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Leptospirosis in Ceylon—Epidemiological and Laboratory Studies*

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INTRODUCTION

Analysis of morbidity statistics in Government hospitals in Ceylon reveals that among the causes of morbidity, Pyrexias of Unknown Origin (P.U.O.) rank second only to the enteric diseases. The contribution of leptospirosis to the group of P.U.O. has not been evaluated. Awareness of this disease in Ceylon was created by Falisevac and Arumainayagam (1959). They wrote, " Since the climatic, geographic and economic and other conditions in Ceylon are similar to those in Indonesia, Malaya and other countries in which leptospirosis is prevalent, one should presume that this disease should be very common in Ceylon as well. On a visit to the General Hospital, Colombo, we saw a case which had the history of 'unicteric leptospirosis'; subsequent serological examination confirmed our suspicion ". Since then Maretic, Arumainayagam, Nityananda, Wickremasinghe and Ratnatunge, (1962), Nityananda (1962), Rajasuriya, Munasinghe, Vitarane, Wijesinghe, Ratnaike and Peiris (1964), Wallooppillai, Markhus and Nityananda (1966) and de Silva, Mendis and Nityananda (unpublished), by their studies have shown that leptospirosis is widely prevalent in this country. The epidemiology of this disease has not been investigated, with the result that the public health importance of it is not fully appreciated. Tjalma and Galton (1965), while discussing the current concepts regarding the public health significance of this disease, observed that "the phenomenal spread and establishment of epizootic leptospirosis among livestock has served to create an enormous reservoir and potential source of human infection ". In 1954 the United States Department of Agriculture estimated an annual loss to their agricultural economy of over 100 million dollars due to leptospirosis.

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

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MATERIALS AND METHODS

Rodents were trapped alive wherever possible, brought alive to the leptospirosis laboratory at the Medical Research Institute, anaesthetized, and the kidney tissues and heart blood were removed under strict aseptic conditions. In the case of cattle and swine, heart blood and kidneys were removed at the time of their slaughter in the Municipal slaughter house, and in the case of dogs the specimens were taken soon after they were gassed at the Municipal dog pound.

Kidney tissues from a total of 1,716 animals were processed. These included 592 rodents of 5 species, 111 unidentified rodents (which included 5 rock squirrels) 359 cattle, 351 dogs, 293 swine, 3 shrews, 5 mongooses, 1 hare and 1 civet cat. The rodents investigated were: *Rattus norvegicus* (the sewer rat) *Gunomys gracilis* (Ceylon mole rat or paddy field rat); *Rattus rattus rufescens* (Ceylon flat country house rat) *Rattus rattus kandianus* (Ceylon hill country house rat) and *Bandicota malabarica* (the bandicoot).

Kidney tissues of all animals were inoculated directly into Fletcher's semisolid medium, and/or ground up, suspended in sterile normal saline and passaged through hamsters. Surface waters were also passaged through hamsters in the search for leptospira.

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

The sera were examined by the microscopic agglutination (agglutination lysis) test, employing as antigens live cultures belonging to 21 serotypes (Wolff, 1954). Agglutination reaction at a titre of 1 : 100 or more was considered significant. The employment of multiple screening antigens revealed a high prevalence of seroreactors.

RESULTS

(i) *Isolation*

The results of isolation and serology are shown in Tables 1 to 4. Several specimens showed cross reactions. Some of these are shown in Table 5.

During this study there were 49 isolations of leptospira from the mole rats, 7 from sewer rats, 2 from bandicoots, 8 from *Rattus sp.* 1 from a shrew and 10 from dogs. These belong to the following serogroups: Icterohaemorrhagiae, Canicola, Javanica, Autumnalis, Hebdomadis, Grippotyphosa and Pomona (Tables 1 & 3).

No leptospira were isolated from the kidneys of cattle and swine and from surface waters.

(ii) *Serological Survey—Random Samples*

a. *River bathers*

Of the 70 sera tested from persons residing in the Ratnapura bazaar area and who bathe regularly in the Kaluganga river, 19 (27.1%) were found positive for leptospiral agglutinins.

b. *Rat pound workers (Rat catchers)*

The sera from 26 of these labourers, who had been engaged in rat catching over several years, were examined and all were negative.

TABLE 1

Leptospira isolated in Ceylon

Serogroup	Serotype	MAN	ANIMAL HOSTS					
			Dog	R.n.	G.g.	B.m.	R.sp.	S.c.g.
Icterohaemorrhagiae	*icterohaemorrhagiae*	+	+	+	+	+	+	+
	gem	+						
Canicola	*canicola*	+						
	malaya	+						
Autumnalis	*autumnalis*	+						
	sumatrana	+						
	bulgarica	+						
	weerasinghe	+						
	alice	+						
	lanka	+						
	bangkinang						+	
Grippotyphosa	*ratnapura*	+						
	grippotyphosa				+			
Hebdomadis	*hebdomadis*		+					
	jeyaweera	+						
	ricardi	+						
Javanica	*javanica*		+		+	+	+	
	ceylonica	+						+
Pomona	*pomona*		+					

R.n. = *Rattus norvegicus* B.m. = *Bandicota malabarica* S.c.g. = *Suncus caeruleus giganteus.*
G.g. = *Gunomys gracilis* R.sp = *Rattus* species

TABLE 2

Leptospira isolated from animals in Ceylon

Animals Examined	No. Examined	No. in which Leptospira were isolated
Rodents		
Rattus norvegicus	18	7
R.rattus rufescens	117	0
Gunomys gracilis	154	49
Bandicota malabarica	100	2
Rattus sp.	105	8
R. r. kandanus	98	0
Insectivores		
Suncus caeruleus giganteus	3	1
Carnivores		
Dogs	351	10

TABLE 3

Distribution of the Leptospira isolated and the seroreactors according to the provinces in Ceylon

Serogroups	Provinces				
	Western	Central	Sabara-gamuwa	North Central	North Western
Icterohaemorrhagiae	+	+	+	—	—
Canicola	+	S	—	S	—
Javanica	+	+	—	+	—
Autumnalis	+	—	++	—	—
Hebdomadis	—	—	+	—	—
Grippotyphosa	—	—	+	—	—
Wolffi	—	—	—	—	S

\+ = Leptospira isolated in small numbers
++ = Leptospira isolated in large numbers
S = Serological evidence only
— = Nil.

c. *Cattle* (*Table* 4)

Though no leptospira have been isolated from cattle, of the 359 cattle sera examined, 108 (30.1%) were positive for leptospiral agglutinins. If so small a sample can yield so many positives, the problem on a national basis must be one of tremendous proportions. The majority of the seropositives were against *wolffi*. All the cattle sera investigated for leptospirosis were also tested for brucellosis and only 2 (0.56%) specimens out of the 359 were seropositive. Therefore the cause of abortion in the cattle in Ceylon requires a more diligent investigation.

TABLE 4.

Positive results of microscopic agglutination tests for leptospiral antibodies on cattle, dog and swine sera in Ceylon

Serotype	Cattle (Total 359)	Dogs (Total 351)	Swine (Total 293)
ballum	5 (I—III)	1 (I)	5 (I—III)
canicola	4 (I—III)	12 (I—V)	17 (I—VII)
icterohaemorrhagiae	5 (I—V)	12 (I—VII)	6 (I—V)
bataviae	3 (I)	—	1 (I)
grippotyphosa	1(I)	—	—
pyrogenes	6 (I—III)	9 (I—III)	8 (I—VII)
autumnalis	7 (I—III)	3 (I—III)	14 (I—III)
pomona	7 (I—III)	—	27 (I—IX)
wolffi	56 (I—V)	—	2 (I—III)
australis	4 (I)	1 (I)	—
tarassovi	13 (I—III)	—	—
LT 117	4 (I)	1 (I)	—
Fort bragg	8 (I—III)	2 (I—V)	7 (I—V)
javanica	6 (I)	6 (I—III)	2 (I—VII)
sentot	4 (I—II)	1 (III)	5 (I—VII)
borincana	22 (I—V)	2 (I—V)	1 (I)
alexi	2 (I)	1 (I)	—
djasiman	4 (I—III)	—	8 (I—VII)
cynopteri	3 (I—III)	—	7 (I—III)
celledoni	2 (I)	4 (I)	1 (III)

Roman numerals indicate the degrees of positivity

1: 100 = I
1: 200 = II
1: 400 = III
1: 800 = IV etc.

Arabic numerals indicate No. of sera giving the agglutination with respect of serotype.

TABLE 5.

Some examples of the cross reactions of the animal sera.

Serotype	Cattle Serum No.					Pig Serum No.					Dog Serum No.			
	33	150	201	213	226	39	161	209	212	221	16	287	292	342
ballum			400			400								
canicola			400			6400	100			400	100	100	6400	100
icterohaemorrhagiae			1600			1600						400		400
pyrogenes			100			6400						100	400	100
autumnalis				400			1600	1600		100	400			
pomona						400	1600	25690	25600	400				
wolffi		400		1600	1600									
LT 117				100	100									
fort-bragg							400	1600	400	400	1600			
javanica	100	100						6400					400	
sentot							400		1600					
borincana	400	100		100	100									
djasiman							400	1600	1600					
cynopteri							400	100						
celledoni	100													

d. *Swine* (*Table* 4)

Of the 293 swine sera examined, 65 (22. 1%) were positive. The majority of the positive reactions were against *pomona* and *canicola*. Apparently leptospirosis in swine is more widespread in this country than hitherto recognised. Though serum samples P-209 and 212 were positive in very high titres- 1 : 25600, no leptospires were isolated from the kidneys of these two animals.

e. *Dogs* (*Table* 4)

From 351 canine specimens processed, there were 10 isolations of leptospira and 31 (8.8%) seropositives. Though the majority of the seroreactors were against *icterohaemorrhagiae and canicola*, none of the isolates belong to this serogroup.

The leptospira isolated from the kidney of dog 16 has been identified as *hebdomadis*, whereas the serum from the same animal was negative against the homologous antigen but positive against *canicola*, *autumnalis*, and *fort-bragg*. Further, the leptospiral isolates from

the kidneys of dogs 29 and 32 have been identified as *javanica* and *pomona* respectively, but the sera from these animals are negative against the full range of antigens including the homologous (Table 6).

TABLE 6.

Results of serotyping of isolates and serology from 3 Dogs: D-16, D-29 and D-32.

	D—16	D—29	D—32
Serology			
canicola	1 : 100	—	—
autumnalis	1 : 400	—	—
fort-bragg	1 : 600	—	—
Other Serotypes	—	—	—
Isolate	*hebdomadis*	*javanica*	*pomona*

DISCUSSION

Leptospirosis is associated with a broad animal host spectrum and is transmitted from the animal reservoir to other animals and man. For many years rats, dogs, and pigs were believed to be the primary world wide animal carriers of leptospira. Today, as a result of extensive studies the known host range has broadened to such an extent that no living agent, either domestic or wild can safely be excluded (Galton, 1966).

The earliest report of Weil's Disease or Leptospirosis icterohaemorrhagiae, in man in Ceylon, was in 1953 (Kahawita, 1954). As knowledge of the disease increased and improved laboratory facilities became available, more cases were diagnosed, confirmed in the laboratory and reported. The majority of the cases were from Ratnapura in the Sabaragamuwa Province, Ragama, Colombo and Kalutara in the Western Province, Matara in the Southern Province, and to a lesser degree from Kandy and Matale in the Central and Anuradhapura in the North Central Provinces (Nityananda, 1967). These reports varied from time to time and from place to place depending on the clinician working in that place. In 1959, the first leptospira serotype *icterohaemorrhagiae*, was isolated from the blood of a patient in Colombo (Nityananda, 1962). Soon after, the same serotype was isolated from the kidney of a sewer rat trapped in the vicinity of that patient's house.

Water, too, has long been recognised as an important vehicle by which pathogenic leptospira are disseminated from animal carriers to human beings. Three classic examples of waterborne infection are the outbreaks described by Schaeffer (1951), Bordoski (1952) and the Kennewicki-Washington state outbreak in 1954 (Sulzer, 1970). A few such episodes have been investigated by the author in Ceylon. In 1964 some members of a group of labourers dredging a canal at Wattala were taken ill with leptospirosis (Walloop-pillai *et al.*, 1966). Some years later at Panadura, a party of 32 volunteers composed of civilians and police personnel worked ankle to knee-deep in water to reclaim marshy land

as part of a 'Shramadana Campaign' (Community Welfare). Out of this number, 12 (37.5%) were subsequently taken ill with leptospirosis (Nityananda, 1967). Further, infection in most cases of leptospirosis investigated at the Ratnapura hospital were attributed to bathing in the Kaluganga river or panning for gems along the jungle streams (de Silva *et al.*, unpublished).

In the survey of possible reservoirs among wild and domestic animals, serological investigation alone may provide useful clues; at the same time it may provide an erroneous or misleading index of infectivity rates, since some seronegative animals may be reservoirs. This limitation is very clearly shown in Table 6, dogs 29 and 32. The total absence of antibodies in carriers as in these dogs is not unknown. Sulzer (1969) stated that the Leptospirosis Unit at Atlanta, once had a laboratory dog that had no antibodies but that leptospires were isolated from the urine of this dog once every month for as long as one year after infection.

The demonstration of *pomona* infection in the dog points to the potential role of the dog in the infection cycle of human and animal leptospirosis. In addition to *pomona* and *icterohaemorrhagiae* (Table 1), dogs can be infected with and can serve as a potential source of infection for numerous serotypes. These include *hebdomadis*, *javanica*, (Table 1), *australis*, *canicola*, *autumnalis*, *bataviae*, *medenensis* and *tarassovi* (Alexander, Gleiser, Malnati and Yoder, 1957).

The negative serological results of the ratpound workers are also of interest. These labourers are employees of the Colombo Municipality and are attached to the Anti-Plague Campaign. They distribute 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 leptospiral isolations were from the mole rats, sewer rats, shrews and bandicoots brought by these workers. These labourers work barefoot with unprotected hands. They are liable to injury and subsequent contamination direct with the urine of these animals during collection and transport. Therefore, one would expect a reasonable percentage of seropositivity among these labourers. These findings are similar to those of Broom (1952) and Wolff (1952) who investigated inhabitants living in rat and vole-infested dumps. Both found that though the rats and voles were carriers of leptospira none of the humans living there showed any evidence of infection. In an earlier study too, Nityananda (1967) had reported that though the sewer rats trapped in the sewers of Colombo were carriers of leptospira, the sewer workers in Colombo did not show any evidence of leptospiral infection. These findings in a way pose the question, what are the factors that control human infection ?

The results of serology of cattle sera show that there is stronger evidence of leptospirosis than brucellosis in the cattle in Ceylon. Therefore, though, Bandaranaike (1966) had stated that the incidence of abortion among the cattle in the state farms in this country was about 5-10% and this was normal in dairying, the cause of abortion among the cattle in the state farms in this country warrants more diligent investigation, for according to him the cause is brucellosis. The observations of Reinhard, Tierny and Roberts (1950), in this

context, is very enlightening; "When abortion is the principal sign in cattle, it may be necessary to obtain laboratory assistance to distinguish leptospirosis from brucellosis, vibriosis or physiological causes".

L. *autumnalis* has been described by workers outside Ceylon as a rare human pathogen (Tjalma and Galton, 1965). In Ceylon, however, of 56 leptospiral isolates obtained from humans in Ratnapura, 26 (46.5%) belong to the Autumnalis serogroup (de Silva *et al.*, unpublished).

The primary serotypes involved in human and domestic and wild animal leptospiral infection appear to be *icterohaemorrhagiae*, *javanica*, *canicola*, *autumnalis*, *pomona*, *hebdomadis* and *grippotyphosa*. The detection of the hitherto unknown new serotypes *ceylonica* (Nityananda and Sulzer, 1969), *lanka* (Nityananda and de Sulzer., 1970), *ratnapura*, *jeyaweera* and *alice* (Cernuha, Kokovin, Saharceva, Nityananda, de Silva, Mendis and Perera, 1968), emphasizes the need for public health, medical and veterinary laboratories to maintain a better surveillance of this disease. Further study of other feral mammals 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 presented so far shows that in Ceylon, rodents, dogs and possibly cattle and swine are reservoirs of leptospira. In addition to the known infecting leptospira strains *icterohaemorrhagiae*, *canicola*, *grippotyphosa*, *javanica*, *hebdomadis*, *autumnalis*, *pomona* and *wolffi*, the following new serotypes; viz; *ceylonica*, *jeyaweera*, *lanka*, *ratnapura* and *alice* were recently isolated involving man and animals. The prevalence of this organism in Ceylon suggests that better surveillance of this disease is needed.

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