

SURVEILLANCE OF LEPTOSPIROSIS AFTER FLOODING AT LOEI PROVINCE, THAILAND BY YEAR 2002

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Abstract. In this surveillance, suspected leptospirosis patients in Loei Hospital, Loei Province were studied by conventional methods of cultivation and microscopic agglutination test (MAT) during July-October, 2002. It was found that 63% of 64 admitted patients and 35% of 34 outpatients were found positive by leptospire cultivation. Antibodies determined by MAT were positive in 78% of 63 admitted patients. Particularly, the five most common agglutinating antibodies were reactive with serovars *bratislava* (57%), *autumnalis* (48%), *new* (38%), *australis* (37%) and *bangkok* (29%). The MAT results of 15 OPD patients were 67% positive with the following five serovars, including *bratislava* (47%), *new* (20%), *bangkok* (7%), *ranarum* (7%) and *australis* (7%). Accordingly, preventive strategies against leptospirosis outbreaks after flooding in Thailand should be undertaken, including the prompt treatment of the disease in this endemic area.

INTRODUCTION

Leptospirosis is a worldwide zoonotic disease, caused by pathogenic *Leptospira* spp. It is most common in tropical countries such as India (Bharadwaj *et al*, 2000), Nicaragua (Trevejo *et al*, 1998) and Thailand. This is due mainly to longer survival of leptospires in environments with warm and humid conditions. However, most tropical countries are also developing countries, and there are greater opportunities for exposure of humans to infected animals or contaminated environments. The disease is seasonal, with peak incidences occurring in the rainy season. The usual portal of entry is through abrasion or via the conjunctiva or intact skin after prolonged immersion in water. Water-borne transmission has been documented and outbreaks of leptospirosis usually occur after flooding (Levett *et al*, 2000). In 2002, Thailand suffered great flooding in many areas, including Loei Province. The purpose of this study was to investigate the prevalence of human leptospirosis in this high risk period.

MATERIALS AND METHODS

Blood collection

Both in-patients (admission) and out-patients (OPD) with clinical diagnosis of leptospirosis at Loei Hospital during July-October 2002 were examined at two different time intervals (day of admission and

about 2 weeks after). All blood samples were then immediately inoculated into vacuum lithium heparinized tubes and gently mixed. This heparinized blood was investigated by either cultivation or microscopic agglutination test (MAT).

Cultivation

Blood samples for cultivation were taken one time at the day of admission. Three drops of heparinized blood were inoculated into 5 ml of EMJH semisolid culture medium and incubated at room temperature for 16 weeks. The cultures were examined by darkfield microscopy at 2, 4, 8, 12 and 16 weeks.

Serological examination

Patient sera were tested by the microscopic agglutination test with 20 reference leptospira serovars (*australis*, *bangkok*, *bratislava*, *autumnalis*, *ballum*, *bataviae*, *canicola*, *cellidoni*, *copenhagen*, *djasiman*, *grippityphosa*, *hedomadis*, *new*, *pomona*, *ranarum*, *sarmin*, *saigon*, *sejroe*, *tarassovi* and *patoc*). Briefly, 50 µl of diluted serum sample (1:100) were added to 96 wells of a plastic microtitration plate. Then, 50 µl of 6-8 day-old liquid medium cultures of living leptospires containing approximately 1×10^8 organisms/ml were added and mixed. The mixtures were then left at room temperature for 3 hours and reactions examined under darkfield microscopy. The criterion for positive reactivity was agglutination of 50% or more of leptospires. The positive sera were further diluted and tested with previous agglutinating serovars. The end resulting titer was the highest serum dilution giving a positive reaction.

Data and statistical analysis

The data were analyzed with one way ANOVA test and the level of significance was set at 0.05.

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RESULTS

Positive cultivation was detected after 4 weeks of observation and a significant increase ($p < 0.05$) was evident with periods of longer observation. The highest cultivation rate was found in samples from hospitalized patients at 16 weeks. The total positive cultivation rate was 53% (52/98 total cases), accounting for 63% (40/64) of admitted patients and 35% (12/34) of OPD patients (Fig 1).

With serological determination, MAT antibodies were detected in small numbers of patients on the first day of diagnosis (8% of hospitalized and negative in OPD patients). Sero-positive cases increased with convalescence to 78% (49/63) of admitted patients and 67% (10/15) of OPD patients (Table 1). The five most prevalence serovars in admitted patients were: *bratislava*, *autumnalis*, *new*, *australis*, and *bangkok* at 57, 48, 38, 37 and 29%, respectively (Table 2). The most prevalent serovars in OPD patients were *bratislava* (47%) and *new* (20%), whereas the other

serovars averaged 7% positivity (Table 3).

Overall, the most prevalent serogroup was identified to be *australis* (serovar *bratislava*) in both groups of patients. On comparison, the admitted group had higher MAT titers (Table 2; 1:800-1:6,400) than the OPD group (Table 3; 1:200-1:400).

DISCUSSION

Leptospirosis is an endemic disease in Thailand because of the common occurrence of flooding during rainy season each year. Although cultivation establishes the definitive diagnosis of bacterial disease, the achievement of leptospire isolation is very low. One factor involved in successful cultivation is a recommended observation time up to 12 weeks. For our investigation, the extended incubation time of up to 16 weeks achieved higher rates of leptospire isolation. The delay cultivation period depended on antibiotic pre-treatment, which was illustrated by an increase in the average incubation time from 7.4 weeks

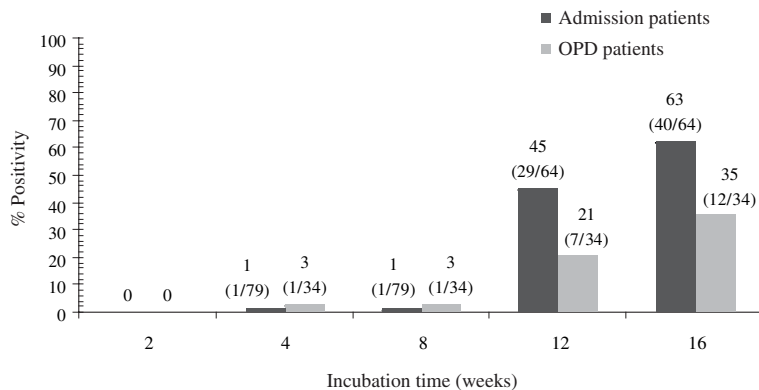


Fig 1 Comparison of percentage culture positivity between admitted and OPD groups. Data above each bar are % and number of positive cases/ total cases.

Table 1

MAT positivity of serum samples from admitted and OPD patients by stage of sample collection.

Group of patients	Time of sample collections	Day of illness (average)	%Positivity (confirm cases/ total)
Admission	Day of admission	1-10 (3)	8 (5/63)
	Then 2 weeks	8-27 (17)	78 (49/63)
OPD	Day of admission	1-14 (4)	0 (0/15)
	Then 2 weeks	15-29 (19)	67 (10/15)
Total	Day of admission	1-14 (3)	6 (5/78)
	Then 2 weeks	15-29 (17)	76 (59/78)

Table 2
MAT results for the 63 patients from the admitted group.

	Outcome serovar	% (No.) Positivity	% Cases positive by titers					
			1:6,400	1:3,200	1:1,600	1:800	1:400	1:200
1	<i>bratislava</i>	57 (36)	42	17	8	11	6	17
2	<i>autumalis</i>	48 (30)	0	10	23	33	7	27
3	<i>new</i>	38 (24)	0	21	25	17	8	29
4	<i>austalis</i>	37 (23)	0	9	34	17	22	17
5	<i>bangkok</i>	29 (18)	0	6	33	11	17	33
6	<i>copenhagen</i>	22 (14)	0	14	7	36	14	29
7	<i>sejroe</i>	16 (10)	0	10	30	30	30	0
8	<i>djasiman</i>	15 (9)	0	11	11	33	33	11
9	<i>saigon</i>	13 (8)	0	25	25	25	13	13
10	<i>ranarum</i>	10 (6)	0	0	17	17	50	17
11	<i>patoc</i>	10 (6)	0	0	0	17	50	33
12	<i>hebdomadis</i>	8 (5)	0	20	0	0	20	60
13	<i>canicola</i>	6 (4)	0	0	0	25	0	75
14	<i>bataviae</i>	6 (4)	0	0	0	0	25	75
15	<i>grippotyphosa</i>	3 (2)	0	0	50	0	50	0

Table 3
MAT results for the 15 patients of the OPD group.

	Outcome serovars	% (No.) Positivity	% Cases positive by titers		
			1:800	1:400	1:200
1	<i>bratislava</i>	47 (7)	0	14	86
2	<i>new</i>	20 (3)	0	33	67
3	<i>bangkok</i>	7 (1)	100	0	0
4	<i>ranarum</i>	7 (1)	0	100	0
5	<i>austalis</i>	7 (1)	0	0	100
6	<i>bataviae</i>	7 (1)	0	0	100
7	<i>canicola</i>	7 (1)	0	0	100
8	<i>copenhagen</i>	7 (1)	0	0	100

to 17.5 weeks after antibiotic treatment (Ellis *et al*, 1985).

The detection rate of MAT antibodies in this study was similar to other reports (Cumberland *et al*, 1999; Bajani *et al*, 2003; Sehgal *et al*, 2003) as approximately 70% on convalescent titers. The MAT levels in admitted patients were higher than OPD patients but it was the same prevalent serovar of *bratislava*. However, different antibody responses among patients could not be explained; therefore, further experiments using animal studies are recommended for future investigation.

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