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Prevalence of Leptospirosis among Rice mill Workers in Tiruchirapalli, South India.

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ABSTRACT

Background: Leptospirosis is one of the emerging and re-emerging neglected tropical bacterial infections, 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, there is an increase in the new cases every year. **Methods:** The present investigation is aimed to assess the presence of leptospirosis among rice mill workers by socio-demographic data, information related to disease, symptoms, animal contact, cleaning procedures and determine the presence of leptospires seroprints by genus specific enzyme linked immunosorbent assay (ELISA) and serovar specific microscopic agglutination test (MAT). **Results & Conclusion:** The Genus specific IgG ELISA was performed for all the serum samples (n=107) and the results were compared with serovar specific MAT by using serial doubling dilutions of serum samples. The results of ELISA showed reactive to 44 samples whereas, MAT showed positive to 32. In MAT, the highest titer value was 1:320 for L. australis and L. grippotyphosa followed by 1:160 for L. autumnalis, L. canicola, L. icterohaemorrhagiae, L. javanica, L. pomona and L. patoc. Screening of this occupational risk group was carried out for the presence of leptospiral seroprints in order to provide appropriate medical check-up and early 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.

Keywords: Leptospirosis, Rice mill workers, Serology, ELISA, MAT.

INTRODUCTION

Leptospirosis is a worldwide zoonoses, particularly observed in tropical and subtropical countries. The infection 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.[1,2] The diseases of same symptoms are interlinked, as the severe forms of leptospirosis mimic typhoid, malaria, dengue and misidentification may occur in different situations. In most situations in the rainy season, the pyrexia of unknown origin (PUO) cases are misdiagnosed due to common clinical manifestations and give false positivity in serological studies.[3,4]

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Investigations with confirmatory and special tests help the clinician to reduce the severity of infection and to recover the patients from chronic morbidity and mortality.[5] Historically, in 1886, Adolf Weil described the clinical manifestations of four different patients with fever, jaundice and renal failure which he defined as Weil's disease.[6]

"A peculiar form of acute infectious disease characterized by jaundice, swelling of the spleen and nephritis"

Later, the causative agent for infectious disease, leptospirosis was identified and clinically compared with yellow fever. Some studies identified the comparativeness of various organisms associated with Weil's disease that are isolated from rats in Japan, Europe and United States. Further, it was evaluated by cross reactivity of serum and new genus was identified as Leptospira.[7]

"Clinically yellow fever is similar to infectious jaundice. The difference existing the two diseases appear to be chiefly those of degree. There is more marked jaundice and less haemorrhage in yellow fever than in infectious diseases"

Leptospirosis has been found everywhere it has been looked for; while some studies consider it an emerging disease there is also the highlighted conception that defines the infection.[8-11]

"Persistent and often under recognized"

Agricultural workers are considered as the main occupational risk groups, who are likely to be exposed to contaminated wet soil and water during their daily activities.[12-14] The animal hosts including rodents, cattle, pigs, dogs, cats and wildlifes) are considered as common reservoirs of leptospires.[15]

Outbreak of human leptospirosis has not previously been reported from the Tiruchirapalli district in Tamil Nadu. But in the year 2014, the risk factors associated with the urban and rural epidemics of leptospirosis in Lalgudi, Tiruchirapalli were well studied.[16] The study area Manachanallur, the rice granary of the district, covers more than 50 villages where many rice mills are located on the outskirts of the town, which process rice cultivated in the surrounding villages.

Rice mill workers form a significant proportion of workers in Manachanallur where they soak rice in large tanks, par-boil, dry and pound the dried rice. The rice mills offer a rich source of food to the rodents and usually constitute their breeding houses, an environment suitable for the survival of leptospires, and a large population of intermediary hosts reared in the same premises, which can be an epidemiological niche for frequent transmission of leptospires. This study was therefore carried out to determine the prevalence of leptospirosis among rice mill workers of Mancahanallur, Tiruchirapalli, South India.

MATERIALS AND METHODS

This study is a cross sectional observational study, involving 107 rice mill workers and 53 age and sex matched non rice mill workers of Manachanallur Taluk, Tiruchirapalli, South India during the period of 5 months from April and August 2018. After getting approval from institutional ethical committee, permission from the rice mill owners and informed consent from the participants were obtained and the blood samples were collected. In the present study, leptospiral diagnosis was restricted to serological study only. The rice mill workers with minimum of 6 months continuous experience were included and the individuals working below 6 months in the rice mill were excluded from the study.

This serological study assessed the efficacy of the IgG ELISA and MAT for diagnosis of leptopsiral seroprints for determining the long term response for epidemiology. Whole procedure was performed according to manufacturer's instruction (Pan Bio). Test sera and controls were diluted in 1:100 in serum diluents and 100µl added into Leptospira (serovar patoc) antigen coated microwell. Then plate was incubated for 30 min at 37°C. After washing the plate with phosphate- buffered saline solution, 100µl of HRP-conjugated anti-human IgG added and incubated for further 30 min at 37°C. Again washing the plate with buffered solution, 100µl of the TMB (tetramethylbenzidine) substrate was added and incubated for 10 min at room temperature. Then reaction was stopped with 100µl of 1M phosphoric acid. The absorbance value of each well was read at 450 nm wave length and reading was interpreted in terms of Pan-Bio units which in turn were calculated by the absorbance of positive control serum, negative control serum and cut-off of calibrators provided by the manufacturer. Pan Bio unit ≥11 was considered positive.[17]

The leptospiral antibodies were demonstrated in the serum by microscopic agglutination test (MAT) (Wolff, 1954). It is the routine and gold standard test and can be done only in the specialized labs where facilities for maintaining the different serovars are available. The reference leptospiral strains maintained in the laboratory 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 list of leptospiral strains is depicted in [Table 1]. All the listed strains were maintained by periodic sub culturing in EMJH semisolid medium.

Table 1: Reference leptospiral strains used for MAT analysis

Serogroup	Serovar	Strain
Australis	Australis	Ballico
Autumnalis	Bangkinang	Bangkinang
Canicola	Canicola	Hond Utrech IV
Grippotyphosa	Grippotyphosa	Moskva V
Hebdomadis	Hebdomadis	Hebdomadis
Icterohaemorrhagiae	Icterohaemorrhagiae	RGA
Javanica	Poi	Poi
Pomona	Pomona	Pomona
Pyrogens	Pyrogens	Salinem
Sejroe	Hardjo	Hardjoprajitno
Semaranga	Patoc	Patoc I
Shermani	Shermani	1342K

[Leptospiral strains were obtained from Regional Medical Research Centre, Port Blair, A&N Islands]

The actively growing cultures were most commonly used as the antigen. The live cultures which desired were maintained in EMJH liquid medium and sub cultured periodically for every 7 days. If the antigen appears too dense, then it was diluted with 1% PBS solution. The serum samples were diluted by using 1% PBS at the pH of 7.2. Serial two fold dilutions of serum were prepared to provide the dilution of 1:10 to 1:10240. If needed further dilutions were also

carried out. Along with the diluted serum sample, equal volume of antigen was added (1:1 ratio). This set up was shaked well and incubated in a dark environment at room temperature for one hour. After incubation, one drop from each well was taken by using micro diluter and examined microscopically under dark field illumination. Microscopic readings were performed by using an agglutination end point of 50%. The most common serovar associated with the highest MAT titre was recorded for the study of seroprevalence.

The MAT was performed strictly by following the standardized protocol by using a panel of antigens representing both ubiquitous and locally prevalent serovars. The titer value of ≥80 is considered as positive. The MAT procedure was repeated if the samples showed a titer value of ≥80. The predominant serogroup was defined as a titer of ≥80 with the maximum titer directed against a single serogroup. Analyses were also performed using a cutoff titer of ≥800.[19] Cases of leptospirosis were excluded from the analysis if patients were seronegative, if maximum MAT titers of <100 were detected, if highest titers were detected against Leptospira biflexa serovar patoc, and if titers of ≥100 were detected, with equal titers directed against >1 serogroup. 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 107 subjects included in this study, 75 (70.1%) were males and 32 (29.9%) were females [Figure 1a]; whereas among control groups 40 (75.4%) were males and 13 (24.5%) were females [Figure 1b].

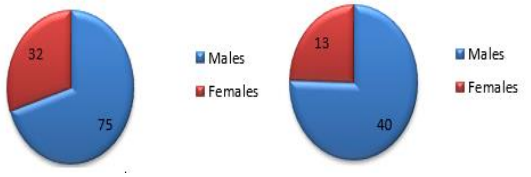

Figure 1: Gender-wise distribution of subjects Figure 1a: Study subjects (Rice mill workers) Figure 1b: Control groups

While analyzing the data, 31.7% belonged to 21 to 30 years followed by 31 to 40 years and 51 to 60 years with 28% and 15.8% respectively. The detailed gender and age wise distribution of study subjects and control groups are depicted in [Table 2].

Table 2: Age and gender wise distribution of study subjects and controls

Age group in years	Study subjects (n=107)		Controls (n=53)	
	Males (n=75)	Females (n=32)	Males (n=40)	Females (n=13)
18 – 20	4 (5.3)	6 (18.8)	2 (5)	1 (7.7)
21 – 30	24 (32)	10 (31.2)	12 (30)	4 (30.8)
31 – 40	24 (32)	6 (18.8)	10 (25)	4 (30.8)
41 - 50	10 (13.3)	4 (12.5)	8 (20)	1 (7.7)
51 - 60	12 (16)	5 (15.6)	5 (12.5)	2 (15.3)
Above 60	1 (1.4)	1 (3.1)	3 (7.5)	1 (7.7)

[Figures in parenthesis denotes percentage]

The animal contacts among the study subjects and controls were recorded and analyzed 87.8% were having contact among study subjects and 96.2% among controls [Figure 2].

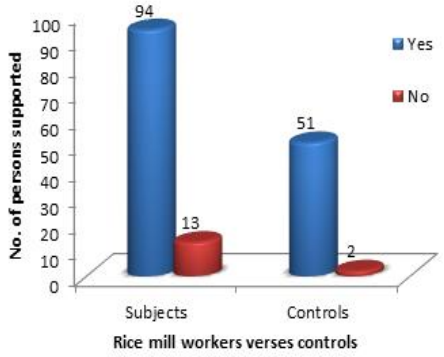

Figure 2: Details of persons having animal contact

The types of animals handled by the subjects and controls are cows, dogs, goats, buffalos and cats. Most of the above said animals are harbouring leptospires and possible to spread or transmit the infection to the humans. The detailed analyses of percentage of subjects who are handling animals were depicted in figure 3. It was observed that 85 rice mill workers and 26 controls had frequent contact with rats and rodents in the working place and residence respectively [Figure 4].

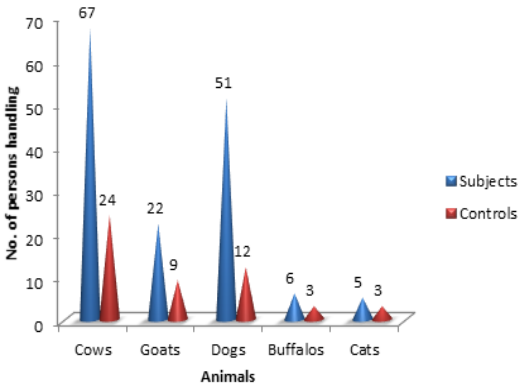

Figure 3: Types of animals verses number of persons handling

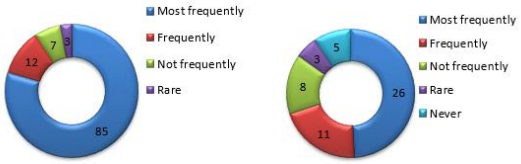

Figure 4: Observation of rats and other rodents in the working environment, Figure 4a: Observation among study subjects Figure 4b: Observation among controls

Meanwhile, the persons included in this study were enquired for the observation of dead rats and other rodents and their urine stains. Most of them answered yes and very few gave no [Table 3].

Table 3: Observational data of dead animals and their urine stains

Criteria	Study subjects (n=107)		Controls (n=53)	
	Yes	No	Yes	No
Observation of dead rats and other rodents	93 (86.9)	14 (13.1)	31 (58.4)	22 (41.6)
Observation of rat/ rodents' urine stain	64 (59.8)	43 (40.2)	14 (26.4)	39 (73.6)

[Figure in parenthesis denotes percentage]

The mode and method of cleaning the working environment were also analyzed and the risks and mode of entry of leptospires to the persons handling the cleaning works was determined. This analysis revealed that 92 members cleaned the work environment (stained floor) with barefoot and hands the details are shown in [Figure 5].

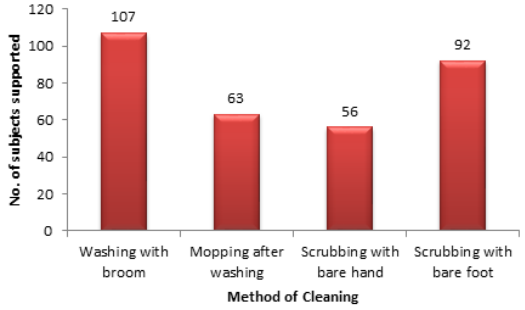

Figure 5: Analysis of method of cleaning

The usage of disinfectants while cleaning (brooming and mopping) the working environment with or without stains were analyzed and depicted in table 4. The usage of antiseptics after cleaning is also furnished in the [Table 4].

Table 4: Description of usage of disinfectant and antiseptics

Criteria	Study subjects (n=107)		
	Yes	No	Rare
Usage of disinfectants while cleaning	63 (57.9)	32 (29.9)	12 (11.2)
Usage of antiseptics after cleaning	31 (29.9)	60 (55.2)	16 (14.9)

[Figure in parenthesis denotes percentage]

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 study subjects, by revealing the past history, one case was recorded as conjunctival suffusion and two with jaundice. The prevalence of various signs and symptoms observed among the study subjects during the past one year is summarized in [Table 5].

Table 5: Percentage frequency of clinical findings from suspected patients

S. No.	Signs and Symptoms	Number of study subjects (n=107)			
		Criteria 1	Criteria 2	Criteria 3	Criteria 4
01.	Fever	12	36	39	20
02.	Head ache	12	34	36	25
03.	Myalgia	12	34	36	25
04.	Arthralgia	10	31	32	34
05.	Jaundice	2	7	4	14
06.	Conjunctival suffusion	1	3	4	17
07.	Nausea	-	2	3	52
08.	Vomiting	8	31	34	34
09.	Oliguria	2	7	4	13
10.	Digestive disturbances	4	16	21	66

[Criteria 1: within 6 – 8 months; Criteria 2: within 8 - 10 months; Criteria 3: 10 months to one year; Criteria 4: more than one year]

The subjects were interviewed for their knowledge about previous history of leptospirosis where suspected and diagnosed for leptospirosis (n=4; 3.7%), suspected alone (n=17; 15.8%), not suspected (27; 25.3%) and no knowledge (59; 55.2%) are recorded. Further the subjects were analyzed for the symptoms related to leptospirosis while suspected as well as suspected and diagnosed (n=21) [Figure 6].

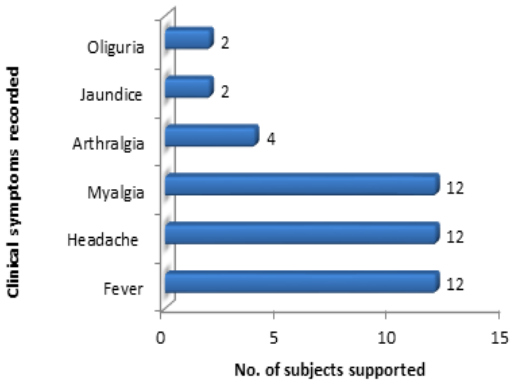

Figure 6: Symptoms related to leptospirosis within two months of exposure (n=44)

The total year of working experience in the rice mill of the subjects included in this study were analysed and calculated. The detailed descriptions of the year of working are depicted in [Figure 7].

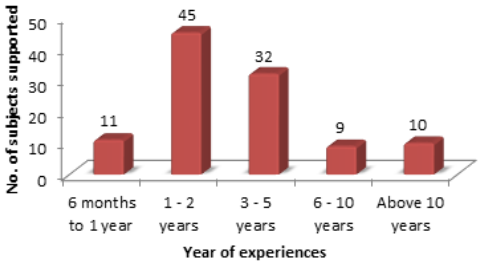

Figure 7: Year of experience among the study subjects (n=107)

The results of genus specific IgG ELISA revealed that 44 (41.1%) serum samples were reactive and 63 (58.9%) were non-reactive [Figure 8a]. While performing IgM ELISA for current infection, two were reactive and 19 were non-reactive out of 21 samples [Figure 8b]

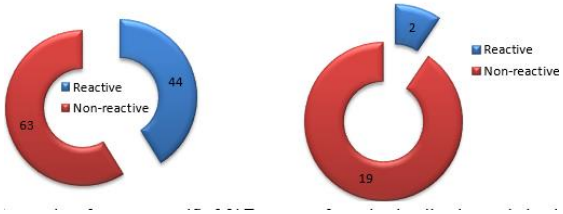

Figure 8a: IgG ELISA reactivity, Figure 8b: IgM ELISA reactivity

The results of serovar specific MAT were performed using live leptospiral cultures as antigen which revealed that 32 (29.9%) serum samples were reactive and 75 (70.1%) were non-reactive [Figure 9a]. While performing with control serum samples, two were reactive and 51 were non-reactive out of 53 samples [Figure 9b].

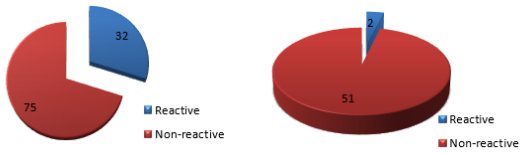

Figure 9a: MAT reactivity among subjects, Figure 9b: MAT reactivity among controls

Among the 107 samples of rice mill 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 107 serum samples, 32 samples showed positive to MAT. Among the serovars, L. grippotyphosa (n=21) was the predominant against which antibodies were present followed by australis, icterohaemorrhagiae etc. The highest antibody titer obtained for L. australis and L. grippotyohosa was 1:320 which are prevalent serovars in Tamilnadu.

The total number of positive cases and its highest titer values for various serovars is depicted in Table 6. Microscopic agglutination of leptospires with antibodies is interpreted in [Figure 10].

Table 6: Serovars involved in 32 MAT positive samples

Serovars	Number of positive samples (n=32)	Highest titer values
L. grippotyphosa	21	1:320
L. icterohaemorrhagiae	17	1:160
L. autumnalis	11	1:160
L. sejroe	8	1:80
L. pomona	8	1:160
L. australis	19	1:320
L. canicola	12	1:160
L. javanica	12	1:160
L. patoc	7	1:160
L. shermani	4	1:80
L. hebdomadis	12	1:80

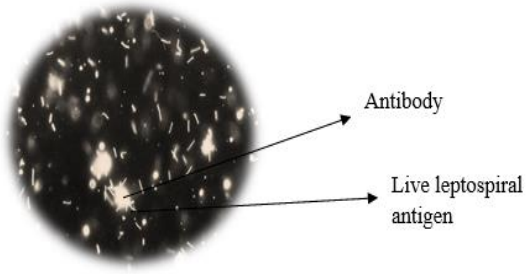

Figure 10: Dark field microscopic view of MAT results

In the case of leptospiral diagnostic methods, the ELISA is described as the prediction of positivity against genus level of leptospires. 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 assessed using the acute and convalescent serum samples. Table 37 depicts the comparative seroprevalence among subjects and controls by using MAT and the ELISA.

Table 7: Comparison of seroprevalence between MAT and ELISA among study subjects and controls

Serum Samples	ELISA		MAT	
	Reactive	Non - reactive	Reactive	Non-reactive
Subjects (n=107)	44 (41.1)	63 (58.9)	32 (29.9)	75 (70.1)
Controls (n=53)	2 (3.8)	51 (96.2)	2 (3.8)	51 (96.2)

[Figure in parenthesis denotes percentage]

While interviewing the subjects, only 32 (29.9%) among 107 informed that the management of the rice mill where they are working arranged medical camps very rarely. They also informed that the last medical camp was conducted 2 years before and no doctors are invited to the working place on any special occasions and routine checkups. All the workers also happily agreed to attend/ participate in the medical camp if arranged.

DISCUSSION

This study was investigated and analyzed from the socio-demographic data provided by the rice mill workers. As per the results obtained, most of the subjects included in this investigation are frequently exposed to rats and other rodents and some of them were even directly exposed to their excreta. This disease has been recognized as an important occupational hazard of agricultural workers and its related manual labourers.[20,21]

As per the clinical manifestations are concerned, most of the study subjects included had fever and headache. Jaundice and conjunctival suffusion are rare observations.[22] The study represents the first systematic serosurvey documenting the occurrence of the seroprints of human leptospirosis in central part of Tamilnadu. It also described the prevalent serovars and clinical presentations.

Leptospirosis was less frequent in children less than 15 years of age and in adults more than 75 years of age because they are having limited contact with infected soil, water and excreta; whereas in this study, the age group of 21 to 40 are recorded in high numbers.[23] The infection mostly affects males as they work in rice mills more frequently than females. The sex difference which has been found in numerous other studies,[24-26] is usually attributed to occupational factors and it tends to vanish if sexes are given equal exposure.[27-29]

The diagnosis of leptospirosis is complex.[30] The ELISA was performed and the results were compared with MAT by using serum specimens collected from the suspected study subjects with leptospiral symptoms. This serological analysis showed higher sensitivity in identifying acute phase leptospirosis.[31]

The application of ELISA and MAT demonstrate the rapid confirmation of results for patients with symptoms suspicious of leptospirosis even when samples with a high proportion with borderline or low titers are tested. As a result, these techniques can be adopted easily and will likely improve the treatment of the patients by allowing a better diagnosis to be made and treatment to be started promptly.[32]

Agglutination tests for the leptospiral antibody were developed soon after the first isolation of leptospires, which occurred >80 years ago. In the early years of diagnosis of leptospiral infection, when few serovars were known, it was customary to include all those serovars known to occur within a region in the antigen panel and to interpret the results of serological testing as being serovar specific.[18,33] Cross reaction serogroups are common,[34,35] as a paradoxical reaction, in which the initial immune response is directed to a heterologous serovar or serogroup.[33,36]

In reference laboratories, a broad range of serogroups has 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 introduced into the population.[19,37] In addition, confounding factor in areas of high endemicity is the possibility of co infection with multiple serovars. The potential for over interpretation of serologic data thus is much greater if only acute phase or early convalescent phase serum samples are available for testing,[33] which was largely observed in this present study.

In the current study, serogroup Grippotyphosa showed the maximum numbers and highest titer of 1:320, which is observed largely in rice mill workers with maximum exposure to the contaminated environment, 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 rice mill workers are more frequently exposed to leptospires than other high risk groups.[38]

Definitive laboratory diagnosis of leptospirosis requires detection of the organism in a clinical samples or a 4-fold or greater increase in MAT titer in the setting of an appropriate clinical syndrome.[39] In our study, seroconversion 1:80 and above was considered positive because endemicity of leptospirosis has not been reported previously in Tiruchirappalli.

CONCLUSION

By this study, the rice mill workers were made aware that their working place is having high risk of leptospirosis. Screening this occupational risk group for the presence of leptospiral seroprints was carried out in order to provide appropriate medical check-up and early 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. When the seroprevalence is high, regular health educational programmes on rodent control and infection control should be provided.

Limitations

1. This study has its own limitations of restricting the selective rice mills whose owners gave permission (not all rice mills in this study area included).
2. The previous clinical data sheet (case sheet) of the infection is not analysed. The seasonal variations are not included.

Acknowledgement

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owners for permitting to utilize their workers for screening leptospirosis.

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