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Leptospirosis in Ceylon - Epidemiological & Laboratory Investigation

by

Dr. Kumaraswamy Nityananda; M.B.B.S.(Cey.). Dip. Bact. (Manch.).
Bacteriologist, Medical Research Institute
Colombo - Ceylon.

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U. S. ARMY RESEARCH AND DEVELOPMENT GROUP
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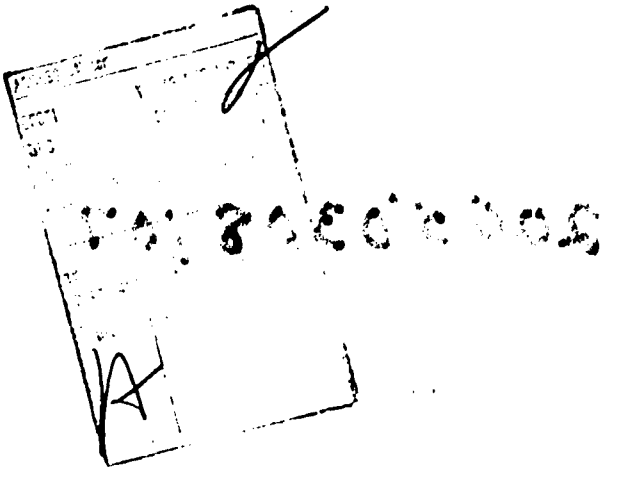


ABSTRACT :

Serological confirmation using DIFCO commercial antigens, was not obtained in every case of leptospirosis even when the organism was isolated from the blood of the patient. One of the possible reasons could have been that the infecting strains in Ceylon were different from those used in the preparation of the DIFCO antigens. Therefore investigations were undertaken to establish the serotypes prevalent and the vectors for them in Ceylon. The methods adopted were i. human and animal tissues and body fluids were cultured direct; ii. tissues from some animals were passaged through hamsters ; iii. surface waters from suspected endemic areas were passaged through hamsters; and iv. serological studies of blood of both humans and animals. Leptospira belonging to the following sero-groups were isolated and vectors for them established; L. javanica L. icterohaemorrhagiae; L.grippotyphosa; L. hebdomadis; L. pomona;and L.autumnalis. The vectors are rodents and dogs. Though large numbers of L. canicola were isolated from patients, no vectors have been established for this . Sera of cattle (30.0 %) and swine (22.1 %) were found to be seropositive for leptospira. The same cattle sera, except for two (2) were seronegative for brucella. These findings are proof that leptospirosis is more widespread among the dairy animals in Ceylon than hitherto recognised and the cause of abortion in the beef herds in our state farms should therefore be more diligently investigated. The isolation of a large number of L. autumnalis and the following hitherto unknown new serotypes, viz: L.icterohaemorrhagiae gem; L.autumnalis lanka; L. grippotyphosa ratnapura; L. hebdomadis javaweera; L. autumnalis alice and L. javanica ceylonica: the first five only in the Ratnapura district is an indication of a possible sylvan reservoir which needs further investigation in addition to the wildlife of the rest of the island.

Introduction

Analysis of the indoor morbidity statistics in government hospitals in Ceylon reveals that amongst the largest groups of morbidity, Pyrexias of Unknown-Origin ranks second to the big group of bowel diseases. The role of leptospirosis in the group of P.U.O. has not been evaluated. Awareness to this disease in Ceylon was created in 1959 (1). Since then Maretic,Z: Arumainayagam,P: Nityananda,K: Wickremasinghe,R.L. and Ratnatunge,P.C.C., 1962 (2): Nityananda,K. 1962 (3): Rajasuriya,K: Munasinghe,D.R.: Vitarane,U.T.: Wijesinghe C.P.de S.: Ratnaike,U.T. and Peiris,O.A. 1964 (4): Wallooppillai,N.J.: Markhus,H.K.N.I. and Nityananda,K. 1966 (5): Nityananda,K. 1967 (6) and Silva,W.A.S.: Mendis,N.M.P. and Nityananda,K. (unpublished) by their studies have shown that leptospirosis is widely prevalent in this country. Yet the epidemiology of this disease has not been investigated, with the result that the public health importance of it is not appreciated. This is reflected very much in the attitude of most public health and veterinary personnel, who refuse to believe that leptospirosis may be prevalent amongst the livestock of this country to a significant degree as to adversely affect the agricultural economy. Tjalma,R.A. and Galton,M.M. in 1965 (7) while discussing the current concepts regarding the public health significance of this disease observed, "the phenomenal apparent spread and establishment of epizootic leptospirosis among livestock has served to create an enormous reservoir and potential source of human infection." In 1954 (7), the United States Department of Aggriculture estimated an annual loss to their aggricultural economy of over 100 million dollars due to leptospirosis.

Dr. A.Bandaranayake, the Deputy Director, Animal Production and Health, in Ceylon states, the incidence of abortion among the livestock in this country is about 5-10 % and this is normal in dairying; the cause according to him is brucellosis (personal communication). Reinhard in 1952 (8) stated that when abortion is the principal sign it may be necessary to obtain laboratory assistance to distiguish leptospirosis from brucellosis, vibriosis or physiological and toxic causes;

Since each leptospiral serotype is believed to have a primary host, it is essential to know the association between the serotypes and their animal hosts in Ceylon before the epidemiology of leptospirosis in this country could be fully understood. This paper describes the work that was conducted to study the vectors and the probable infecting strains of leptospira present in Ceylon.

History of Leptospirosis in Ceylon

The available earliest evidence in record of Weil's Disease having been diagnosed in Ceylon, is in 1953 (9). As the knowledge of the disease increased and with the availability of improved laboratory facilities more cases were diagnosed, confirmed in the laboratory and reported. The majority of the cases were from Ratnapura in the Sabaragamuwa Province, then Ragama, Colombo and Kalutura in the Western Province, Matara in the Southern Province, to a lesser degree from Kandy and Matale in the Central and Anuradhapura in the North Central Province. These reports varied from time to time and place to place depending on the clinician working in thatplace. In 1959 (3) the first leptospira L.icterohaemorrhagiae was isolated from the blood of a patient in Colombo and soon after from the kidney of a sewer rat trapped in the vicinity of that patient's home. Since then upto 19 leptospiral serotypes belonging to 7 serogroups have been isolated and incriminated as the causative agent of leptospirosis in man and/or animals in Ceylon.

L.autumnalis, the elusive and confounding agent of Fort-bragg fever, is described by some workers out side Ceylon as apparently rare as a human pathogen. In Ceylon however, 56 leptospiral isolates obtained from humans in Ratnapura have been identified and 26 (46.4%) of these belong to the L.autumnalis serogroup.

Source of Infection

For many years rats, dogs and pigs were believed to be the primary animal carriers of leptospira. Today as a result of extensive studies the host range (10) has broadened to such an extent that no living agent, both domestic and wild, can safely be excluded. In addition to animals water too has long been recognised as an important vehicle by which pathogenic leptospira are disseminated and infection transferred from animal carriers to human beings. Three classical examples of water being the source of infection are, the outbreak of Fort-bragg fever in 1942 (11) and those described by Schaeffer in 1951 (12) and Bordjoski in 1952 (13).

A few such episodes have been investigated by the author in Ceylon too. In 1964 (5) some members of a gang of labourers dredging a canal at Wattala were taken ill with leptospirosis. In 1967 at Panadure a party of volunteers composed of civilian and police personnel worked ankle to knee deep to reclaim marshy land, as part of a Shramadana-Campaign (community-

welfare). Out of these, I2 (37.5%) were subsequently taken ill with leptospirosis. Further the majority of the patients investigated at the Ratnapura hospital stated that bathing in the river Kaluganga or panning for gems along the jungle streams as the probable source of their infection.

Materials and Methods

a. Materials

Rodents were trapped alive wherever possible, brought to the leptospirosis laboratory at the Medical Research Institute, anaesthetised and opened up under strict aseptic conditions. Blood was obtained from the heart and kidney plugs taken with sterile pasteur pipettes. Heart blood and kidneys from cattle and swine were taken at the time of their slaughter in the municipal slaughter house in Colombo. Heart blood and kidneys from stray dogs were taken soon after these animals were gassed at the municipal dog pound.

The rodents investigated were; Rattus norvegicus (R.n.) - the sewer rat: Gunomys gracilis (G.g.)- Ceylon mole rat, also known as the paddy field rat: Rattus rattus rufuscens (R.r.r.) - Ceylon flat-country house rat: R.r.kandyanus (R.r.k.) - Ceylon hill-country house rat: Bandicoota malabarica (B.m.) - bandicoot and Suncus caeruleus giganteus (S.c.g.) - the shrew, which is a member of the carnivores family.

b. Methods

i. Kidney tissues of all animals were inoculated direct into Fletcher's semi-solid medium, and/or ground up, suspended in sterile normal saline and passaged through hamsters.

ii. Blood was examined for evidence of leptospiral agglutinins from, (a) rat pound workers who are of occupational risk: (b) daily river bathers at Ratnapura which is a suspected endemic area and, (c) cattle, dogs and pigs.

iii. Surface waters from suspected endemic areas (Ratnapura and Ragama) were passaged through hamsters. These hamsters which were inoculated, if they died between the 6th and I4th day after inoculation, were opened up and their liver tissue inoculated into Fletcher's semi-solid medium. No leptospira were isolated from these waters.

Findings

a. Isolations. Kidney tissues from a total of 1611 animals were processed. These included, 592 rodents of 5 species, 3 shrews, 359 cattle, 351 dogs, 293 swine, 5 rock-squirrel, 2 red-mongoose, 2 grey-mongoose, 1 mongoose, 1 hare, 1 civet cat, and 1 field rat. The number of leptospira isolated is shown in table I.

Table I. Leptospira isolated from rodents, shrews and dogs in Ceylon.

	rodent species						dogs
	R.n.	G.g.	R.r.r.	R.r.k.	B.m.	S.c.g.	
Number examined	18	154	117	98	100	3	351
number of leptospira isolated	7	49	0	0	2	1	10

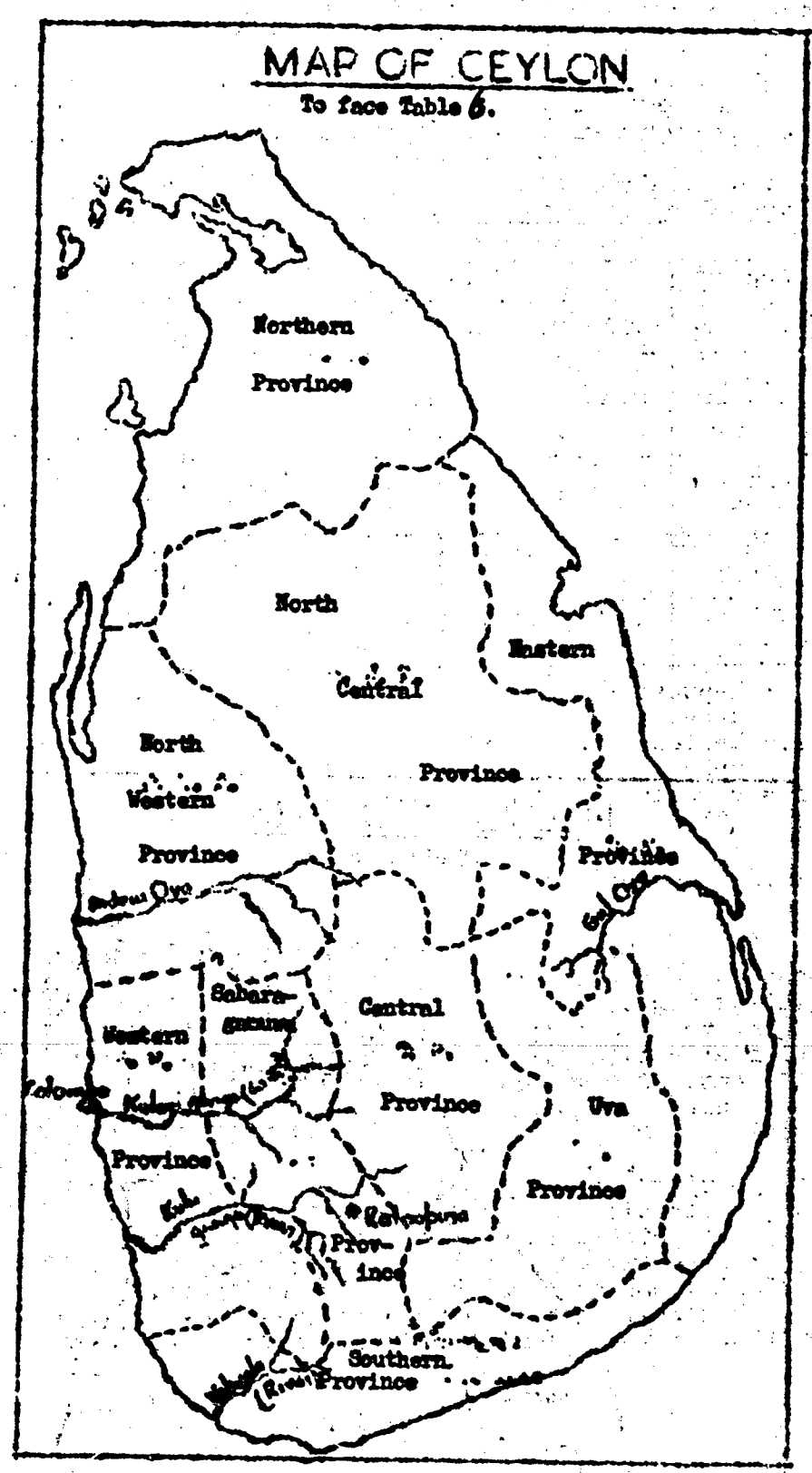
105 unidentified rodents were also examined and 8 isolates were obtained from these.

Table 2. Distribution of the leptospira isolated in Ceylon according to source and sero-group.

Source	sero-groups						
	ict.	can.	jav.	aut.	heb.	grip.	pom.
Human	+	+	±	+	+	+	o
R.n.	+	o	o	o	o	o	o
G.g.	+	o	+	o	o	o	o
B.m.	o	o	+	o	o	o	o
S.c.g.	o	o	±	o	o	o	o
Rodents unidentified	+	o	+	+	o	o	o
Dogs	+	o	+	o	+	o	+

MAP OF CEYLON

To face Table 6.



ict. = icterohaemorrhagiae: can. = canicola: jav. = javanica: aut. = autumnalis: heb. =hebdomadis: grip. = grippotyphosa: and pom. = pomona.
+ = leptospira of that serogroup isolated from that source.
o = leptospira not isolated.
± = two different isolates, but belonging to the same new serotype - L. jav. ceylonica, (14).

Table 3. Distribution of the leptospira isolates according to the provinces in Ceylon.

Name of province	Serogroups							
	ict.	can.	jav.	aut.	heb.	grip.	pom.	wolf.
Western	i/s	i/s	i	i	o	o	i/s	o
Central	i	s	i	o	o	o	o	o
Sabaragamuwa	i	o	o	ii	i	i	o	o
North - western	o	s	i	o	o	o	s	o
North - central	o	o	o	o	o	o	o	s

i = leptospira of that serogroup isolated in that area.
ii = leptospira of that serogroup isolated in that area in large numbers.
s = serological evidence only.
o = nil.

b. Serological survey

Sera were examined for the presence of leptospiral agglutinins by the microscopic agglutination (agglutination lysis) test employing live cultures belonging to 21 serotypes, as antigens. Agglutination reaction at a titre of 1:100 or more was considered significant. The employment of multiple screening antigens revealed a significantly high prevalence of sero-reactors.

i. Rat pound workers (rat catchers). The Colombo Municipal Council maintains a regular anti-plague campaign in which a permanent labour force distributes or lays rat cages in the city's sewers, surface drains and house gardens, collect these cages the following morning with the trapped live rodents, transport them in vans to the central rat depot and destroy

them. Most of the Ceylon mole rats, sewer rats, shrews and bandicoots from which there have been a high percentage of leptospira isolations by the author, were supplied alive from this campaign. These labourers walk bare-footed and work with their unprotected hands. They therefore are liable to injury and subsequent contamination directly with the urine of these rodents during collection and transport or indirectly from the polluted floor boards of the vans. Therefore one would expect a reasonable percentage of seropositivity among these labourers. The sera from 26 of these labourers who had been engaged in this work over several years were examined and all were seronegative. These findings are similar to those of Dr. J.C.Broom (15) and Dr. Jan W.Wolf (15), who investigated inhabitants of rat infested dumps. Both found that though the rats in those areas were carriers of leptospira, none of the human beings living there showed evidence of infection.

ii. Random samples Blood was examined from persons residing in Ratnapura bazzar area, who bathe regularly in the Kaluganga river. Of the 70 sera tested for leptospira agglutinins 19 (27.1%) were seropositive.

iii. Cattle Though no leptospira has been isolated from the kidneys of cattle and swine, there is strong serological evidence that both these are carriers of leptospira in Ceylon.

Out of 359 cattle sera examined 108 (30.0%) were positive for leptospira agglutinins (Table 4). If so small a sample can yeild so many positives the problem on a national basis must be one of tremendous proportions. The majority of the seropositives were against L.wolffi. All the cattle sera investigated for leptospirosis were also tested for brucellosis and only 2 specimens out of the 359 (0.5%) were positive. Therefore the cause of abortion in the cattle in Ceylon requires a more diligent investigation.

iv. Swine Of the 293 swine sera examined 65 (22.1%) were seropositive (Table 5). The majority of the positive reactors were against L.pomona and L.canicola. Apparently leptospirosis in swine is more widespread in this country than hitherto recognised. Though serum samples P-209 and P-212 were positive in very high titres, 1:25600, it was unfortunate that no leptospira were isolated from the kidneys of these two animals.

Table 4 Some of the results of serology of Cattle sera (Ce

Specimen Nos.	C 4	C 8	C 11	C 13	C 17	C 28	C 33	C 48	C 85	C 108	C 111	C 119	C 120	C 150	C 151	C 157
Antigen		100														
ballum		200			100											
canicola		100														
ictero																
bataviae			100					100								
grippo																
pyrogenes																
autumnalis									400							
pomona	100			400								400				
wolffi		400	1600	100	100					100	100		100	400	100	400
australis																
tarassovi								400		100	100					
LT.117																
fort bragg	100														400	
hardjo																
Javanica						100	100						100	100		
scntot									100						100	
borincana						100	400						100	100		100
alexi																
djasiman												1600				
cynopteri																100
celladoni							100									

ictero = icterohaemorrhagiae. grippo = grippotyphosa

rology of Cattle sera (Ceylon)

08	C 111	C 119	C 120	C 150	C 151	C 157	C 164	C 201	C 206	C 213	C 214	C 216	C 217	C 219	C 226	C 227	C 228	C 236	C 247	C 259
								400												
								400												
								1600												
							100													
							100	100												
														100		100		400		
		400																		
00	100		100	400	100	400			200	1600	400	400	400	400	1600		100		400	10
										400						100	100			
00	100						100		100											
										100					100					
					400													100		
			100	100																
					100															
			100	100		100			400	100	100		100		100		100		400	
										100	1	100								
		1600																400		
						100														

grippo = grippotyphosa

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Table 5 Some of the Results of serology of swine ser

Spesimen Nos.	P 4	P 11	P 26	P 35	P 39	P 41	P 44	P 45	P 46	P 66	P 76	P 87	P 90	P 97	P 106
Antigen															
ballum					400						400				
canicola	400			100	6400			1600	1600	1600	1600				
ictero					1600		100								
bataviae															
grippo															
pyrogenes	100				6400	100									
autumnalis						100						400	100		
pomona		400	6400		400		6400						1600	400	1600
wolffi															
australis															
tarassovi															
LT.117															
fort bragg															
hardjo															
javanica															
sentot															
borincana															
alexi															
djasiman						400									
cynopteri															400
celladoni				400											

ictero = icterohaemorrhagiae. grippo = grippotyphos

ne sera.

P 7	P 106	P 115	P 125	P 151	P 161	P 164	P 172	P 194	P 209	P 212	P 214	P 221	P 223	P 232	P 234	P 239	P 253
		100															
		1600										400					
		100			100		100										
																	100
		1600															
			100	100	1600				1600			100					100
00	1600				1600		1600	1600	856 00	25600	400	400	1600	100	1600	1600	
						400											100
					400				1600	400		400					400
									6400								100
				100	400	100	400			1600							
											100						
			100	100	400				1600	1600				400			
	400				400				1600		400		100				

9a

otyphosa.

Table 6 Some of the Results of Serology of Dogs' sera.

Specimen Nos.	D 8	D 14	D 16	D 19	D 21	D 24	D 35	D 72	D 93	D 115	D 119	D 130	D 131	D 150	D 161	D 162
Antigen																
ballum														100		
canicola	400	100	100					1600						100		100
ictero							1600			1600						
bataviae																
grippo.																
pyrogenes							400									
autumnalis			400	400					100							
pomona																
wolffi																
australis																
tarassovi																
LT. 117													100			
fort bragg			1600						100							
hardjo																
Jav. nica										400				100		
sentot																
borincana												100	1600			
alexi															100	
dgasiman																
cynopteri																
ccelladoni					100	100										

ictero = icterohaemorrhagiae. grippo = grippotyphosa.

32	D 193	D 194	D 246	D 287	D 292	D 294	D 303	D 304	D 308	D 316	D 327	D 328				
		100				[illegible]										
)	100			100		400		100	100							
			1600	400	6·00		100		1600		400	100				
	100		100	100		100		100	400							
		100														
					100											
	100				400				100	100						
			400													
	100				100											

10a

v. Dogs In the case of dogs 351 specimens were processed. There were 10 isolations of leptospira and 31 (8.8%) seropositives (Table 6). Though the majority of the seroreactors were against L.canicola none of the isolates belong to the canicola serogroup.

Table 7. Results of serotyping of isolates and serology from 3 dogs: D-16, D-19 & D-32.

Specimen No.	Results of serology				isolate identified as
	can.	aut.	Pt.br.		
D-16	100	400	1600	negative against all others.	L.hebdomadis
D-29	negative against all serotypes.				L.javanica
D-32	- do -				L.pomona

The leptospira isolated from the kidney of dog -16 has been identified as L.hebdomadis whereas the serum from the same animal is negative against the homologus antigen, but positive against L.canicola, L.autumnalis and L.Fort bragg antigens. Further the leptospirae isolates from the kidneys of dog-29 and 32 have been identified as L.javanica and L.pomona respectively, but the sera from these animals are seronegative against the full range of antigens belonging to the 21 serotypes including the homologus.

Discussion

Leptospirosis is associated with a broad animal host spectrum and is transmitted from the animal carriers to other animals and man. In the investigation of possible reservoirs among wild and domestic animals, serological investigation alone may provide useful clues; at the same time they may provide an erroneous or misleading index of infectivity rates, since some seronegative animals may be carriers. This limitation is very clearly shown in table 7: Dogs - 29 and 32. The total absence of antibodies in carriers as in these dogs, though cannot be satisfactorily explained, has been met with. Mrs. Sulzer from the leptospirosis unit of the National Communicable Diseases Centre in Atlanta states, 'we once had a laboratory dog that had no antibodies but we isolated leptospira from the urine of this dog for once a month for as

long as one year after disease' (personal communication).

Almost all homes in Ceylon are rat infested, either with R.r.rufuscens or R.r.kandyanus. From the findings of this study it is evident that the above two species of rattus are not vectors for leptospira in Ceylon; a finding of great relief indeed. The demonstration of L.pomona infection in the dog prompts the potential role of the dog in the infectious cycle of human and animal leptospirosis. In addition to L.canicola, L.icterohaemorrhagiae and L.pomona, dogs can be infected and can serve as a potential source of infection with numerous other serotypes. These include L.hebdomadis, L.javanica (Table 2); L.australis, L.autumnalis, L.bataviae, L.medinensis and L.hyos 1957 (16).

While L.icterohaemorrhagiae, L.javanica, L.canicola, L.autumnalis, L.hebdomadis, L.grippotyphosa, L.pomona and L.wolffi appear to be the primary serotypes involved in human and domestic animal infection in Ceylon, the detection of new serotypes hitherto unknown; viz: L.ictero. gem.(17), L.jav. ceylonica, L.grippo. ratnapura (18), L.hebdo. jayaweera (18), L.autum. alice (18) and L.autum.lanka (19), emphasizes the need for public health, medical and veterinary laboratories to maintain a greater awareness to this disease. Further studyof the new feral mamals will be necessary to determine the prevalence of infection in other parts of the island and to determine their role in the epidemiology of this disease.

Summary

The evidence established so far shows that in Ceylon, rodents, dogs, cattle and swine are carriers of leptospira. The infecting strains are L.icterohaemorrhagiae, L.canicola, L.grippotyphosa, L.hebdomadis, L.javanica, L.autumnalis, L.pomona and L.wolffi.

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11. SUPPLEMENTARY NOTES	12. SPONSORING MILITARY ACTIVITY
	U.S. Army R&D Group (Far East) APO San Francisco 96343

13. ABSTRACT

Investigations were undertaken to establish the serotypes prevalent and the vectors for them in Ceylon. The methods adopted were: i. human and animal tissues and body fluids were cultured direct; ~~ii.~~ tissues from some animals were passaged through hamsters; iii. surface waters from suspected endemic areas were passaged through hamsters; and ~~iv~~ serological studies of blood of both humans and animals. Leptospira belonging to the following sero-groups were isolate and vectors for them established; L. javanica; L. icterohaemorrhagiae; L. grippotyphosa; L. hebdomadis; L. pomona; and L. autumnalis. The vectors are rodents and dogs. (Author)

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Unclassified

Security Classification

14. KEY WORDS	LINK A		LINK B		LINK C	
	ROLE	WT	ROLE	WT	ROLE	
Leptospirosis						
Epidemiology						
Serology						
Reservoir						
Population						
Vectors						
Soil						
Water						
Ceylon						