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# EPIDEMIOLOGICAL STUDY ON THE SEROPREVALENCE OF LEPTOSPIROSIS IN SALEM AND NAMAKKAL DISTRICTS, THE MAJOR CENTRE FOR LIVESTOCK IN TAMILNADU

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Abstract -

## INTRODUCTION

Leptospirosis is a zoonotic disease prevalent in South India (Ratnam, 1994; Muthusethupathi, *et al.* 1995 and John, 1996). Clinical manifestations of this disease ranges from fever, myalgia, conjunctival suffusion to severe life threatening illness due to involvement of multiple organ system e.g. hepatic, renal, central nervous system etc. (Varaiya *et al.* 2002). Pulmonary complications occurring early in the disease with high case fatality rate have been reported (Singh *et al.* 1999; Goncalves *et al.* 1994). In Tamilnadu, majority of the leptospirosis outbreak occurs during monsoon (Ratnam, 1994 and Singh, 1999). Sumathi *et al.* (1997) had reported a higher incidence of leptospirosis among persons treated in government hospital in Chennai. Variya *et al.* (2002) had reported the outbreak of leptospirosis in and around the city, Mumbai. Jayakumar *et al.* (2004) had reported the seroprevalence of leptospirosis among the people involved in agricultural, livestock and veterinary activities in the city, Chennai. As leptospirosis is one of the dangerous infections diseases in South India, an attempt has been made to investigate the seroprevalence of leptospirosis among people involved in agricultural, veterinary and

livestock occupation in Namakkal and Salem districts of Tamilnadu. In Salem and Namakkal districts of Tamilnadu, livestock workers are in a higher proportion. These two districts are the major poultry centre in India. Hence the present study to analyse the association between leptospirosis and occupation in these districts as a first attempt.

## MATERIAL AND METHODS

The study area, Salem and Namakkal districts are in the North-West area of Tamilnadu (Fig. 1). More than 60% of people living in these area are agricultural and livestock workers. Most of the agricultural workers are also doing work in poultry farms. Few are exclusively doing livestock work. So the people involved in agriculture and livestock activities were coupled as integrated farm workers. In the present study, an interview was conducted using a questionnaire to analyse their health problem in relation to leptospirosis infection. The sample includes the age group 1 to 70 years. During the study period January 2003 to April 2003, blood samples were collected from these people and serum was tested for leptospirosis using Microscopic Agglutination Test (MAT).

The antigens used for MAT study contained EMJH liquid medium of 7 days old culture with a minimum density of  $2-3 \times 10^8$  cells/mL. The MAT was preferred with a battery of five live serovars (Australis, Autumnalis, Louisiana, Icterohaemorrhagiae, Grippityphosa and a non pathogenic Patoc 1). The serum sample from suspected cases were doubly diluted in a sterile phosphate buffered saline (PBS) (pH 7.2). About 20  $\mu$ l of sterile PBS at pH 7.2 was added to each well in microtitre plate. To the first well, 20  $\mu$ l of the 1/10 diluted sera was added with the help of micropipette to make the serum dilution as 1/20. Then double fold serial dilution of serum was made to all the wells, with the help of micropipette. To all the wells, with the help of micropipette, 20  $\mu$ l of the battery of locally significant serovars of *Leptospira* was added. After adding the antigen, the dilution of the serum was 1/20, 1/40, 1/80, 1/160 and so on. To promote antigen-antibody reaction, the microtitre plate was kept at room temperature for 2 hours the serum antigen mixture was examined under a dark field microscope for agglutination. For observation, one drop of sample from each well was transferred to a clean dry microscopic slide and examined under dark field microscope with 20x objective. The titre is the dilution gives 50% agglutination, leaving 50% of the cell free and it was compared with a control suspension of leptospire dilution 1:2 in phosphate buffered saline without serum.

## RESULTS

Among the 160 people studied, 150 persons suffered with fever, myalgia, diarrhoea, jaundice, conjunctivitis and renal complications and 10 were healthy and normal persons. Of the 150 persons with health problems, 90 were males and 60 were females. Among them integrated farm workers were 37.5%, 28.13% were livestock workers, 28.13% were sewage workers and 6.24% were involved in other activities. The various health problems observed among the 150 patients are given in Table 1.

Out of the 160 samples screened, seventy persons (43.75%) had leptospiral infection as per MAT Test. The relationship between occupation and leptospiral positivity is given in Table 2.

Among the 60 integrated farm workers screened, 35 persons (58.3%) were positive for leptospirosis and 16 persons (35.5%) doing livestock work were positive out of the 45 cases studied. In a sample of 45 sewage workers, 18 (40%) were positive

leptospirosis. Ten per cent of cases were from other occupations. Out of the seventy leptospiral cases identified, 50% were integrated farm workers and 22.85% were livestock workers. This study indicated that the integrated farm workers were more prone to leptospiral infection than the others. Sewage workers were also affected considerably (25.71%).

Levett (2001) reported that the human infections might be acquired through occupational, recreational or avocational exposures. Waitkins (1986) reported occupation as a significant factor for human leptospirosis. Direct contact with infected animals accounts for most infection in farmers, veterinarians, abattoir workers, meat inspector, rodent control workers and other occupations, which require contact with animals (Chan *et al.* 1987; Campagnolo *et al.* 2000 and Terry *et al.* 2000). Ratnam *et al.* (1993a) reported a high seroprevalence of leptospirosis among agricultural workers. The high incidence of leptospirosis among integrated farm workers indicated that the field condition and the animals they handled during farming practices had induced leptospiral infection. In the study area, livestock workers were less affected by leptospirosis when compared to the integrated farm workers. In these area, agricultural workers were not only involved in agricultural activities, but are also doing livestock related work. such occupational combination encouraged high incidence of leptospirosis. Hence, occupation plays a major role in deciding leptospiral infection and a significant relation ( $P > 0.05$ ) was observed between occupation and leptospirosis infection.

From the Table 3, it is clear that the female integrated farm workers were much affected (78.1%) than the male workers. Among the livestock workers, male workers were affected highly (37.5%). Among sewage workers also, males (43.3%) were more affected than the females. However the variation in the incidence of leptospirosis in male and female workers are not statistically significant ( $P < 0.05$ ).

An age wise classification of the leptospiral positive cases (Table 4) showed that the persons in the age group 51 - 70 years were more prone to leptospiral infections. Out of the 70 leptospirosis cases identified, 55.6% were in the age group of 51 - 70 years. However no statistically significant correlation was observed between leptospirosis infection and age group ( $P < 0.05$ ). Long exposure duration and reduction of immunity on aging process could pave way for easy infection.

The clinical analysis of the leptospiral cases

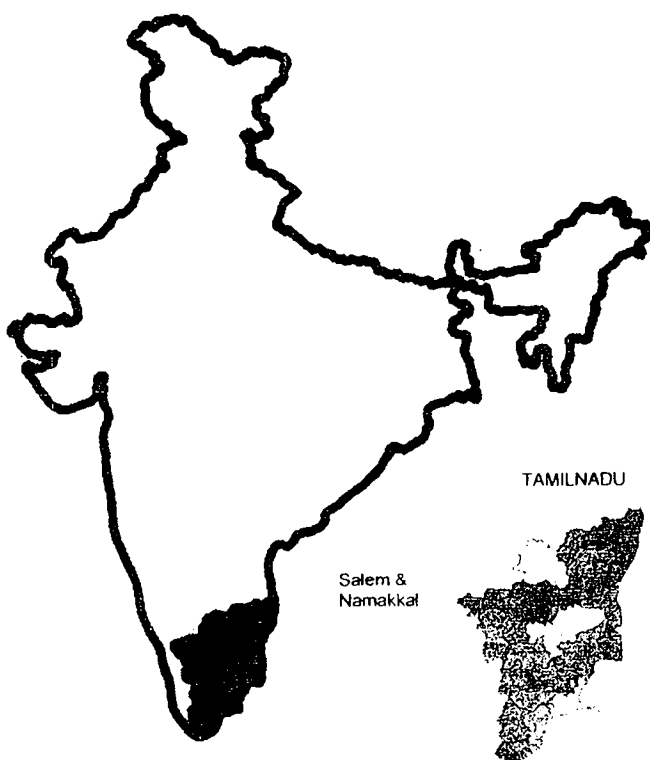


Fig. 1 Location map of Salem and Namakkal District, Tamil Nadu

(Table 5) indicated that the common symptom for leptospirosis is fever and jaundice. An indepth study of integrated farm workers infected with leptospirosis and the type of serovar involved were given in Table 6. Five serovars were observed in the serum sample of the integrated farm workers. They were Louisiana (11%), Autumnalis (8.5%), Australis (5.7%), Icterohaemorrhagiae (2.8%) and Grippityphosa (2.8%). The antibody present in the sample against serovars was found individually or in mixed proportion. In the integrated farm workers, mixed serovars were observed in 68.8% of the positive cases.

From this study it is clear that the five types of serovars are highly prevalent in the study area. Of the different serovars, Louisiana was the most commonly (11.4%) recorded organism. It was followed by Autumnalis (8.5%), Australis (5.7%) and

Table 1. Percentage distribution of various health problems among workers in Salem and Namakkal Districts.

Health Problems	Percentage occurrence
Fever / Jaundice	33.57
Fever / Headache	18.13
Fever / Myalgia	11.88
Diarrhoea / Myalgia	11.25
Conjunctivity	11.25
Renal Complication	4.38
Other Symptoms	6.25
No Symptoms	3.12

others. Ratnam *et al.* (1993a) had reported a high incidence of Autumnalis infection among the slum workers in Chennai city. Ratnam *et al.* (1993b) had also reported that the Autumnalis were found to be the most frequent serovar in agricultural workers. Natarajaseenivasan *et al.* (1997) had reported equal distribution of Autumnalis and Pomona in cattle and are predominance of Icterohaemorrhagiae in human population of South India. In contrary, the present investigation informs the predominance of the serovar Louisiana among the integrated farm workers in Salem and Namakkal districts.

The MAT antibody titre analysis of the serum of livestock workers showed the high incidence of Louisiana serovar (18.8%). This was followed by Icterohaemorrhagiae (12.5%), Grippityphosa (6.24%) and Autumnalis (6.2%). In the livestock workers also mixed representation of the different serovar was observed (56.25%). The highest occurrence of the serovar Louisiana among them is suggestive of the livestock as the natural reservoir host for these serovars of *Leptospira*.

Weekes *et al.* (1997) studied the seroepidemiology of leptospirosis on the Island of Barbados and reported Icterohaemorrhagiae as the most common predominant serogroup followed by the serogroup Australis. Among the sewage workers, both Louisiana and Icterohaemorrhagiae were equally represented (11%). The other serovars viz. Autumnalis, Australis and Grippityphosa were also in equal proportion (3.5%). The mixed serovars were

Table 2. Incidence of leptospirosis in relation to occupation

S. No.	Occupation	Number of Workers	Number of positive cases	Percentage of positive cases	Total Percentage
1.	Integrated Farm Workers	60	35	58.3	50.00
2.	Livestock Workers	45	16	35.5	22.85
3.	Sewage Workers	45	18	40.0	25.71
4.	Other activities	10	1	10.0	1.42

Table 3. Incidence of leptospirosis in relation to sex

S. No.	Occupation	Male specimens			Female specimens				
		Total no. of samples	No. of positive	Test %	Total positive%	Total no. of samples	No. of positive	Test %	Total positive %
1.	Integrated farm workers	15	10	66.6	26.3	45	25	55.5	78.1
2.	Livestock workers	40	15	37.5	39.4	5	1	20.0	3.1
3.	Sewage workers	30	13	43.3	34.2	15	5	33.3	15.6
4.	Other activities	5		Nil	Nil	5	1	20.0	3.1

Table 4. Incidence of leptospirosis in relation to sex

S. No.	Age Group	Occupation				Total No. of Samples	Sero positive sample	Test %	Total %
		Integrated farm workers	Livestock workers	Sewage workers	Other Activities				
1.	Child (1-10 years)	4	1	Nil	Nil	5	1	20.0	1.42
2.	11-25 years	11	10	15	5	41	12	29.6	17.14
3.	26-50 years	15	9	12	1	37	18	48.4	25.71
4.	51-70 years	30	25	18	4	77	39	50.6	55.71

Table 5. Clinical features in samples

S. No.	Clinical features	Occupation				Total No. of sample	Sero positive sample	Test%	Total%
		Integrated farm workers	Livestock workers	Sewage workers	Other Activities				
1.	Fever/Headache	26	14	15	1	56	41	75.9	58.57
2.	Rigors/Headache	10	5	11	7	33	11	33.3	17.11
3.	Fever/Myalgia	5	8	6	Nil	19	7	36.8	10.00
4.	Renal Complication	5	7	5	1	18	7	38.9	9.80
5.	Other Symptoms	3	3	2	Nil	8	4	50.0	1.46
6.	Diarrhoea/Myalgia	10	5	3	Nil	18	4	22.2	9.71
7.	Conjunctivitis	1	2	5	2	10	3	30.0	4.20
8.	No Symptoms	1	1	Nil	Nil	2	Nil	0.0	0.00

Table 6. Serodiagnosis of leptospirosis using MAT antibody titre among integrated farm workers

S. No.	Serovar	MAT Titre						Total positive	Positive percentage
		1:80	1:160	1:320	1:640	1:1280	1:2480		
1.	Louisiana	-	3	-	-	-	1	4	11.4
2.	Autumnalis	-	2	-	1	-	-	3	8.5
3.	Australis	-	-	1	1	-	-	2	5.7
4.	Icterohaemorrhagiae	1	-	-	-	-	-	1	2.8
5.	Grippityphosa	-	-	-	-	1	-	1	2.8
	Subtotal	1	5	1	2	1	1	11	31.4
6.	Mixed	8	6	5	2	2	1	24	68.6
7.	Total	9	11	6	4	3	2	35	100

Table 7. MAT antibody titre of all clinical samples

S. No.	Serovar	MAT Titre						Total positive	Positive percentage
		1:80	1:160	1:320	1:640	1:1280	1:2480		
1.	Louisiana	4	1	-	1	-	3	9	12.8
2.	Icterohaemorrhagiae	-	-	1	2	-	-	3	4.2
3.	Autumnalis	3	-	1	1	-	-	5	7.1
4.	Australis	1	2	1	1	-	-	5	7.1
5.	Grippityphosa	-	1	-	1	1	-	3	4.2
	Subtotal	8	4	3	6	1	3	25	35.7
6.	Mixed	16	12	9	3	4	1	45	64.2
7.	Total	24	16	12	9	5	4	70	100

observed in 61.5% of the cases studied. In the persons involved in other occupation, only mixed population of the different serovars were recorded.

The present study indicates that the leptospirosis is a serious threat to the integrated farm workers. The usual portal of entry of this microbe is through abrasion or cut in the skin or via the conjunctiva: infection may take place via intact skin after prolonged exposure to water (Levett, 2001). As integrated farm workers are associated with dairy animals and soil environment, they were found to be the major occupational risk group in the present study.

Kuriakose *et al.* (1997) while reporting leptospirosis in the Kerala State of India identified Autumnalis, Australis and Icterohaemorrhagiae as the most common serogroups.

The comparison of MAT antibody titre value observed for the all the five serovars also confirmed a high titre value for Louisiana followed by Icterohaemorrhagiae, Autumnalis, Australis and Grippityphosa (Table 7).

The epidemiological study on the seroprevalence of leptospirosis among integrated farm workers, livestock workers and others exposed the fact that leptospirosis has close link with occupation. Hence leptospirosis is an occupational disease, which need an immediate attention.

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