan, 15% had antibody against a wide variety of serotypes. Sardjito & Zuelzer (1929) suggested that an acid pH in the soil and surface water accounted for the low incidence of leptospirosis in Indonesian ricefield workers. In general, the pH of Malayan ricefields is between 4.5 and 5.0,[1] and *L. javanica* survives for very short periods in water with a pH below 6.0.[2] But in the Bukit Meriam ricefields the pH was neutral or slightly alkaline and the salinity was quite low. We have no information about the soil and water in the other ricefields.

There are few other occupations among Malayan agricultural workers which are likely to expose them to a high risk of leptospirosis. When *R. jalorensis* is very numerous in an oil-palm estate, the conditions are generally dry and the incidence of leptospirosis in this species is apparently not high. A case in an engineer engaged in bridge building in Malaya was reported by Keal (1957).

The risk in the forest

Under normal conditions, only a few persons, such as forest workers, are at risk in the Malayan forest, and 6 of 17 (35%) Chinese woodcutters tested were found to have antibody. During the recent military operations, however, a very large number of troops were exposed. In a study of pyrexias of unknown origin in British troops, the United States Army Medical Research Unit found that 41% were due to leptospirosis. McCrumb et al. (1957) concluded on epidemiological grounds that the vast majority of these infections were acquired in the forest. Fig. 3 shows the relative incidence of the various serogroups in these human infections[3] compared with the incidences in various habitat groups of animals. With the notable discrepancy that the high incidence of Pyrogenes infections of the troops is unaccounted for, the two distributions which most closely resemble one another are those of the humans and of the forest rats, which fits the epidemiological conclusions of McCrumb et al.

In the Bentong camps, where there was a considerable outbreak among troops, the rats trapped were almost all *R. exulans*; 12% of 110 animals had evidence of infection and where the infecting serogroup was identified it was uniformly of the Canicola serogroup. This was certainly not a predominant group in the troops and their Canicola infections could easily be accounted for by the 5% proportion of this serogroup in the forest rats.

The low number of about six rats per acre in forest (see above) does not seem sufficient to account for the high infection rate in the troops. If forest rats have an excretion rate even of the order of 10%, there would only be one infected rat in two acres. There may, however, be small islands of enzootic infection. Troops operating in forest regularly patrolled for a number of years probably bivouacked in clearings (which in the Malayan forest are usually abandoned aboriginal settlements) close to streams

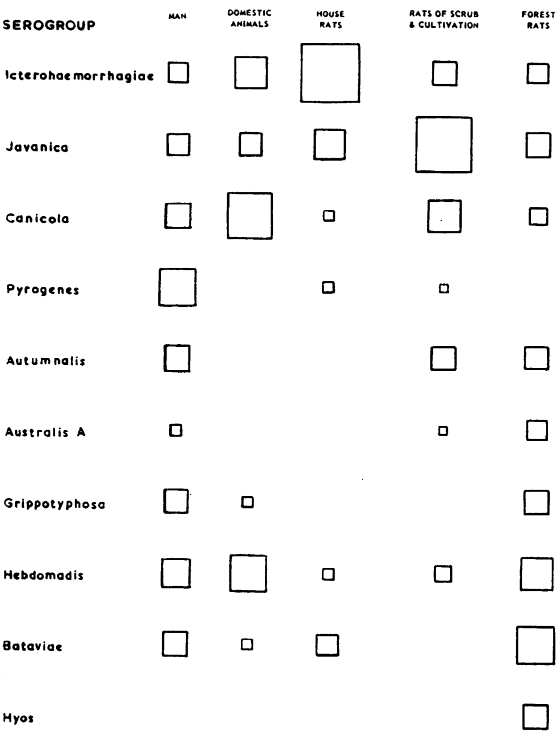
[1] Personal communication from G. W. Arnott, B.Sc., Soil Chemist, Department of Agriculture, Federation of Malaya.

[2] See the article by Smith & Turner on page 35 of this issue.

[3] The proportions of serogroups in the human infections were compiled from the culture identifications made by Broom on duplicate cultures sent to him by the United States Army Medical Research Unit and they correspond to the strains described by Alexander et al. (1957).

FIG. 3

RELATIVE INCIDENCE OF LEPTOSPIRAL SEROGROUPS IN MAN, DOMESTIC ANIMALS AND THE DIFFERENT HABITAT GROUPS OF RATS



where there is often a dense local rat population. When a "jungle fort" was made in one of these abandoned clearings deep in the forest the men complained that everywhere they looked there were rats, and that any container left exposed overnight would contain urine in the morning. Soon after occupation of the fort there was a sharp outbreak of leptospirosis. Unfortunately, rats could not be obtained from this site as helicopter transport could not be spared at the time for this purpose.

Accepting, therefore, that the troops acquired their infections in the forest—and leptospirosis was five times as common in operational units as in support units (McCrumb et al., 1957)—the difference in incidence found by them between troops operating in West Pahang (47 cases per 1000 men at risk) and in South Selangor (where it was about a fifth of this incidence) must be accounted for either by very great differences in the incidence of leptospirosis in the rats or by differences in the nature of the forest. We have no direct information about either, but one very suggestive difference in the distribution of the forest is shown by the relative amounts of forested and deforested land in the two areas.

TABLE 6

DISTRIBUTION OF TYPES OF TERRAIN IN WEST PAHANG AND SOUTH SELANGOR

	Land below 1000 feet contour			Land over 1000 feet contour
	Swamp	Cultivation	Forest	
West Pahang	1 %	12 %	50 %	37 %
South Selangor	12 %	47 %	26 %	15 %

Cultivation and settlement usually commences on the flat land and the valley bottoms, and the hills are the last to be cleared and settled, so that in a mosaic of cultivated and forested land, the bulk of the forest will be on the drier hillsides and hilltops, while only in land which is very little cultivated will there be forested valleys.

Wyatt-Smith [1] has compared the two areas [2] of McCrumb et al., and the distribution of cultivation, forest and swamp is shown in Table 6.

Table 7 shows the relative amounts of forest (primary forest or belukar riparian fringe) and cultivated land in the two areas when only dry land (excluding swamp) below 1000 feet (or about 300 m) is considered.

TABLE 7

DISTRIBUTION OF FOREST AND CULTIVATION IN DRY LAND BELOW 1000 FEET ABOVE SEA LEVEL IN WEST PAHANG AND SOUTH SELANGOR

	Forest	Cultivation
West Pahang	81 %	19 %
South Selangor	36 %	64 %

Clearly a much higher proportion of forest has been felled for development in South Selangor than

[1] Unpublished observations of J. Wyatt-Smith, Senior Forest Research Officer, Federation of Malaya.

[2] "West Pahang" taken as Map Sheets 3B4, 3B8, 3B12, 3C5, 3C6, 3C9 and 3C10 and "South Selangor" as Map Sheets 3B15, 3B16, 3F3, and 3F4 in the 1-inch-to-1-mile Malayan Topographical Sheets (Hind 1035). These correspond to the areas in Fig. 4 of McCrumb et al.

in West Pahang, and it would be fairly safe to deduce that there is a correspondingly lower proportion of valley bottoms under natural forest in the former. If, therefore, infection is related to, say, camping in forest by the sides of streams, then infections are more likely in West Pahang than in South Selangor.

An alternative explanation could, of course, be based on the larger amount of swamp in South Selangor. We know very little about conditions in fresh-water swamp forest. It might be argued that swamp water is an obvious source of leptospiral infection, but we do not know whether infected animals occur in such forest, and swamp (as distinct from swampy areas of otherwise dry forest) may be comparatively free of infection.

Differences in serotype complexity in different habitats

The diminution of numbers of species of animals from forest, through scrub and cultivation, to town was discussed above (page 16). The reduction in the number of serogroups on the scale from forest to town which might be expected to parallel this does not seem to occur: 12 serogroups occurred in forest rats compared with 8 in rats of scrub and cultivation and 7 in town rats. There is, however, a notable difference in the number of predominating serogroups in the different habitats. In house-rats 65% of infections were due to one serogroup (Icterohaemorrhagiae) and 80% to two (Icterohaemorrhagiae and Javanica); in rats of scrub and cultivation 60% of infections were due to one serogroup (Javanica) and 78% to two (Javanica and Canicola); in contrast, the highest proportion of infections in forest-rats due to one serogroup was 23% (Bataviae) and at least seven serogroups are required to account for 80% of infections. In domestic animals 83% of infections were due to three serogroups. So there is an indication that the same gradation of species complexity occurs from forest to town as with animals and, indeed, with plants.

Predation as a cause of incidental infections

Predators large enough to eat infected rats or mice may become infected by so doing. There was no good experimental evidence of this mechanism until van der Hoeden [1] recently reported that when white mice infected with *L. ballum* were fed to hedgehogs a high proportion of the latter became infected. Vysotskii et al. (1958) found high incidences of leptospirosis among foxes and raccoons.

Although the mechanism seems established, its importance in Malaya is difficult to assess. Cats and dogs may obviously be infected sometimes in this way as may civets—*Paradoxurus hermaphroditus* is, however, mainly fruit-eating. The water-snake *Acrochordus*, although large enough to eat rats, apparently eats only fish. *Acrochordus* is almost entirely aquatic while *Homalopsis* is only partially so. *Acrochordus* is more likely to have prolonged contact with the muddy bottom where leptospires may tend to accumulate (van Thiel, 1937).

[1] Personal communication from Professor J. van der Hoeden, Israeli Institute for Biological Research, Ness-Ziona, Israel, to J. C. Broom

CONCLUSIONS

Although evidence of leptospiral infections has been found in a wide variety of vertebrate species in Malaya, maintenance of the organisms in nature appears to depend mainly or entirely on rats. By comparison of excretion rates and antibody rates in rat species, a distinction has been made between highly efficient and poorly efficient maintenance species: species with almost equal rates probably remain infective for life while those with much higher antibody rates than excretion rates probably have fleeting infections. Different serotypes were found to vary widely in their ability to cause antibody formation in rats, so that, for example, with *L. hyos*, which does not stimulate antibody formation in its maintenance hosts, the criterion could not be applied.

Infections of man, domestic animals, and probably of all other wild species than rodents are incidental infections which do not contribute to the long-term maintenance of leptospires in nature, although domestic animals may maintain leptospires for quite long periods. Longer-ranging species, including man, may be important in introducing infection to previously uninfected places and host communities.

Transmission is probably usually through contaminated water, although direct urinary contamination may occur within the nests of rodents and in human habitations. Predators may become infected by eating infected animals.

Human infections in towns are probably usually from the house-rat which, although it has a low excretion rate, is numerous and in close contact with man. Other important species are *R. exulans*, the palm civet, and, near seaports, *R. norvegicus*.

Leptospirosis does not seem to be an occupational hazard for agricultural workers in Malaya, although it is not clear why ricefield workers in close contact with highly infected *R. argentiventer* do not frequently become infected.

The widespread existence of leptospirosis in the Malayan forest was revealed when large numbers of troops operated there. The chief risk of infection is probably in forested valleys near streams, especially in clearings.

Evidence was found of practically every serogroup of leptospires in Malaya and the complexity of strains seems to be greatest in forest. The distribution of specific serogroups in different species and localities and their detailed serological identification and comparison will be discussed in later papers.

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RÉSUMÉ

La leptospirose en Malaisie, étudiée dès 1928, a suscité un regain d'intérêt depuis la deuxième guerre mondiale, du fait que les troupes participant aux opérations militaires ont été fortement infectées. Cette maladie est actuellement reconnue comme l'une des principales causes des maladies fébriles souvent non identifiées. Les auteurs ont entrepris une enquête épidémiologique de grande envergure, afin de déterminer quels étaient les hôtes animaux entretenant l'infection, et quelles conditions favorisaient le passage de l'infection de l'animal à l'homme. De nombreuses espèces animales ont été capturées par diverses méthodes, dans des hameaux forestiers, des prairies, des rizières, des villes et des villages.

Résumant leurs résultats, les auteurs concluent que le maintien de la leptospirose en Malaisie est assuré essentiellement par les rats, bien que de nombreux vertébrés aient été trouvés infectés. Certaines espèces de rats sont plus aptes que d'autres à entretenir l'infection. Ce sont celles dont le taux d'excrétion et le taux d'anticorps sont à peu près équivalents; les animaux excrètent des leptospires pendant toute leur vie. D'autres espèces jouent un rôle moins actif dans le maintien de l'infection; leur taux d'excrétion est faible et l'infection, chez elles, n'est que passagère. Parmi les rongeurs en contact avec l'homme examinés durant cette enquête, *Rattus rattus diardi*, le rat domestique de Malaisie, avait un taux d'infection de 3%, *R. norvegicus*, confiné dans les ports, de 34%, la civette *Paradoxurus hermaphroditus*, de 19%, *R. argentiventer*, des rizières, de 29-53%.

En Malaisie, presque tous les sérotypes de leptospires connus ont été rencontrés. 104 souches ont été isolées.

L'infection de l'homme, des animaux domestiques, et celle de vertébrés autres que les rongeurs, est accidentelle, et ne contribue pas de façon appréciable à la persistance de l'infection dans la nature. Une étude sérologique parmi les animaux domestiques a révélé chez les chèvres la proportion la plus élevée d'animaux porteurs d'anticorps antileptospires (28%), et chez les bovidés la proportion la plus faible (4%). Ces hôtes d'occasion peuvent cependant jouer un rôle en introduisant l'infection dans des zones indemnes où se trouvent les rongeurs réceptifs. L'urine des animaux infectés, ou l'eau souillée, contamine directement ou indirectement l'homme. Dans les villes, l'infection se fait par le rat domestique, qui compense son faible taux d'excrétion par son abondance et son étroite promiscuité avec l'homme.

La leptospirose ne paraît pas être une maladie professionnelle des ouvriers agricoles de Malaisie, et il est même difficile d'expliquer pourquoi les travailleurs des rizières, en contact étroit avec des *R. argentiventer* fortement infectés, ne sont pas plus fréquemment contaminés.

L'existence de la leptospirose dans les forêts malaises s'est révélée au cours de la deuxième guerre mondiale, où la maladie a représenté 41% des cas de maladies fébriles d'origine inconnue. C'est dans les vallées boisées près des cours d'eau, dans les clairières en particulier où pullulent les rats, que les risques d'infection sont les plus graves.

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