

Short Communication

Leptospirosis among Rice Mill Workers of Salem, South India

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SUMMARY: Leptospirosis is not commonly reported from the Salem district in central Tamil Nadu in India. In October 2000, a rice mill worker, who had fever and jaundice, tested positive in leptospiral IgM enzyme-linked immunosorbent assay (ELISA). Microbiological and serological investigations were performed on the patient, a sample population of the rice mill workers, and the animal and rodent populations living in the same premises. *Leptospira* was isolated from the patient about 2 weeks after the onset of symptoms when he had recovered from illness following a course of doxycycline. The isolate was serovar Icterohaemorrhagiae of serogroup Icterohaemorrhagiae. The patient also showed a fourfold rise in titers in microscopic agglutination test (MAT) and IgG ELISA. The rice mill workers had a seroprevalence rate of 68.3%, which was significantly higher than that among a control group consisting of persons engaged in other occupations. Serological studies conducted among cattle, dogs, cats, and rats showed seroprevalence rates of 52.9%, 50.0%, 66.6%, and 52.1%, respectively. Leptospire were isolated from two rats, but the isolates were lost during subculturing and could not be characterized. The most predominant serogroup identified by MAT was Autumnalis for rice mill workers and all animal populations. The other serogroups that reacted in MAT were Icterohaemorrhagiae, Australis, Grippotyphosa, and Patoc. Although Australis and Grippotyphosa showed agglutination in the case of human samples, none of the animals had detectable titers to these serogroups. The rice mills of Salem, having large rodent populations, various animals living in close proximity, a wet environment, and unprotected exposure of the workers to the environment, constitutes an ideal setting for transmission of leptospirosis and could be an epidemiological niche of leptospirosis.

Leptospirosis is a widespread zoonosis that affects humans on all continents, in both urban and rural contexts, and in temperate and tropical climates (1). Human beings get the infection either from rodent hosts or from domestic animals. Environment contaminated with the urine of carrier animals is usually the immediate source of infection (2). Because of the ability of the leptospire to survive in the environment and infect human beings, leptospirosis is a potential health hazard of occupational groups exposed to the environment.

Many places in South India are known to be endemic to leptospirosis. These include Chennai and Madurai in the state of Tamil Nadu, Kolenchery, and Kochi in the state of Kerala and some areas in the state of Karnataka. Human leptospirosis has not previously been reported from the Salem district in Tamil Nadu. Salem town, the headquarters of the district, is located on the foothills of the Yercaud hills in Central Tamil Nadu. There are many rice mills on the outskirts of the town, which process rice cultivated in the surrounding villages. Rice mill workers form a significant proportion of the factory workers in Salem. In the mills, they soak rice in water pumped into large tanks, par-boil it in boilers, dry it by spreading it in the sun, and pound the dried rice in pounding machines. Thus they have constant contact with water. The rice mills offer a

rich source of food to the rodents and usually constitute their breeding houses.

In October 2000, a 36-year-old male worker of one of the rice mills (ADMRM rice mill) fell ill with fever and jaundice. He was hospitalized and the hospital reported that his serum sample showed positive results for leptospira IgM enzyme-linked immunosorbent assay (ELISA). Since leptospirosis was very rare in Salem, we carried out a microbiological and epidemiological investigation of leptospirosis among the rice mill workers in Salem town. The owners of the mill stay in a house attached to the mill. They have a cattle shed and also rear several cats and dogs on the mill premises. Investigations were also carried out among the animals living on the mill premises.

The worker who fell ill had fever, body aches, headache, and conjunctival suffusion. He developed jaundice after a few days and was hospitalized. The hospital reported that his serum sample showed positive result in leptospira IgM ELISA. He was treated with doxycycline at 200 mg per day for 1 week and was discharged from the hospital after 10 days.

Two weeks after the onset of illness, we collected a blood sample and a urine sample from the patient. By this time, the patient had already been discharged from the hospital. A second blood sample was collected 2 weeks after collecting the first sample. Blood samples were collected from 329 other rice mill workers in Salem town. Twenty-nine blood samples

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were collected from persons who are engaged in other occupations. Blood samples were also collected from 34 cattle, four dogs and nine cats living in the premises of ADMRM rice mill. Twenty-three field rats were caught in the rice fields surrounding the mill.

Isolation of leptospires was attempted from the blood and urine samples of the patient and from the kidney samples of the rats. The blood sample from the patient was inoculated into EMJH semisolid medium (3). His urine sample and rat kidney samples were inoculated into EMJH semisolid medium containing 2% rabbit serum and 100 $\mu\text{g/ml}$ of 5-fluorouracil (3,4). The cultures were examined every 10 days up to 6 months. Positive samples were subcultured into EMJH semisolid media.

Serum was separated from all the blood samples and stored at -20°C until use. Microscopic agglutination test (MAT) was performed on the samples using ten live leptospiral strains as antigens. The strains belonged to the serogroups Australis (strain Jez-Bratislava), Autumnalis (Akiyami A), Ballum (Mus 127), Bataviae (Swart), Canicola (Hond Utrecht IV), Icterohaemorrhagiae (RGA), Grippotyphosa (Moskva V), Hebdomadis (Hebdomadis), Pomona (Pomona), and Saprophytic Semarang (Patoc I). MAT was performed using doubling dilutions starting from 1:20. Positive samples were titrated up to end titers. A titer of 1:80 or above was considered as positive in the case of human and animal samples except for rats. In the case of rats, a titer of 1:20 or above was considered positive (5-7).

IgG ELISA was done using ELISA plates prepared in-house. Plates were coated with heat-killed whole cell antigen prepared from the Patoc I strain using the standard procedure (8). Serial twofold dilutions of the sera in phosphate buffer saline with tween (PBST) were added to the wells and incubated for 1 h. The plates were then washed with PBST and incubated again with 100 μl of peroxidase-labeled anti-human IgG conjugate (Sigma Chemical Co., St. Louis, Mo., USA) for 1 h at 30°C . After washing the plates thoroughly, 100 μl substrate (One OPD, 2HCl, tablet in 10 ml of 0.05M phosphate citrate buffer, pH 5.0) was added and the plates were kept in darkness for 30 min. Finally 50 μl of stopping solution (sulphuric acid) was added and the OD was read at 492 nm in an ELISA reader.

The urine culture of the patient yielded leptospires, which were identified as belonging to serogroup Icterohaemorrhagiae of serovar Icterohaemorrhagiae. MAT performed on the first and second blood samples showed titers of 1:640 and 1:5120 against Icterohaemorrhagiae. IgG ELISA showed titers of 1:1280 and 1:5120 on first and second samples, respectively.

Kidney cultures of two of the rats also yielded leptospiral isolates. However, they were lost during subcultures and hence could not be characterized. We used selective medium containing 5-FU to prevent growth of other bacteria. This selective medium is known to yield lower culture positivity rates than non-selective EMJH media. An earlier study had shown that leptospiral isolate from the kidney of a lamb could not be passaged beyond the first subculture (9).

Of the 329 rice mill workers who were tested for sero-positivity, 266 (80.9%) were males and 63 (19.1%) were females. They were in the age group of 15-56 years with a median age of 28 years for males and 24 years for females. MAT showed that 225 rice mill workers (68.3%) showed positive. IgG ELISA was positive for 234 (71.1%). Control group had 20.6% and 24.1% positivity in MAT and ELISA, respectively. The difference in the MAT positivity rates among rice mill workers and controls was statistically significant ($\chi^2=52.85$, $P < 0.001$). The titers observed in MAT and ELISA among the controls were lower than that of the rice mill workers. Serogroup Autumnalis (34.9%) was the predominant reacting serogroup in MAT. The other serogroups were Icterohaemorrhagiae (24.2%), Grippotyphosa (17.4%), and Australis (16.5%) and mixed equals (6.7%) (Table 1). The common mixed equals were Autumnalis and Icterohaemorrhagiae. Although the isolate obtained from the patient was Icterohaemorrhagiae, MAT results indicated that Autumnalis was the dominant infecting serogroup among the rice mill workers (34.9%), field rats (26.6%), cattle (23.5%), dogs (25.0%), and cats (33.3%). IgG ELISA showed greater sensitivity as compared to MAT (Table 2) and was significant at a level of 1% by χ^2 test.

The study was conducted because the hospital reported positive-IgM ELISA results for a rice mill worker who was admitted with fever and jaundice. We could isolate leptospires from his urine sample even after the patient had recovered from his illness and had been discharged from hospital. He

Table 1. Seroprevalance of leptospirosis among the rice mill workers and different animals with the highest titer as determined by MAT

Serogroup	Rat 12/23 (52.1%)	Cat 6/9 (66.6%)	Dog 2/4 (50%)	Cattle 18/34 (52.9%)	Rice mill workers 225/329 (68.3%)	Human control 6/29 (20.6%)
Australis	-	-	-	-	16.5% (1:1280)	-
Autumnalis	26.6% (1:160)	33.3% (1:320)	25.0% (1:320)	23.5% (1:1280)	34.9% (1:10240)	20.6% (1:80)
Canicola	-	16.6% (1:80)	25.0% (1:80)	-	-	-
Grippotyphosa	-	-	-	-	17.4% (1:640)	-
Icterohaemorrhagiae	21.7% (1:80)	16.6% (1:80)	-	-	24.2% (1:1280)	-
Pomona	-	-	-	-	-	-
Patoc I	-	-	-	11.7% (1:160)	-	-
Mixed Equals	4.34% (1:80)	-	-	5.8% (1:80)	6.7% (1:320)	-

Table 2. Distribution of antibody titers as determined by MAT and ELISA in rice mill workers and their controls

Titer	Rice mill workers (n=329)*				Control (n=29)			
	MAT (+ve 225/329)		ELISA (+ve 234/239)		MAT (+ve 6/9)		ELISA (+ve 7/29)	
	Number	%	Number	%	Number	%	Number	%
1:80	43	13.1	7	2.1	6	20.6	5	10.3
1:160	80	24.3	21	6.4	–	–	2	6.8
1:320	33	10.0	86	26.1	–	–	–	–
1:640	32	9.7	27	8.2	–	–	–	–
1:1280	19	5.8	29	9.0	–	–	–	–
1:2560	7	2.1	36	10.9	–	–	–	–
1:5120	9	2.7	13	3.9	–	–	–	–
1:10240	2	0.6	6	1.8	–	–	–	–
1:20480	–	–	9	2.7	–	–	–	–
Total	225	68.3	234	71.1	6	20.6	7	24.1

*Significant at 1% by using χ^2 test.

had been treated with doxycycline for a week. Compared to other tetracyclines, excretion of doxycycline in urine is low (10). This may be the reason for the survival of leptospires in the kidneys of the patient even after a week of treatment with this drug. At the same time, the possibility of doxycycline resistance among leptospires cannot be ruled out.

Although leptospirosis is not commonly reported in Salem, the seroprevalence rates among the rice mill workers and animal populations indicate that it would be occurring fairly frequently at least among the rice mill workers. Perhaps, because of the difficulty in making a clinical diagnosis in the case of uncomplicated leptospirosis, these cases might have been overlooked by the clinicians. Even among the controls, the seroprevalence rate was high (20.6%), indicating that the area is endemic for leptospirosis. Seroprevalence studies among occupational groups such as slaughterhouse workers (11), laboratory animal house workers (12), and agricultural workers of Tamilnadu (7) have shown similar high prevalence rates.

All the animal species studied showed seroprevalence rates in excess of 50%. However the role of these animals in the transmission cycle of leptospirosis in the area cannot be assessed without more detailed studies. Isolation of leptospires from rats indicates that they act as sources of infection for the domestic animals and perhaps to humans. Autumnalis and Icterohaemorrhagiae are the predominant infecting serogroups in the case of all the animal species and human beings. Although the isolate from the patient was serogroup Icterohaemorrhagiae, MAT results indicate that Autumnalis infection was more common than Icterohaemorrhagiae. Rice mill workers had MAT titers against Australis and Grippotyphosa as well, which were not seen in any of the animals studied. Some other animal species such as bandicoots or pigs might be playing a role in the transmission of such serogroups.

Earlier studies performed in Tamil Nadu, based on isolation and serology among human beings, had shown that Autumnalis was the dominant infecting serogroup (6,13). The ADMRM rice mill is surrounded by rice fields with a large population of field rodents. The mill owners keep domestic animals in the mill premises itself. After the harvest, the rodents depend on the rice mills for their food and hence there is a chance of contamination of the water used for soaking rice and the wet soil by rat urine. The mill workers are constantly exposed to the environment and the water used for soaking rice. They usually do not wear any protective

footwear or gloves while working. These working conditions and work habits of the rice mill workers put them under risk of leptospiral infection. The significantly higher seropositivity rates among them compared to the control group indicate that work in the rice mill is a significant risk factor for leptospiral infection. The rice mills of Salem, which offers an abundant source of food for the rodents, an environment suitable for the survival of leptospires, and a large population of intermediary hosts in the form of cattle, dogs, and cats reared in the same premises, can be an epidemiological niche for frequent transmission of leptospires.

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