

Isolation and serological evidences in suspected leptospirosis cases in and around Chennai

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Abstract: Leptospirosis is a zoonotic infection, which occurs mostly in persons venturing in aquatic environment, like farmers and sanitary workers. The objectives of the study were to isolate leptospires and to screen for leptospiral antibodies by MAT and IgM and IgG plate ELISAs to ascertain leptospirosis among suspected leptospirosis cases in and around Chennai. 198 cases of suspected leptospirosis cases attending government and private hospitals in Chennai, were included for this study. Blood samples collected in triplicate at an interval of 7 days from each case, were subjected to culture and leptospiral antibody detection by MAT and IgM and IgG plate ELISAs. The corresponding urine samples were screened for culture only. Out of the 198 cases, 118 (59.5%) were positive by MAT and 124 (62.6%) were positive by IgM and IgG plate ELISAs. Only two isolates of leptospira were possible, that too in urine only. One of the isolates belonged to serogroup *Canicola* and the other to *Autumnalis*. To conclude, leptospirosis is endemic in and around Chennai and so proper identification, diagnosis and treatment, apart from prophylactic measures are required.

Keywords: Leptospirosis, Isolation, *Canicola*, *Autumnalis*, MAT, Plate ELISA

1. Introduction

Incidence of human leptospirosis has been reported throughout the world, mainly from tropical and temperate zones. In Malaysia, McCrumb *et al* (1957), confirmed leptospira cases (1). Damude *et al* (1979), undertook a retrospective study of 220 cases recorded between 1968 and 1974 in Barbados West Indies (2). Hardjo serogroup was found to be the most common among positive cases of leptospirosis in a study in Israel by Shenberg *et al* (1982) (3). Heisey *et al* (1988), reported incidence of human leptospirosis from Thailand (4). Between 1980 and 1982, a longitudinal study of leptospiral agglutinins in subjects, 5 years of age and above, was undertaken in Barbados by Everard *et al* (1990) (5).

In India, leptospirosis was reported in Andaman islands, as early as in 1881. Later, Sehgal *et al* reported few positive cases in 1995 (6). In mainland, as early as 1906, Tucker (1907) reported typical Weil's disease in the crowded quarter of Bombay (7). In Tamil Nadu, 9 cases of human leptospirosis was reported in Kodaikanal hill station as early as 1928 by Turkhud (8). Though there are reports of serological evidences of Leptospirosis in Chennai (Ratnam

et al 1982) (9), there are very few documentations of isolation of leptospira from human cases(10). By keeping all these in mind, we have undertaken this work, so as to know, there was any variation in the infecting serogroup of leptospira by way of serological evidences and also mainly to isolate leptospires from human cases to know the predominant serogroup/s in Chennai.

2. Materials and Methods

The work is part of my PhD thesis being carried out in the Institute of Microbiology, Madras Medical College, Chennai, India. The degree (PhD) has already been awarded by Tamil Nadu Dr. MGR Medical University, Chennai.

2.1. Blood

5 ml of venous blood collected aseptically from all of the 198 cases selected for the study. Three samples were collected from each patient at an interval of 7 days. Immediately, 2-3 drops of whole blood was inoculated in to media bottles, with pores made and sealed on the cap, having a rubber washer beneath, through the needle. The

remaining blood was centrifuged at 2000 rpm and the serum separated for the demonstration of leptospiral antibodies by Microscopic agglutination test (MAT), using live as well as formalinized leptospiral Ag and by IgM and IgG plate ELISAs by coating the microtitre plates with genus specific formalinized patoc antigen. EMJH semisolid and Fletcher's semisolid were the media used for isolation. The culture bottles were subcultured every week looking for growth up to a maximum of 2 months, before declaring negative for growth.

2.2. Urine

Urine samples were mainly collected in the second and third week of infection from every case. With the help of sterile syringe and needle, 1-2 drops of undiluted midstream urine was inoculated in to EMJH semisolid and Fletcher's semisolid, in the same way as for blood sample.

Both the blood and urine cultures were incubated at

room temperature in dark.

3. Results

Out of the total of 198 cases screened for leptospiral antibodies by MAT (golden standard), 118 (59.5%) were positive. Australis was the most predominant serogroup, giving the highest titres in 52 (44%) of the 118 MAT positive cases, followed by Autumnalis 38 (32%), Canicola and Icterohaemorrhagiae in that order (Table-1).

The highest titre of 1:10240 was detected among 13 cases against Australis serogroup followed by Autumnalis in 12 cases (Table-2). While screening by IgM and IgG plate ELISAs, 124 (62.6%) cases were positive for both IgM and IgG antibodies. Though the highest titre of 1:10240 was observed in 43 cases for IgG antibodies, it was only in 3 cases for IgM antibodies (Table-3).

Table 1. Serogroup prevalence by MAT among suspected leptospirosis cases (No. of MAT positive cases 118)

S. No.	Serogroup	No. of positive cases	% positivity
1.	<i>Australis</i>	52	44.0
2.	<i>Autumnalis</i>	38	32.2
3.	<i>Icterohaemorrhagiae</i>	13	11.0
4.	<i>Canicola</i>	11	09.3
5.	<i>Pomona</i>	04	03.4
	Total	118	100

Table 2. Highest MAT titres against each serogroup used, in MAT positive cases.

Serogroup	40	80	160	320	640	1280	2560	5120	10240	Total	%
<i>Australis</i>	-	-	-	-	2	12	10	15	13	52	44.0
<i>Autumnalis</i>	-	-	-	-	-	7	13	6	12	38	32.2
<i>Icterohaemorrhagiae</i>	-	-	-	-	-	2	4	3	4	13	11.0
<i>Canicola</i>	-	-	-	-	-	3	1	5	2	11	09.3
<i>Pomona</i>	-	-	-	-	-	1	1	2	-	4	03.4
Total	-	-	-	-	2	25	29	31	31	118	100

Table 3. Highest IgM and IgG ELISA titres in positive cases of suspected leptospirosis (No. of positive cases 124).

Antibody	40	80	160	320	640	1280	2560	5120	10240	Total	%
IgM	-	1	8	20	17	29	24	22	3	124	100
IgG	-	-	-	3	8	12	20	38	43	124	100

We got only 2 culture positives from urine samples. One of the isolates (code PAI-1) was identified as belonging to serogroup Canicola, strain Hond Utrecht IV (Fig-1) and the other (code N2) as Autumnalis, strain Akiyami A (Fig-2), by Monoclonal antibody technique, courtesy from Royal Tropical Institute, Leptospira Reference Laboratory, Amsterdam, the Netherlands. The pathogenicity test revealed positivity with both the strains (Plate 1, 2, 3 and 4). Both the culture positive cases were also positive for MAT and IgM and IgG plate ELISAs. The highest titre of 1:5120 against Canicola and 1:10240 for Autumnalis were reported for the first and second cases respectively.



Plate 1. Leptospirosis case showing yellowishness of eye and skin rashes on the lips.

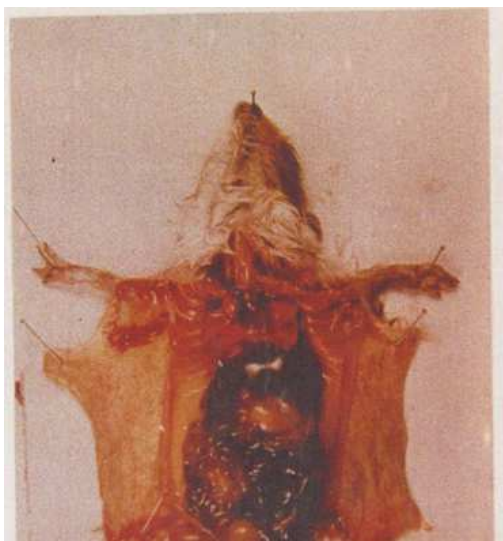


Plate 2. PAI-1 strain, *Canicola* -jaundice and haemorrhage.



Plate 3. N2 strain, *Autumnalis* - lacrimation, mild jaundice and haemorrhage from nostril.



Plate 4. N2 strain, *Autumnalis* - haemorrhages and mild jaundice.

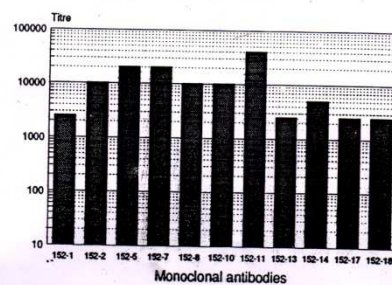
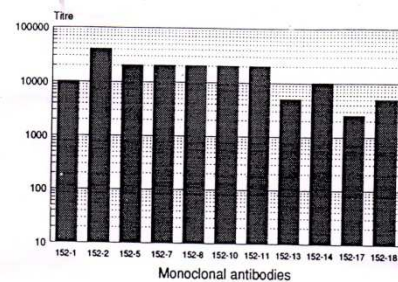


Fig 1 Top: Strain Madras PAI -1

Bottom: Strain Hond Utrecht IV

Serovar canicola / Canicola

Serovar canicola / Canicola

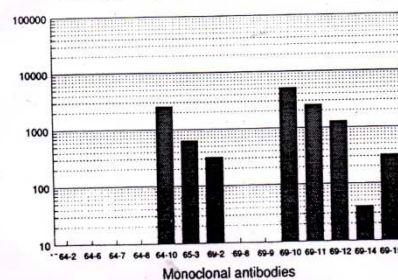
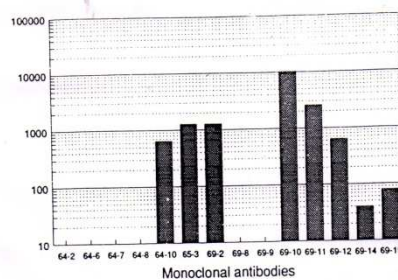


Fig 2 Top: Strain Madras N2 / Autumnalis

Bottom: Strain Akiyami A / Autumnalis

Serogroup Autumnalis

Serogroup Autumnalis

We failed to get any isolations from blood samples.

4. Discussion

Our study indicated 59.5% of case positive for leptospirosis by MAT, which portrays the endemicity of the infection in and around Chennai. This observation is substantiated by few of earlier works done by Ratnam *et al* (10, 11, 12). The emergence of *Australis* as the most predominant serogroup infecting the positive cases of leptospirosis is in correlation with one study by Ratnam *et al*, 1993 (12). The predominance of *Australis* is a new finding, as it indicates a shift in the epidemiology of leptospirosis in Chennai, as most of the earlier reports suggested, *Autumnalis* as the predominant serogroup. In our study *Autumnalis* came second to *Australis*. Although, among the two isolations we got from urine samples of MAT positive cases, one of them was of *Autumnalis* serogroup. The second isolate of *Canicola* serogroup, was the first of its kind being reported from India, at least when the work was being carried out.

IgM and IgG plate ELISAs have shown more positive cases (62.6%) than MAT, though MAT remained the golden standard for the confirmative leptospiral Ab detection. IgM Ab appeared first as evidenced by IgM ELISA and possibly peaked during the first week and then dropped down. The IgG Abs steadily increased and attained highest level in the third sampling and appeared to maintain that level for quiet sometime. Similar observations were also recorded by one of our earlier studies also (13). Recent confirmed cases of leptospirosis in Chennai were reported by few authors (14, 15).

Summary

To summarise and conclude, as per our study, Chennai is endemic for leptospirosis. The emergence of *Australis* as the predominant serogroup infecting leptospirosis cases is a new finding, as it alters the epidemiological status of the region, warranting, more further studies to elucidate the predominant serogroups, which will help in formulating the panel of serogroups for MAT test, as well as to derive an apt vaccine for leptospirosis in future. This observation is also strengthened by the isolation of *Canicola* from one of the positive cases in our study.

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