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Seroprevalence of leptospiral antibodies among municipal service workers in Tiruchirapalli, South India

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Abstract

*Problem: Leptospirosis is emerging and re-emerging neglected tropical bacterial infections that are attracting greater attention from the public health and medical communities. Due to increase in urbanization and conversion of rural setup to urban, poor sanitation and unawareness of the risk sources increasing the infectious diseases recorded every year. The objective of this study is to determine seroprevalence of leptospiral antibodies and distribution of Leptospira serovars presented in sample of municipal service workers in both railway and residential areas. **Methodology:** The Genus specific ELISA was performed for all these serum samples [(n=199) – (103 residential municipal workers and 96 railways municipal workers)] and the results were compared with serovar specific MAT by using serial doubling dilutions of serum samples. **Findings:** The results of MAT showed reactive to 92 subjects. In MAT, the highest titer value was 1:320 in L. grippityphosa and L. australis among residential municipal workers; whereas 1:320 was found as the highest titre in L. autumnalis and L. australis among railway municipal. Other serovars were also found reactive but at low value. In our study, seroconversion to 1:80 and above was considered positive because endemicity of leptospirosis has not been reported previously in the study area. **Conclusion:** This could be an indication that sewage workers are more frequently exposed to leptospires than other high risk groups and they have been routinely screened for leptospirosis also in order to avoid the multiorgan dysfunction and failure.*

Keywords: 1 Leptospirosis, 2 seroprevalence, 3 municipal service workers

Introduction

Leptospirosis is an anthro-po-zoonotic disease, observed largely in tropical and subtropical countries. The infection due to this spirochete in humans is caused by either direct contact with infected animals, or by indirect contact via contaminated environment. The animal hosts are thus considered as reservoirs for human infection (Natarajaseenivasan *et al.*, 2004; Prabhu *et al.*, 2015).

The diseases of same signs and symptoms are interlinked, as the severe forms of leptospirosis including typhoid, malaria, dengue and misidentification may occur in different situations. During rainy season, pyrexia of unknown origin (PUO) cases are misdiagnosed due to the common clinical manifestations and provide false positivity in serological studies (Rathinamsivakumar *et al.*, 1996; Marotto *et al.*, 1999). In order to control and prevent leptospirosis, appropriate screening both clinical and laboratory investigations in acute stage itself. It also helps to recover the patients from chronic disease state (multiorgan dysfunction and multiorgan failure) and death (Cohen, 2002). The observation of clinical manifestations among patients with fever, jaundice and renal failure are defined as Weil's disease (Cordova *et al.*, 2012) and quoted with "A peculiar form of acute infectious disease characterized by jaundice, swelling of the spleen and nephritis".

Leptospirosis is an emerging and re-emerging infectious disease mainly during rainy season which is also the highlighted the conception that defines the infection that often under recognized but persistent (Bennett, 1996; Levett, 1999; Meites *et al.*, 2004; Vijayachari *et al.*, 2008).

The workers who are closely in contact with the contaminated animal urine are considered as the main occupational risk groups (municipal workers, farmers, butchers, veterinarians etc), who are likely to be exposed to contaminated water and wet soil during their daily activities (Tangkanakul *et al.*, 2000; Dreyfus *et al.*, 2014; Della *et al.*, 2015). The animal hosts including rodents, cattle, pigs, dogs, cats and wildlife are considered as common reservoirs of leptospire that spread frequently to humans (Costa *et al.*, 2015).

The outbreak of human leptospirosis has not previously been reported from the Tiruchirapalli district of Southern India. But in the year 2014, the risk factors associated with the urban and rural epidemics of leptospirosis in Ialgudi, Tiruchirapalli were well studied (Prabhakaran *et al.*, 2014). The study area has many municipal workers on the outskirts of the town, where they are largely exposing to the contaminated drainages and garbage which are rich source of leptospiral dwelling. But the municipal workers are neglected and not considered for screening leptospirosis globally.

Tiruchirapalli Corporation was facing unrelieved problems regarding high rat population especially in railway tracks and residential areas (Prabhusaran *et al.*, 2015). Furthermore, urban rats are prolific breeders with average weight up to one kilogram and may produce up to fifteen pups a year. Even though lot of rat and rodent control measures are taken, this issue is still a rising one. Eradication of rats indirectly may prevent spreading of *Leptospira* which is commonly transmitted by rats (Prabhakaran *et al.*, 2014; Prabhusaran *et al.*, 2015). This situation becomes crucial for any work activities which involve environmental sanitation and rat catching campaign. Thus, the railway tracks cleaning sanitary workers and other municipal workers have to ensure safe working environment and proper procedures should be followed by workers and individuals with high probability of exposure to leptospirosis. In order to address this issue, this study aimed to determine the seroprevalence of leptospiral antibodies among municipal service workers is being undertaken. Furthermore, the association between seropositivity of leptospiral antibodies with job category and animal contacts of municipal service workers will also be determined in this study.

Materials and Methods

This is a cross-sectional observational study carried out in the municipal and residential outskirts of Tiruchirapalli, South India. A total of 199 municipal service workers (103 railway and 96 residential) were included and study was conducted between July and October 2019. The Institutional ethics committee clearance was obtained (Ref. No. 636/TSRMMCH&RC/ME-1/2019 - IEC No. 003 dated 17.07.2019). After informing study participants about the objectives of the study and assuring the confidentiality of their data,

written informed consent was obtained from all the participants (municipal workers – railway and residential). The municipal workers with minimum of 6 months continuous experiences in railways and residential areas are included in this study.

The case study proforma including socio demographic data, work site associated informations, animal handling, cleaning procedures were collected. Blood samples (approximately 5ml) were collected from all the municipal workers and serum was separated and stored at -20°C till testing. The serum samples were further tested for the presence of serological imprints by following enzyme linked immunosorbent assay (ELISA -genus specific) and microscopic agglutination test (MAT -serovar specific). The sera of municipal workers and controls were diluted in 1:100 using phosphate buffered saline (PBS) and 100µl of non-pathogenic *Leptospira patoc* (semaranga) was coated in the 96 microtiter well and the mixture incubated for 30 minutes at 37°C in dark condition. After saline wash, 100µl of HRP-conjugated anti-human IgG was dispensed and further incubated at 37°C for 30 minutes. The substrate tetramethylbenzidine (TMB) (100µl) was added and incubated at room temperature for 10 minutes and the reaction was stopped with 100µl of 1M phosphoric acid. The absorbance value was read at 450nm and the reading was interpreted as Pan-Bio units which were calculated by the absorbance of positive and negative control serum where the unit ≥ 11 was considered positive (Bajani et al., 2003).

The serovar specific leptospiral antibodies can be demonstrated by microscopic agglutination test (MAT) using live leptospiral antigens (Wolff, 1954) where the reference leptospiral strains maintained in the laboratory which were obtained from Regional Medical Research Centre (ICMR), WHO collaborating centre for diagnosis, reference, research and training in leptospirosis, Port Blair, Andaman and Nicobar Islands, India. The leptospiral strains included in this study are Australis, Autumnalis, Canicola, Grippityphosa, hebdomadis, Icterohaemorrhagiae, Javanica, Pomona, Patoc, Pyrogenes, Sejroe and Shermani, and were maintained by periodic sub culturing in EMJH semisolid medium.

The test serum samples were diluted using 1% PBS at the pH of 7.2 where doubling dilution from 1:10 to 1:10240 was prepared; equal volume of antigen was added (1:1 ratio). It was shaken well and incubated at dark, room temperature for one hour. After incubation, one drop of each well was taken by using micro diluter and observed under dark field microscope. The readings were performed by using an agglutination end point of 50% associated with the highest MAT titre was recorded for the study of seroprevalence.

The titer value of ≥ 80 is considered as positive and the predominant serogroup was defined with maximum titer. The sensitivity of the MAT was defined as the proportion of isolates of a single serovar correctly predicted by the corresponding serogroup that was the predominant reactive serogroup in the convalescent phase serum or in the acute phase sample obtained most recently. The specificity of MAT serologic analysis was defined as the proportion of patients with a predominant serogroup whose isolate was of the corresponding serovar.

Results

Socio-demographic details

Among the 103 and 96 residential (ReMW) and railway municipal workers (RaMW) included in this study, 74 (71.8%) and 62 (64.5%) were males, and 29 (28.2%) and 34 (35.5%) were females respectively. The age groups were also well analyzed thereby 31 to 50 years of subjects were highly prone to the infection. Among the residential municipal workers, 25.2% were belonged to 31 to 40 years followed by 41 to 50 years and 51 to 60 years with 44.7% and 16.5% respectively. The detailed gender and age wise distribution of subjects of both railways and residential workers were depicted in table 1.

The residential location details of the subjects of both railway and residential municipal workers were recorded and analyzed thereby equal numbers of both rural (52/103) and urban (51/103) background were observed among residential workers, whereas maximum railway workers were resided in urban

environment (66/96). The animal contacts among the residential and railways workers were recorded and analyzed thereby 94.2% of residential and 87.5% of railways workers were having frequent animal contacts.

The types of animals handled by the subjects were cows (102), goats (109), dogs (70), pigs (32), cats (36), sheeps (57), buffalos (22) and donkeys (9). Most of the above said animals were harbouring leptospire and possible to spread or transmit the infection to the humans. It was also recorded the frequency of observing rats and other rodents in the working place and residence by the subjects; thereby 97/103 residential municipal workers and 84/99 railway municipal workers frequently observed the same.

Simultaneously, the observation of rodents and other small animals that are highly responsible for spreading leptospirosis were recorded by asking a simple yes or no question. Among the subjects, 199 were observed rats and 92 observed bandicoots in their working environment. Meanwhile, the persons included in this study were enquired for the observation of live and dead rats and other rodents and its exact locations; thereby most of the residential municipal workers answered ditches followed by garbages and field areas; whereas the railways workers frequently observed in food area followed by railway tracks, toilets and coaches.

While handling the dead rodents in the working and residential environments, most of the subjects answered that they handle the animals in barehand followed by holding plastic covers followed by old clothes and newspapers. Very few were informed that they were using gloves (Figure 1).

The mode and method of disposing the dead animals by the both residential and railway municipal workers were also determined thereby the maximum persons threw the dead animals in the ditches (n=109) directly followed by garbages (n=132). Very few persons were found to be eco-friendly that they buried the dead animals (n=4). The leptospirosis infection history were also collected from both the municipal workers thereby maximum were not aware about the disease and its infectious state. Out of 199 subjects, only 9 (4.5%) were diagnosed and 30 (15.1%) were suspected but non-reactive. The mode and method of cleaning the working/ contaminated environment were also analyzed thereby the risks and mode of entry of leptospire to the persons handling the cleaning was determined. By this analysis, 92 members revealed that they cleaned the stained floor with barefoot.

The usage of disinfectants while cleaning (brooming and mopping) the working environment with or without stains were well analyzed thereby 84 out of 199 using disinfectants. Most of them (179) were not using antiseptics after cleaning the contaminated environment, thus awareness and education related to handwashing and antiseptics usage will be encouraged in future. All the subjects who are included in this study were analyzed for the past clinical history related to leptospirosis like fever and head ache. Along with pyrexia, the other co-morbid illnesses were also assessed and recorded. The next common symptom was myalgia followed by arthralgia. Among the cases, one case was recorded as conjunctival suffusion and two with jaundice. The various signs and symptoms observed are summarized in table 2.

The total year of working experiences in the municipal field were also analyzed and calculated thereby the maximum subjects have the experiences between 11 and 25 years. As per the methodology to be concerned, the individuals working below 6 months were excluded from the study. The results of genus specific IgG ELISA were performed thereby 73 (70.9%) serum samples were reactive and 29.1% were non-reactive among residential municipal workers. While performing ELISA among railways municipal workers, 56.2% and 43.8% were found reactive and non-reactive respectively (Table 3) among railway municipal workers.

The results of serovar specific MAT were performed using live leptospiral cultures as antigen thereby 52 (50.5%) serum samples among residential municipal workers were reactive and 49.5% were non-reactive (table 4). While performing with railway municipal workers' serum samples, 40 (41.7%) were reactive and 56 (58.3%) were non-reactive (table 4). Among the 103 serum samples of residential municipal workers participated in this study, all had single sampling only. The common serovars against which antibodies were present included Australis, Autumnalis, Canicola, Hebdomadis, Icterohaemorrhagiae, Javanica, Grippotyphosa, Sejroe, Pomona, Pyrogenes, Shermani, Patoc. Out of the total 103 serum samples, 52 samples

showed positive to MAT. Among the serovars, *L. grippityphosa* (n=21) was the predominant against which antibodies were present followed by australis, icterohaemorrhagiae and so on. The highest antibody titer obtained for *L. australis* and *L. grippityphosa* was 1:320 and it was already proved as a prevalent serogroup on Tamilnadu.

Among railway municipal workers who participated in this study, 40 (41.7%) were reactive and 56 (58.3%) were non-reactive by MAT. The common serovars against which antibodies were present included Australis, Autumnalis, Hebdomadis, Icterohaemorrhagiae, Javanica, Grippityphosa, Sejroe, Patoc. Out of the total 96 serum samples, 40 samples showed positive to MAT. Among the serovars, *L. autumnalis* (n=17) was the predominant against which antibodies were present followed by icterohaemorrhagiae, australis and so on. The highest antibody titer obtained for *L. autumnalis* and *L. icterohaemorrhagiae* was 1:320 and it was already proved as a prevalent serogroup on Tamilnadu. The total number of positive cases and its highest titer values for various serovars was depicted in Table 4.

In the case of leptospiral diagnostic methods, the ELISA is described as the prediction of positivity against genus level of leptospire. But the MAT is a gold standard method which confirms the leptospiral infections by using a battery of live leptospiral antigens. The sensitivity and specificity were well assessed using the acute and convalescent serum samples. The data available in the table 3 impregnated the comparative seroprevalence among the two different study subjects by using MAT and the ELISA.

While interviewing the subjects, only 32 (16.1%) among 199 informed that the authoritative and officials where they are working arranged medical camps very rarely. They also informed that the last medical camp was conducted 2 years ago and no doctors are invited to the working place for any special occasions and routine checkups. All the workers also happily agreed to attend/ participate in the medical camp if arranged.

Discussion

This study analyzed in detail from the socio-demographic analysis of the infectious risk groups (municipal workers). Most of the subjects included in this study are frequently exposed to rats and other rodents even some of them were directly exposed to their excreta. This disease has been recognized as an important occupational hazard of municipal workers, agricultural workers and its related manual labourers (Sharma et al., 2000).

As far as the clinical manifestations are to be concerned, most of the study subjects included have supported to fever and headache. Jaundice and conjunctival suffusion are the rare observations (Gerding *et al.*, 1997). The study represents the first systematic serosurvey documenting the occurrence of the seroprints of human leptospirosis in central part of Tamilnadu especially inclusion of the municipal workers as a sole occupational risk groups.

The infection is mostly affects males while this was not highly observed in female groups. The gender differences and variations which have been found in numerous other studies (Faine, 1994; Brown and Levett, 1997; Heath *et al.*, 1965) is usually attributed to occupational factors and it tends to vanish if genders are given equal exposure (Merien *et al.*, 1992; Park *et al.*, 1989; Prabhakaran *et al.*, 2017). In this study it was observed that the hand washing after exposed to the contaminated environment is higher among females than males; also identified that usage of PPEs is more among females. While interview, the male population told that the usage of gloves is not comfortable and no officials guided them to wear PPEs.

If the number of year of experiences increases, then the exposure towards the infectious materials also gets increased. Thus, in this study it was proved that the individuals who are having more years of experiences in the municipal environment showed higher seropositivity than the lesser experienced municipal workers. The same were also described in other studies (Vijayachari et al., 2008; Wasinski, 2011; David and Levett, 2015).

The diagnosis of leptospirosis is complex (Levett and Whittington, 1998), where ELISA was performed and the results were compared with MAT by using serial serum specimens collected from the

suspected patients with leptospiral infection and higher sensitivity is identifying in acute phase leptospirosis (McBride *et al.*, 2007). The application of ELISA and MAT demonstrate the rapid confirmation of patients with symptoms suspicious for leptospirosis. As the result these techniques can be obtained shortly after drawing of the blood sample, application of the assay will likely to be improved and the treatment of the patients by allowing a better diagnosis to be made and treatment to be started promptly (Smits *et al.*, 2001; Raagavendhar *et al.*, 2019).

In the early days of diagnosis, when few serovars were known and included for MAT, it was customary to include all those serovars known to occur within a region in the antigen panel for the interpretation of the serological testing and defined as being serovar specific (Turner, 1968; Wolff, 1954). Cross reactions serogroups are common (WHO, 1967; Sulzer and Jones, 1978), as a paradoxical reactions, in which the initial immune response is directed to a heterologous serovar or serogroup (Torten, 1979; Turner, 1968).

The broad range of serogroups have been used in the MAT to maximize the probability of detecting an immune response to a serovar not expected, either because it has not yet been isolated or because a previously known serovar has been found in the population (Faine, 1982; Gochenour, 1953). The perplexing factor in areas of high endemicity is the possibility of co-infection with multiple serovars where the potential for over interpretation of misidentifying of serologic data is much greater if only acute phase or early convalescent phase serum samples are available for testing (Turner, 1968) which was largely observed in this present study.

In the current study, serogroup Grippityphosa was dominated with the highest titer of 1:320, which is observed largely in municipal workers with maximum exposure to the contaminated environment (drainages and garbage dumping areas), and rest of the high risk groups showed a maximum titer of 1 in 160 and very few with the lowest titer of 1 in 80. This could be an indication that sewage workers are more frequently exposed to leptospire than other high risk groups (Sharma *et al.*, 2006).

Definitive laboratory diagnosis of leptospirosis requires detection of the organism in a clinical specimen or a 4-fold or greater increase in MAT titer in the setting of an appropriate clinical syndrome (Ismail *et al.*, 2006). In our study, seroconversion to 1:80 and above was considered positive because endemicity of leptospirosis has not been reported previously in the study area.

Conclusion

By this study, municipal workers were made aware that their working place is having high risk of leptospirosis. It was screened the occupational risk group for the presence of leptospiral sero-prints in order to provide appropriate medical check-ups and earliest treatment. Significantly higher prevalence rates in rice mill workers compared to control group is identified, indicating that working in the rice mill is a significant risk factor for leptospiral infection. The educational programme for rodent control and infection control is provided to all subjects included in this study in order to provide general informations for getting rid from the infections.

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Table 1: Age and gender wise distribution of subjects of both railway and residential

Age group in years	ReMW (n=103)		RaMW (n=96)	
	Males (n=74)	Females (n=29)	Males (n=62)	Females (n=34)
21 - 30	6 (8.1)	3 (10.3)	2 (3.3)	2 (5.9)
31 - 40	19 (25.7)	7 (24.2)	21 (33.8)	14 (41.2)
41 - 50	32 (43.2)	14 (48.3)	26 (41.9)	8 (23.5)
51 - 60	14 (18.9)	3 (10.3)	11 (17.7)	7 (20.6)
↑ 60	3 (4.1)	2 (6.9)	2 (3.3)	3 (8.8)

[Figures in parenthesis denoted percentages]

Figure 1: Methods of handling of dead rodents

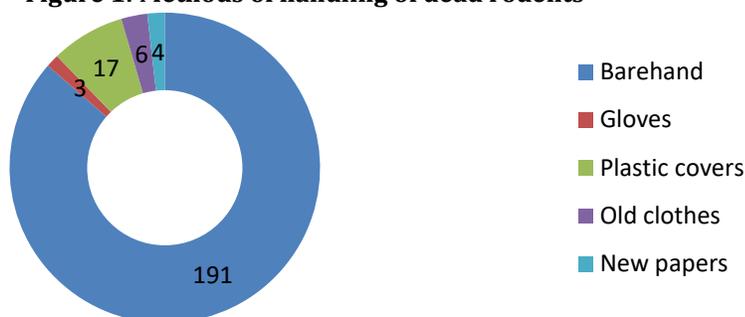


Table 2: Frequency of clinical findings observed among municipal workers

S.No.	Signs and Symptoms	Number of subjects (n=199)							
		ReMW (n=103)				RAMW (n=96)			
		C-1	C-2	C-3	C-4	C-1	C-2	C-3	C-4
01.	Fever	12	34	37	20	11	31	35	19
02.	Head ache	12	32	34	25	10	32	32	22
03.	Myalgia	12	32	34	25	12	29	31	24
04.	Arthralgia	10	29	30	34	10	27	29	30
05.	Jaundice	2	7	4	14	2	7	4	14
06.	Conjunctival suffusion	1	3	4	17	1	3	4	17
07.	Nausea	-	2	3	12	-	2	3	12
08.	Vomiting	8	14	13	21	8	14	13	17
09.	Oliguria	2	7	4	13	2	7	4	13
10.	Digestive disturbances	4	11	14	32	11	31	35	19

[C-1: Criteria 1 - within 2 months; C-2: Criteria 2 - within 6 months; C-3: Criteria 3 - 6 months to one year; C-

4: Criteria 4 - more than one year]

Table 3: Reactivity of genus specific ELISA and MAT

Serology	Re MW (103)	Ra MW (96)
IgG ELISA		
Reactive	73 (70.9)	54 (56.2)
Non-Reactive	30 (29.1)	42 (43.8)
MAT		
Reactive	52 (50.5)	40 (41.7)
Non-Reactive	51 (49.5)	56 (58.3)

[Figures in parenthesis denoted percentages]

Table 4: MAT positive data

Serovars	Residential municipal workers (n=103)		Railway municipal workers (n=93)	
	Number of positive (n=52)	Highest titre value	Number of positive (n=40)	Highest titre value
<i>L. australis</i>	19 (36.5)	1:320	12 (30)	1:320
<i>L. autumnalis</i>	11 (21.2)	1:160	17 (42.5)	1:320
<i>L. canicola</i>	12 (23.1)	1:160	-	-
<i>L. grippotyphosa</i>	21 (40.4)	1:320	-	-
<i>L. hebdomadis</i>	12 (23.1)	1:80	12 (30)	1:80
<i>L. icterohaemorrhagiae</i>	17 (32.7)	1:160	14 (35)	1:160
<i>L. javanica</i>	12 (23.1)	1:160	12 (30)	1:160
<i>L. patoc</i>	7 (13.5)	1:160	7 (17.5)	1:160
<i>L. sejroe</i>	8 (15.4)	1:80	5 (12.5)	1:80
<i>L. shermani</i>	4 (7.7)	1:80	-	-

[Figures in parenthesis denoted percentages]