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LEPTOSPIROSIS IN WEST MALAYSIA – EPIDEMIOLOGY AND LABORATORY DIAGNOSIS*

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Summary

Serological surveys and clinical studies have shown that leptospirosis is highly endemic in W. Malaysia. About 30 different serotypes of leptospires have been isolated and all the known serogroups have been represented in the agglutinins found in positive sera.

Of afebrile people throughout W. Malaysia, 12.7% were positive for SEL antibodies. These were found in ages ranging from 4 to 60 years and above. Males (83.4%) had a much higher antibody rate than females (16.6%) end Indians lead with a rate of 5.6%, followed by Malays with 3.3% and Chinese with 2.8%.

Oil palm workers, rubber estate workers and labourers in rural and forest areas were most highly infected, and office workers, housewives and underground (lode) tin miners, least infected. Rural residents had a 16.4% antibody rate compared with 5.6% in town residents.

Results of surveys done in 5 rubber estates and in the Kelantan and Perlis padi planters were discussed.

A recent ten-year (1969–78) observation of clinical leptospirosis showed a 19.6% confirmation of suspected cases with males (93.3%) markedly predominating over females (6.7%). Indian and Malay cases were more common than Chinese, and the age group 20 to 40 years was most frequently affected.

An interesting difference was noticed between the distributions of the presumptive infecting serogroups observed in 1958–1968 and those observed in 1978.

An account of the different methods of laboratory diagnosis was also given.

Leptospirosis is highly endemic in West Malaysia. About 30 different pathogenic serotypes of leptospires have been isolated(1) and clinical cases have been extensively reported in civilian(2, 3) as well as in military groups(1, 4).

EPIDEMIOLOGY

Antibody studies

A serological survey was conducted from 1961 through 1971 on 4,646 afebrile persons of different age, sex, racial and occupational groups in various states of W. Malaysia. 592 (12.7%) were positive for leptospiral SEL (Sensitized Erythrocyte Lysis) antibody. The SEL test was evaluated as an epidemiological tool for human serological survey(5).

SEL antibodies were acquired as early as four to six years of age in rural areas. These antibodies persist through the older age groups at much the same levels (Table 1). As SEL antibodies last for about two years only(5) this indicates constant reinfection throughout life, even up to 60 years and above, the repeated booster effect conferring a high state of immunity to the general population.

Males (83.4%) were more commonly infected than females (16.6%) and Indians had the highest antibody prevalence rate (44.1%) compared with those of Malays (33.9%) and Chinese (22.0%). This corresponds to the racial distribution among the 18 occupational groups studied (Table II) in which the occupations most widely exposed to leptospirosis were held by Indians(6).

The highest antibody rates (23.2% to 32.6%) were found among oil palm estate workers, rubber estate workers and hospital staff(6). Moderately high rates (13.0% to 17.9%) were observed in labourers, the army, tin miners (of open cast mines), farmers and padi planters. Shop owners, policemen and the veterinary

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TABLE I

LEPTOSPIRAL SEL ANTIBODY RATIOS IN DIFFERENT AGE GROUPS

Age Groups	No. Examined	Positive	Per Cent Posirive
0 – 10	193	19	9.8
11 – 20	781	99	12.7
21 – 30	1,571	191	12.2
31 – 40	1,080	111	10.3
41 – 50	673	90	13.4
51 – 60	331	42	12.7
60 or more	190	16	8.4
Total	4,819	568	11.8

TABLE II

SEL ANTIBODY DISTRIBUTION AMONG 18 OCCUPATIONAL GROUPS IN WEST MALAYSIA (IN ORDER OF FREQUENCY)

Group	No. Examined	No. Positive	% Positive
Oil palm estate workers	92	30	32.6
Hospital staff	47	12	25.5
Rubber estate workers	427	99	23.2
Town cleansing labourers	459	82	17.9
Malaysian armed forces	290	50	17.2
School children	176	30	17.0
Tin miners'	122	20	16.4
Farmers	204	30	14.7
Anti-malarial labourers	246	32	13.0
Padi planters	259	37	14.2
Shop-owners	172	21	12.2
Policemen	154	18	11.7
Veterinary staff	463	54	11.6
School teachers	53	5	9.4
Housewives	1,201	64	5.3
Office workers	120	6	5.0
Tin miners[2]	136	2	1.5
Fishermen	25	0	0
Total	4,646	592	12.7

1. Of open cast, dredge and gravel pump mines.
2. Of a lode or underground mine.

staff had rates of 11.6% to 12.2%; and school teachers, housewives, office workers and tin miners of underground or lode mines had the lowest rates (1.5%to 9.4%).

Five rubber estates were surveyed viz. in Gua Musang (Kelantan), Puchong (Selangor), Tangkak (Johore), Sungei Choh (Selangor) and Batu Tiga (Selangor). Very distinct and significant differences in the antibody prevalence ratios were found between the first three (42.6% to 45.9%) and the last two estates (0%to 3.3%). The first three estates were small and closely adjacent to forest areas and were easily invaded by the highly infected rats from them. The latter two were very large concerns with workers living in quarters in the centre of the estates and therefore at a much greater distance from the forest. The main species of rats in the rubber estates is R. *jalorensis* which is normally arboreal in habitat and therefore do not usually come in contact with rubber estate workers, unlike the condition in oil palm estates where they are strongly attracted to the oil palm fruit and are very numerous at ground level when the fruit is cut down and stored(7).

Rural residents had a 16.4% antibody rate compared with 5.6% of town residents surveyed(7).

Leptospirosis in the Ricefields of W. Malaysia

Leptospirosis has been closely associated with rice cultivation. High proportions of leptospiral cases were found in the ricefields of Asia. In West Malaysia, clinical leptospirosis is uncommon among padi planters in Kelantan (1.4% of cases examined) but common in Perlis (10.2%).

Field studies were conducted in five Kelantan ricefields on two separate seasons, wet and dry(8). SEL antibodies were surveyed in the ricefield workers and chemical analyses of water and soil samples collected from the areas under study were performed.

The overall SEL antibody prevalence ratio of the Kelantan padi planters was 14.2% with a higher figure for the wet season (24.2%) and a lower, for the dry season (7.2%).The SEL titres were low in level and no cumulative increase in incidence with age was detected. The pH values of the Kelantan soil and water samples were very low. The mean pH of water and soil were 5.9 and 5.2 respectively during the rainy season and that of soil alone was 4.8 during the dry season. The soil type was mainly clay which has been found in the laboratory to adsorb leptospires and render them helpless.

It may be suggested that the low frequency of clinical leptospirosis in Kelantan padi planters was due to acquired immunity through infection by leptospirosis which had been rendered relatively avirulent because conditions in the ricefields proved unfavourable for their growth and multiplication, although they had been excreted at a high rate into the ricefields by the R. ***argentiventer*** rat.

The attack rate of leptospirosis in padi planters in the State of Perlis, however, was found to be significant higher. Table III shows the incidence of these clinical cases from 1975 to 1978. This occupational group formed 40.8% of the total confirmed cases of lepto spirosis. This exceeded considerably the figure for Kelantan padi planters which was only 2.6% of total positive cases. The water and soil conditions of the Perlis ricefields have yet to be examined.

The presumptive infecting serogroups encountered in the Perlis cases from 1975 to 1978 were (in order of frequency): pomona(49),

TABLE III

CLINICAL LEPTOSPIROSIS IN PERLIS PADI PLANTERS (1975–1978)

Year	*Padi Planters Positive*	*Total Positive*	*% of total Positive*	*Total Examination*
1975	24	41	58.5	124
1976	22	45	48.9	140
1977	16	53	30.2	241
1978	16	52	30.8	257
Total	78	191	40.8	762

pyrogenes(46), ballum(21), javanica(17), icterohaemorrhagiae(15), canicola(14), cynopteri(8), hyos(7), grippotyphosa(5), hebdomadis(3) and batavia(1).

CLINICAL LEPTOSPIROSIS IN W. MALAYSIA (1969–78).

In a recent ten-year (1969–78) observation of clinical human leptospirosis, of 1,738 suspected cases examined, 339 (19.5%) were confirmed positive. Male cases (93.3%) predominated greatly over female cases (6.7%). Most of the cases were between 20 to 40 years of age. Racially, 66.5% were Malay, 15.3% were Chinese, 16.4% Indian and 1.8% others. However, the attack rates per 100,000 population (based on the 1976 census) were higher in Indians (4.4) and Malays (4.1) than in the Chinese (1.6). These figures are consistent with those of the antibody survey which indicate that the groups most highly exposed to leptospirosis were governed mainly by occupation.

The majority of cases were from rural areas and occurred at the rainy season during the last quarter of each year, often extending into January of the next year. The occupational group most greatly affected, especially from 1975 to 1978, was padi planters in Perlis, as mentioned in the foregoing.

The presumptive infecting serogroups encountered in 1978 as compared with those from 1958 to 1968(3) are shown in Table IV. It is interesting to note that after an interval of 10 years the serogroup distribution has changed. For example, autumnalis and hebdomadis have dropped from top position to bottom; and javanica, pomona and ballum which were lowest in distribution in 1968 have climbed to the top. The other serogroups appeared to have remained much the same in distribution. The four most common serogroups encountered in 1978 were also the most common ones found to infect the padi planters in Perlis from 1975 to 1978.

LABORATORY DIAGNOSIS

Microscopic examination

Darkfield examination of alkalinised urine and cerebro-spinal fluid, which have been concentrated by centrifuging may reveal leptospires, but only rarely. Fluorescent antibody (FA) technique may also be employed to detect

TABLE IV

PRESUMPTIVE INFECTING SEROGROUPS IN WEST MALAYSIA

	1978		*1958–1968*	
Presumptive Infecting Serogroup	***No. Cases***	***Order of frequency***	***No. cases***	***Order of Frequency***
javanica	61	1	4	9
pomona	44	2	4	10
pyrogenes	34	3	31	3
ballum	28	4	2	11
canicola	20	5	26	4
icterohaemorrhagiae	18	6	21	5
bataviae	14	7	20	6
cynopteri	13	8	NT	NT
hyos	12	9	NT	NT
australis	6	10	15	7
grippotyphosa	6	11	5	8
autumnalis	4	12	38	1
hebdomadis	4	13	34	2

NT = Not Tested

the organisms if they are sufficiently plentiful in the specimen but this method is limited as the FA scope is not available in many laboratories.

Culture

This is best done at the bedside. Two drops of blood freshly drawn early in the disease, or alkalinised urine collected during the 2nd or 3rd week of illness are inoculated into Fletcher's (semi-solid) medium and/or Korthof (liquid) medium. The inoculated specimen is then examined under darkground illumination every fortnight for 6 weeks for the presence of leptospires.

Animal inoculation

Guinea pigs or weanling hamsters are inoculated intra-peritoneally with blood taken early in the illness. They are bled 7 days later from the heart and the heart blood cultured into suitable media and examined for 6 weeks for the presence of leptospires.

Serology

(a) Microscopic agglutination test.

The antigens used for this test may be live or formalinised. With live (L. *interrogans)* antigens, the test is more sensitive but hazardous. It is serotype-specific and a battery of antigen suspensions including at least one serotype from each of the 16 known serogroups is required. With live L. ***biflexa*** antigen, the test is safer and genus-specific (please see below). Formalinized antigens which are usually pooled in the test are safer to use but are less sensitive. Results are read under darkground illumination.

(b) Macroscopic agglutination test.

This is done with pooled antigens on a slide and results read with the naked eye. The antigens are available commercially but the results of this test are too variable and not reliable enough. Moreover, interpretation of the results can be difficult due to several factors, one of which is cross-reaction.

(c) "Genus-specific" test.

These include the (i) complement-fixation test, (ii) sensitized-erythrocyte lysis (SEL or HL) test, (iii) indirect immunofluorescence test, (iv) biflexa agglutination test and (v) indirect haemagglutination (IHA) test.

As only one antigen of non-pathogenic strain is used, the test is very much simpler than the serotype-specific tests and is safe. Diagnosis of leptospirosis can be obtained irrespective of the infecting serotype. The agglutinins in positive sera may subsequently be typed in a serotype-specific test, if necessary.

Our laboratory employs the SEL test for serological surveys and the biflexa agglutination test (using live antigens) for diagnosis of current illness employing paired sera(9). The IHA has the advantage of detecting specific IgM in the early sera of patients with recent infection. It is currently being evaluated in this laboratory and will replace the biflexa agglutination test if found suitable.

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