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THE IMPORTANCE OF LEPTOSPIROSIS IN MALAYA

By Dora S. K. Tan, M.B., B.S.
Virus Research Officer, Insitute for Medical Research,
Kuala Lumpur, Malaysia.

INTRODUCTION

Although the significance of leptospirosis as a major cause of febrile diseases in civilians and military personnel in Malaya has been established for the past 10 years (Broom, 1953; Robinson and Kennedy, 1956; McCrumb *et al.*, 1957) it would appear that this fact is far from being adaquately recognised by general practitioners and government medical officers in this country. Danaraj (1950), Trimble (1954) and Turner *et al.* (1959) pointed out that many cases of leptospirosis escaped recognition either because the actual clinical features of leptospirosis did not always conform with the generally accepted picture of it (i.e. Weil's disease) or because clinicians failed to consider it in the differential diagnosis of febrile illnesses. All three papers were published in local journals, but apparently escaped the attention they deserve.

The main purpose of this paper is to emphasise the following points: (a) leptospirosis is much more common in Malaya than is generally realised (b) leptospirosis can be mild and may even be subclinical and deceptive (c) leptospirosis can be diagnosed easily by a relatively new serological test called the *S*ensitized *E*rythrocyte *L*ysis (SEL) test (see below) or by culture methods.

LEPTOSPIROSIS IS COMMON IN MALAYA

Studies of Malayan leptospirosis have revealed a high prevalence of leptospiral antibodies in human beings, domestic and wild animals — especially in wild rodents (Wisseman *et al.*, 1955; Smith *et al.*, 1961; Annual Reports of the IMR, 1955 —). Moreover, the presence of a multiplicity of leptospiral serotypes, about 30 in number, in Malaya was determined by serologic and cultural studies by Alexander and his colleagues (1955 and 1957).

Out of a recent study of 584* cases of pyrexia of unknown origin (PUO) over a period of 4½ years (June 1958 to December 1962) 173 (29.6%) were found to be positive, the diagnosis being based on blood cultures, significant serological (SEL) titres, or both (table I). Increases in SEL titres of 16-fold or more in paired sera were observed by Chang *et al.* (1957) in his study of 40 proved cases of leptospirosis. A similar increase was found in 74.0% of the cases considered as

TABLE I.

Results of the test for leptospirosis in 173 positive patients

Titres	No.	%		Remarks
16-fold or more increases	128	74.0		Diagnostic
Positive cultures	14	8.0	89.4%	,,
* Less than 16-fold increases in paired convalescent sera	11	6.3		,,
Stationary Titres				
320 or 640	3	1.7		
1280	5	2.9	11.6%	Presumptive positive
† 5120 or more	12	6.9		

* The intervals between the dates on which the sera were taken were less than 4 days. The SEL titres range from 320 to 5120 or more.

† Maximum dilution employed in test.

positive in our study. Not all paired sera were obtained at optimal times during the course of the illness and sera with high titres (320 to 5120 or more) but increases of less than 16-fold (often 4-fold or more) were encountered in 11 patients from whom the blood specimens were taken during convalescence and at less than 4 days' interval apart. Twenty cases showed no rise in titre but the titres were high (320 to 5120 or more) and were therefore presumably indicative of recent infection (Cox, 1957). We have therefore regarded this last group as one of "presumptive positive" cases.

Leptospires were isolated from blood specimens of 14 patients in this study. Out of 12 of these sent to Dr. L. H. Turner of the Wellcome Laboratories of Tropical Medicine in London for identification, 11 have been identified to-date. The remaining 2 were not available from the laboratory which isolated them. The serotypes of the 14 isolates and the localities from which they were obtained are outlined below:

Kuala Lumpur: *caniloca* (2), *pomona* (1)
Tampin: *canicola* (1)
Bentong: *pyrogenes* (1), *autumnalis* (1)
Klang: *pyrogenes* (1)
Mentakab: *autumnalis* (1)
Malacca: *pyrogenes* (3), unidentified (2)
Penang: unidentified (1).

Table II shows the localities from which paired specimens of the PUO patients were received. The results would seem to indicate that an overall proportion of nearly 1 in 3 PUO cases in the Federation can be considered as being due to leptospirosis. The proportion, however varies from locality to locality. While in Kuala Lumpur and some other areas the proportion is approximately 1 in 5, in certain areas it is considerably higher — e.g. it is 1 in 2 in Melaka and 3 in 5 in Penang. The figures for places other than those commented on are too small for any valid conclusions to be drawn. It is regretted that an urban/rural analysis is not yet available owing to lack of data.

Observation was made also of the occupations of patients diagnosed as having leptospirosis (table III). It is noteworthy that rubber estate workers are apparently very highly infected. So also is a group classified as labourers in which are included those dealing with sewage, drainage, town-cleansing, forestry and anti-malarial work. It is unfortunate that full details are not available for the purpose of sub-grouping the large number of labourers listed as "Miscellaneous." Farm workers and the army being constantly exposed to leptospirosis have understandably high seropositivity rates of infection. The relatively high rates obtained for police personnel, however, probably apply only to the field force and not to the police force in general. The results shown against the category "business" would appear to be surprisingly high in view of the fact that business executives would not normally be exposed to infection to the same extent as outdoor workers. However a possible explanation for this relatively high percentage of positive cases is the inclusion of rural hawkers and keepers of shops selling sundry goods in this category. These 2 groups of "business" people have a relatively high rate of exposure to infection, the former understandably so, while the latter are constantly being exposed to infection from the usually high rodent population in their shops. The relatively common occurrence in housewives may be attributed to the outdoor chores like farming, padi planting, woodchopping which many rural housewives in Malaya have to perform while their husbands are engaged in some other occupation elsewhere. As only a few padi planters were investigated this group is included under the Unemployed/Miscellaneous category.

The incidence of leptospirosis with respect to sex, racial and age groups is summarised in table IV. The occurrence in males, though higher than in females, is not markedly so. The same remarks regarding incidence in housewives may be repeated here. In addition to this is the fact that women and girls form a very considerable proportion of the labour force in rubber estates and tin mines. The differences noted in the results for different racial groups have no statistical significance, and the incidence may be regarded as having no racial bias. The rates occurring among the age-groups ranging from 11 to 60 have also been determined to be essentially the same. However, the number of patients examined in the age groups 0-10 and over 60 are too small for any valid conclusions to be drawn from them. Moreover samples of blood taken from these groups tend to be selective, since only patients considered well enough are subjected to additional investigations of this nature.

* Some of the cases are included in the results of the preliminary report by *Turner et al.*, (1959), Med. J. Malaya, 14, 83-98.

TABLE II

Incidence of Leptospirosis in PUO Cases in different parts of Malaya

Place	June '58—Dec. '59		1960		1961		1962		June '58—Dec. '62		% pos.
	Pos.	No. Exam.	Pos.	No. Exam.	Pos.	No. Exam.	Pos.	No. Exam.	Pos.	Total No. Exam.	
Alor Star	—	—	1	1	—	—	4	5	5	6	—
Batu Gajah	—	—	0	3	1	3	—	—	1	6	—
Batu Pahat	—	—	—	—	1	1	1	2	2	3	—
Bentong	2	8	—	—	—	—	—	—	2	8	—
British Mil. Hosp.	8	15	0	3	0	2	—	—	8	20	40.0
Butterworth	—	—	1	2	1	1	—	—	2	3	—
Grik	—	—	—	—	0	2	—	—	0	2	—
Ipoh	—	—	—	—	2	4	4	6	6	10	60.0
Jelebu	—	—	0	4	—	—	—	—	0	4	—
Johore Bahru	—	—	2	3	0	1	—	—	2	4	—
Kampar	—	—	2	14	1	6	—	—	3	20	15.0
Klang	1	3	—	—	1	1	0	1	2	5	—
Kluang	0	7	0	7	0	2	—	—	0	16	0
Kota Bahru	—	—	2	2	2	3	—	—	4	5	—
Kuala Kangsar	—	—	1	3	—	—	1	4	2	7	—
Kuala Krai	0	1	0	4	—	—	—	—	0	5	—
Kuala Kubu Bahru	—	—	0	3	3	10	—	—	3	13	23.1
Kuala Lipis	1	4	0	4	1	6	2	16	4	30	13.3
Kuala Lumpur	4	37	7	20	6	25	9	50	26	132	19.7
Kuala Pilah	—	—	—	—	—	—	1	4	1	4	—
Kuantan	0	1	0	1	0	1	—	—	0	3	—
Kulim	—	—	—	—	3	11	—	—	3	11	27.3
Lumut	—	—	0	3	1	4	1	1	2	8	—
Melaka	5	15	12	17	19	41	11	21	47	94	50.0
Mentakab	2	8	2	7	—	—	0	2	4	17	23.5
Pekan	—	—	0	1	0	1	—	—	0	2	—
Penang	0	2	4	7	11	15	7	11	22	35	62.8
Port Dickson	0	5	1	1	—	—	—	—	1	6	—
Raub	1	14	0	8	—	—	—	—	1	22	4.5
Seremban	1	6	—	—	1	2	0	2	2	10	20.0
Sungei Lembing	—	—	1	1	4	17	5	25	10	43	23.2
Sungei Patani	—	—	—	—	1	2	—	—	1	2	—
Taiping	0	1	—	—	2	2	4	7	6	10	60.0
Tampin	1	4	—	—	—	—	—	—	1	4	—
Tapah	—	—	0	1	0	5	—	—	0	6	—
Telok Anson	0	1	0	1	0	1	0	5	0	8	—
	26	132	36	121	61	169	50	162	173	584	29.6

TABLE III.

Incidence of Leptospirosis in PUO cases by occupation

Group	No. Positive	No. Examined	% positive
Rubber Estate workers	28	56	50.0
Labourers (miscellaneous)	43	97	44.3
Tin Miners	3	7	42.8
Farm Workers	5	14	35.7
Police	5	14	35.7
Army	21	70	30.0
Business	6	22	27.3
Housewives	7	27	25.9
Unemployed / Miscellaneous	44	180	24.4
School children	8	50	16.0
Office workers	3	39	7.7
Hospital staff	0	8	0
Total	173	584	29.6

TABLE IV.

Incidence of leptospirosis in PUO cases by sex racial and age groups

Group	No. positive		No. examined		% positive
Males	149	173	476	584	31.4
Females	24		108		22.6
Indians	47	173	132	584	35.6
Chinese	56		182		30.8
Malays	55		224		24.5
Other Races	15		46		32.6.
0 — 10 years of age	1	173	25	584	4.0
11 — 20 ,, ,, ,,	30		117		25.6
21 — 30 ,, ,, ,,	65		200		32.5
31 — 40 ,, ,, ,,	42		126		33.3
41 — 50 ,, ,, ,,	18		62		29.0
51 — 60 ,, ,, ,,	13		40		32.5
Over 60	4		14		28.5

LEPTOSPIROSIS CAN BE MILD AND DECEPTIVE

Although a fair proportion of the patients investigated had the signs and symptoms indicative of leptospirosis, especially in the military group, the majority of the cases investigated for leptospirosis might well have been diagnosed as influenza, acute respiratory disease, gastritis, dengue, malaria, typhoid or infective hepatitis. Out of the 584 cases examined, 243 had fever alone with no other clinical manifestations. Of these 35 were found positive. The clinical features of the 173 positive cases were analysed and the results are summarised in table V. Seven of these cases were manifested as meningitis, 2 as encephalitis and 3 as broncho-pneumonia.

Fever. Fever was present in every case

TABLE V.

Clinical features in 173 cases of leptospirosis
(in descending order of frequency)

Feature	No. positive	% of total positive
Fever	173	100.0
Muscle pain	83	48.0
Conjunctival Injection	74	42.8
Jaundice	70	40.0
Muscle tenderness	68	39.3
*Gastric symptoms	46	26.6
Chills/Rigors	39	22.5
Headaches	35	20.2
Palpable Liver	27	15.6
Tender Liver	19	11.0
Palpable spleen	9	5.2
Haemorrhagic signs	5	2.9
Cough	5	2.9
Diarrhoea	5	2.9
Sore Throat	4	2.3
* Anorexia/Nausea/Vomiting		
Renal function		
Abnormal urine analysis (Proteinuria & casts)	38	22.0
Raised blood urea (48-222 mg. %)	20	11.6
Other manifestations of leptospiral infection		
Meningitis	7	4.0
Bronchopneumonia	3	1.7
Encephalitis	2	1.1

and it varied from 99.2° to 104°F. The majority of cases had mild temperatures of 99.2° to 100°F.

Conjunctival injection and headache. It is surprising to note that in a large proportion of cases these two clinical features were recorded as being absent. One explanation is that most of these cases belong to the 3rd and lowest degree of severity usually called "benign leptospirosis" (Alston and Broom, 1958) in which the "onset may be sudden with fever, muscular pains, sore throat, slight or no jaundice and slight or no nephritis." Neither headache nor conjunctival injection is mentioned as typical symptoms here although both these are characteristic features of the first and second degrees. Another possible explanation is that these signs and symptoms were not specifically noted or recorded. This mild degree of leptospirosis could easily be mistaken for influenza. The serotypes causing this mild degree of illness are usually *canicola, grippotyphosa,* hebdomadis, hyos, pomona,

sejroe and ballum, all except the last of which have been isolated in Malaya, (Trimble, 1954; Alexander *et al.*, 1955 and 1957).

Clinical jaundice. This sign was seen in less than 50% of cases which confirms the findings of previous investigators (Danaraj, 1950; Turner *et al.*, 1959). The jaundice was usually mild in severity and some doubt was even expressed in a number of cases owing to the fact that the sclera of many anicteric Indian patients tend to assume a yellowish tinge.

Gastric symptoms. These appear higher on the list than generally expected and may often lead to mis-diagnosis. The usual symptoms are anorexia, nausea and vomiting.

Chills/Rigors. These two terms are usually used to mean the same feature and are therefore considered together. They were noted in only 22.5% of the cases. Presumably, most of the fevers were mild.

Haemorrhagic signs. Two of these 5 patients had blood-streaked sputum. In the remaining 3 cases mild petechial rash was observed. No case of epistaxis was recorded although it is suspected that this sign would have been quite common if sought for.

Hepatomegaly and splenomegaly. Both enlargements were mild and were usually 1-2 finger breadths below the costal margin. The spleen was never tender but the liver was tender in 11.0% of the cases.

Abnormal urine analysis. Albuminuria, white cells and casts were noted in 22.0%. It is suspected that the occurrence of this feature is actually much higher than that recorded here as complete laboratory data were frequently not submitted with each specimen. The haematological data were omitted in most returns and therefore cannot be included here.

Raised blood urea. Seventeen cases (11.6%) were reported to have raised blood urea levels of 48-222 mgm.%. Here again owing to lack of complete laboratory data it is possible that the actual number might be higher than that recorded. Several positive cases, however, had normal blood urea values of 35-47 mg.%. This was also noted by other investigators (McCrumb *et al.*, 1957).

DIAGNOSIS OF LEPTOSPIROSIS

(a) Serological.

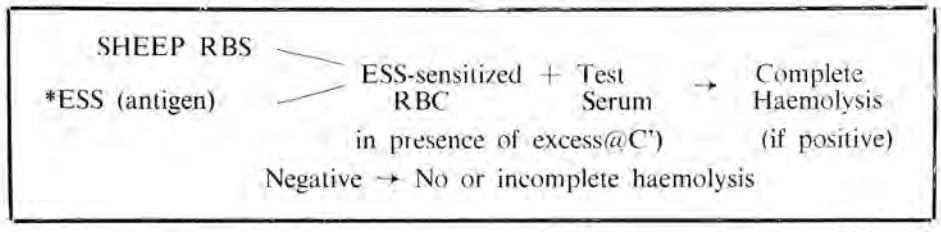
The agglutination test has been the standard method of diagnosis for several years. The patient's sera are tested for antibodies which will agglutinate suspensions of living or formolized leptospires of known serotypes. Unfortunately cross-reactions are common and the sera often agglutinate a variable number of serotypes in addition to the causative one. Moreover, because there are so many serotypes in Malaya the possibility of false negative results occurring can only be avoided if the sera are tested against a battery of serotypes representing the known sero-groups: this procedure is tedious and time-consuming. It is therefore not a practical test for routine diagnostic purposes.

Numerous complement-fixation (CF) tests have been employed using antigens prepared by various methods. However, although the range of serotypes required in the test is much reduced, the sensitivity and specificity of different preparations of antigens tend to vary.

Recently an antigen capable of sensitizing human erythrocytes so that they agglutinate in the presence of leptospiral antibodies, was extracted from leptospires (Chang and McComb, 1954). Subsequently Cox (1955) described a haemolytic modification of this technique which was more sensitive in the detection of leptospiral antibody. This test is named the *Hæmolytic* (HL) test or the Sensitized *E*rythrocyte *L*ysis (SEL) test. The latter description, being more explicit, is favoured by the IMR.

The principle of the SEL test is as follows: (see diagram).

The antigen, prepared by chemical extrac-



* *E*rythrocyte *S*ensitizing *S*ubstance @ Complement

tion of leptospires is mixed with a suspension of normal sheep red cells. The antigen is absorbed to the cell surface thereby sensitizing the cells so that they lyse when exposed, in the presence of excess complement, to serum containing leptospiral antibody. The antigen is aptly described as *E*rythrocyte *S*ensitizing Substance (ESS).

As the SEL test detects genus-specific antibody, any leptospiral infection can be diagnosed regardless of the serotype of the causal agent. As in most serological tests, however, detection of a significant rise in titre is necessary for conclusive diagnosis, and it cannot be stressed too often that both acute (taken as early in the illness as possible) and convalescent sera (taken 10-14 days later) are (minimum) required specimens for the tests.

The SEL test has been evaluated extensively with good results in America (Cox *et al.*, 1957), in Australia (Chang *et al.*, 1957) and also in this laboratory (unpublished). As far as is known, the use of antibiotics in the treatment of leptospirosis does not alter the development of SEL antibodies in the patient but this problem requires further investigation.

A faster method of diagnosis, the Fluorescent Antibody (FA) technique is being tested and evaluated but it will be some time before it can be used for routine diagnosis.

(b) Cultural.

In the past it was usual to inoculate body fluids into laboratory animals which were then observed for febrile response and development of jaundice as well as for death with characteristic autopsy findings. The most severe limitation of this method is the extreme range in virulence for laboratory animals encountered in different serotypes of leptospires and even in strains of the same serotype. The usefulness of animal inoculation is nowadays limited to the isolation of leptospires from contaminated material, such as urine. Even this has been supplanted by a recent method (Menges *et al.*, 1958 and 1960) for isolation of leptospires by direct culture not only of urine obtained aseptically by bladder tapping, e.g. in animals, but also of voided urine which is usually contaminated. In this method contamination was successfully controlled by a simple dilution technique in which serial (10-fold) dilutions of the urine were inoculated into the media. The specific medium and the relatively low incubation temperature (28°-30°C as opposed to 37°C) may not favour the growth of bacteria, especially in the higher dilution where the numbers may be small.

Usually, however, the patient's blood taken early in the illness (1st-5th day of disease preferably) and not the urine is employed: it is cultured directly into Fletcher's (Fletcher, 1928) and/or Korthof's medium, (Korthof, 1932). Only 1-2 drops of bloods are inoculated into each of 3 tubes containing about 5 cc of the medium. Leptospires are rarely detectable before the 7th day and usually around the 14th day of incubation. Cultures must be incubated for at least 28 days before they may be safely discarded as negative.

Most of the blood specimens sent from various parts of the Federation reach the laboratory after 12 hours or so and are not cultured until then. It is suspected that many of the specimens found negative might have given positive results, especially those from patients with a positive SEL test, if they had been cultured immediately after they were obtained, i.e. AT THE BEDSIDE.

SUMMARY

Although established some 10 years ago as a major cause of febrile disease in the Federation of Malaya, leptospirosis has not received recognition as such by most local medical practitioners.

The following 3 points are emphasised:

(a) Leptospirosis is common in Malaya. A recent study of 584 PUO cases over 4½ years revealed 173 positive cases (29.6%). The incidence in the different localities, occupations, races, sex and age groups was also studied.

(b) Leptospirosis can be mild and deceptive. The clinical features of the 173 positive cases are analysed. Some of the cases were manifested as meningitis (7) or encephalitis (2) and some as bronchopneumonia (3). Thirty-five had fever alone with no other clinical manifestations.

(c) Leptospirosis can be diagnosed easily by the SEL test and by direct culture into Korthof's or Fletcher's medium. The SEL test is described and its advantages over other serological tests explained. The importance of submitting paired specimens for the serological test is stressed. Methods for culture of blood and urine directly into media, replacing the technique of animal inoculation are briefly described. The value of culturing patient's blood at the bedside is pointed out.

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