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Leptospirosis Seroprevalence among Blue Metal Mine Workers of Tamil Nadu, India

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Abstract. Leptospirosis is mainly considered an occupational disease, prevalent among agriculture, sewage works, forestry, and animal slaughtering populations. However, putative risk to miners and their inclusion in the high-risk leptospirosis group remain in need of rigorous analysis. Therefore, a study was conducted with the objective to assess the leptospirosis seroprevalence among miners of two districts of Tamil Nadu, India. A total of 244 sera samples from Pudukkottai miners (124) and Karur miners (120) were analyzed by microscopic agglutination test. Antibodies to leptospires were detected in 94 samples giving an overall seroprevalence of 38.5%. The seroprevalence was higher among Pudukkottai miners (65.3%) when compared with Karur miners (10.8%). Seroprevalence among control population (13%) was significantly less than that of the Pudukkottai miners marking a possible high-risk population group distinction. Subject sera most commonly reacted with organisms of the serogroup Autumnalis, and the pattern was similar in carrier animals of the study areas. Two leptospires were isolated from kidney samples of rats. The prevalence of Autumnalis among rodents and humans source tracked human leptospirosis among the miners. The study also determined that Pudukkottai miners are subjected to high-risk challenges such as exposure to water bodies on the way to the mines (odds ratio [OR] = 10.6), wet mine areas (OR = 10.6), rat infestation (OR = 4.6), and cattle rearing (OR = 10.4) and are thus frequently exposed to leptospirosis compared with Karur miners. Hence, control strategies targeting these populations will likely to prove to be effective remediation strategies benefiting Pudukkottai miners and workers in similar environments across occupations.

INTRODUCTION

Leptospirosis is a zoonotic disease caused by pathogenic spirochetes of the genus *Leptospira.* It affects both domesticated and wild animals.[1] Infected animals excrete leptospires in their urine, and direct or indirect exposure to the contaminated urine causes leptospiral infection in humans.[2] Direct *Leptospira* infection occurs through penetration of impaired skin barrier and ingestion of food contaminated with urine of infected rats, whereas indirect infection occurs by inhalation of contaminated fluid aerosols, as occurs in slaughterhouses. There are over 250 known leptospiral serovars arranged in 25 serogroups with pathogenic *Leptospira* predominant for the human and animal infection. *Leptospira* infection affects liver, kidney, lungs, heart, and leads to hepatic disease, kidney failure, myocardial infection, and pulmonary hemorrhage syndrome.[3,4] Leptospirosis is an emerging disease in India and other developing countries, and it mainly affects field workers exposed to stagnant water. A large number of studies are available pertaining to leptospiral exposure, seroprevalance, and associated risk factors among field workers including farmers, sewage workers, and rice mill workers from India.[5–7] However, the *Leptospira* threat to mining workers putative seroprevalence, and the associated risk factors are unknown. Thus in this study, seroprevalence and risk factors among Pudukkottai and Karur district mine workers of blue metal and quartz feldspar were analyzed.

METHODS

Study site. The study was conducted in the Pudukkottai and Karur districts of Tamil Nadu, India. Pudukkottai, located between 9°50′ and 10°40′N latitudes and 78°25′ to 79°15′E longitudes, is wide spread with an aerial extent of about 4,663 km^2 and a 2011 census population of 1,618,725 (rural—1,304,800 and urban—313,925). Blue metal is the commonly available mineral of Pudukkottai. Karur district is located between 10°55′35″N latitude and 77°50′38″E longitude with 2,895.57 km^2 area and a 2011 census population of 1,064,493. The common economic minerals available are milky to glassy quartz varieties and potash feldspar with 12% potash. Both the districts receive rainfall from northeast monsoon and temperature ranges between 19.1°C and 37.1°C.

Sample size. Phenotypically healthy mining workers were enrolled for the study for subsequent collection of blood and urine samples. The purpose and procedures of the study were explained to the study subjects, and informed written consent was obtained from all the participants. Subjects were excluded if they had fever within the previous 2 weeks or if they declined participation. This study was approved by Institutional Ethics Committee (IEC) of Bharathidasan University, Tiruchirappalli, India (Ref No. DM/2010/101/13). Sample size was estimated as per expected prevalence with a relative precision of 20% or an absolute precision of 6% and with 95% confidence limits.[8] A total of 124 and 120 samples were collected from Pudukkottai and Karur districts, respectively. In addition, three control subjects (healthy white-collar workers) from each mine workers were selected from the respective study areas to match for age (±5 years) and sex to make a statistically age-matched case–control study. Blood samples were also collected from 86 cattle, 15 dogs, and 29 goats reared around Pudukkottai mines. Twenty-three field rats (*Rattus norvegicus*) caught from the same mine and its surrounding areas were also included. Sera were stored at −80°C until processed.

Isolation of *Leptospira*. Leptospires were isolated from urine samples of the mine workers and the kidney samples of rats. They were inoculated into Ellinghausen–McCullough–Johnson–Harris (EMJH) semisolid medium containing 2%

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rabbit serum and 100 μg/mL of 5-fluorouracil.[9] The cultures were examined every 10 days for up to 6 months. Positive samples were subcultured in EMJH semisolid medium and characterized.[10]

Serological analysis. Sera samples were tested for the presence of antileptospiral antibodies using microscopic agglutination test (MAT) as per standard procedures.[9] A panel of 12 reference strains including Australis (serovar Australis, strain Jez-Bratislava); Autumnalis (Autumnalis, Akiyami A); Ballum (Ballum, Mus 127); Bataviae (Bataviae, Swart); Canicola (Canicola, Hond Utrecht IV); Icterohaemorrhagiae (Icterohaemorrhagiae, RGA); Grippotyphosa (Grippotyphosa, Moskva V); Hebdomadis (Hebdomadis, Hebdomadis); Javanica (Poi, Poi); Pomona (Pomona, Pomona); Pyrogenes (Pyrogenes, Pyrogenes); and Sejroe (Hardjo, Hardjoprajitno) were used. Reference strains were obtained from World Health Organization Reference Center for Leptospirosis, Regional Medical Research Center, Port Blair, and maintained in EMJH medium with periodical subculture at medical microbiology laboratory. Cultures used as antigen were 7 days old and auto agglutination and contamination free. Each serum was tested in doubling dilutions, starting from 1 in 20. The endpoint titer for each positive serum was determined, and a titer of 1 in 160 or more against any of the serovar was considered positive.[8]

Survey about potential risk factors. Interviews both from the miners and control subjects were performed, and a standardized questionnaire was administered to obtain information on potential exposures to sources of leptospiral infection. The rigorous survey was designed to obtain detailed information about the study subjects as they pertain to contact with leptospiral reservoirs.[8,11]

Statistical analysis. Univariate odds ratios (ORs) for the potential risk factors were calculated by matched analysis, 95% confidence intervals were calculated using maximum likelihood ratio method,[12] and analysis was performed with EpiInfo (Version 6.04; CDC, Atlanta, GA).[13] Biologically plausible first-order interaction terms were listed. If the test suggested (at 5% level) the ORs to differ significantly, then those factors were used for further analysis. Tests of statistical significance were based on difference in log likelihoods and all *P* values were two sided.

RESULTS

A total of 244 serum samples from miners of Pudukkottai (124) and Karur (120) were tested by MAT. The anti-leptospiral seroprevalence among the control population was observed to be 13% for Pudukkottai and 9.1% for Karur. Alarmingly, anti-leptospiral antibodies against one or more serogroups of leptospires were found in 65.3% (81/124) and 10.8% (13/120) of serum samples from Pudukkottai and Karur miners, respectively. In Pudukkottai, the seropositivity was observed to be increased among the age group of 20–40 years with females (35.6%) being more affected than males (16%). In Karur, no difference in seropositivity was observed between males and females (23%). The seroprevalence among the miners and controls was found to be statistically significant in Pudukkottai ($X^2 = 149.072$, $P < 0.0001$) and not for Karur ($X^2 = 0.332$, $P = 0.5703$) (Table 1). In Pudukkottai, Autumnalis (23.4%) was found to be the commonest infecting serogroup followed by Australis (10.5%), and Icterohaemorrhagiae (8.9%). In Karur, the highest frequency serogroups were Icterohamorrhagiae (4.5%), Autumnalis (2.5%), Grippotyphosa (1.6%), and Australis (1.6%).

TABLE 1
Leptospirosis seroprevalence in Pudukkottai and Karur mining workers

Serogroup	Pudukkottai n (%)		Karur n (%)	
	Miners (*N* = 81)	Controls (*N* = 63)	Miners (*N* = 13)	Controls (*N* = 43)
Australis	13 (10.5)	16 (3.3)	2 (1.6)	17 (3.6)
Autumnalis	29 (23.4)	19 (3.9)	3 (2.5)	6 (1.3)
Ballum	6 (4.8)	1 (0.2)	–	–
Bataviae	3 (2.4)	2 (0.4)	–	–
Canicola	9 (7.3)	5 (1)	–	1 (0.2)
Grippotyphosa	7 (5.6)	2 (0.4)	2 (1.6)	4 (0.9)
Icterohaemorrhagiae	11 (8.9)	17 (3.5)	5 (4.5)	13 (2.7)
Javanica	–	–	–	–
Pomona	3 (2.4)	1 (0.1)	1 (0.8)	2 (0.4)

Univariate analysis found the risk of acquiring leptospirosis among Pudukkottai miners to be significantly associated with house dwellings near bodies of water (OR = 22.8), rat infestation (OR = 7.3), open defecation (OR = 6.6), and swimming (OR = 6.9) (Table 2). No particular significant risk factors were found to be associated with Karur miners for acquisition of leptospirosis (Table 3). Therefore, the environmental risk factors associated with leptospirosis among Pudukkottai miners were assessed on comparison with Karur. The significant environmental risk factors specifically associated with the risk of acquiring leptospirosis among Pudukkottai miners were found to be water bodies on the way to the mines (OR = 10.6), wet mine area (OR = 10.6), rat infestation (OR = 4.6), and cattle rearing (OR = 10.4) (Table 4).

To further understand the source of human leptospirosis in Pudukkottai, samples were included from domestic animals including dogs (*N* = 15), goats (*N* = 29), and cattle (*N* = 86) (Table 5). Since rat infestation was a significant risk factor associated with leptospirosis, field rats (*N* = 23) were trapped and used in the study.

Cattle (45.3%) from the study area showed the highest prevalence of leptospirosis, followed by rodents (39.1%), dogs (26.7%), and goats (24.1%). The predominant serogroups encountered were Autumnalis (27.1%) in rodents, Pomona (20.9%) in cattle, Canicola (13.3%) in dogs, and Javanica (10.3%) in goats. From 23 field rats, 2 leptospiral isolates from kidney were obtained and characterized. One was identified as serogroup Autumnalis and another as serogroup Javanica. Isolation was unsuccessful from miners' samples and other carrier animals.

DISCUSSION

The state of Tamil Nadu, India, is known to be endemic for leptospirosis with majority of the population being exposed to occupational risks. Upsurges are always observed post monsoon with considerable mortalities.[3,8,14] The tropical climate of India favors the survival, maintenance, and transmission of leptospires to human from the environment through carrier animals or by direct contact. Leptospires thrive in water-logged areas after heavy rainfall, and exposure to these contaminated environments poses a risk of infection among humans. The chances of humans contracting leptospiral infection are significantly higher among occupational-risk communities. This study attempted to estimate the seroprevalence

TABLE 2
Univariate matched ORs, CIs, and *P* values of potential risk factors associated with leptospirosis among miners from Pudukkottai

Parameters	Cases (*N* = 81)		Controls (*N* = 63)		OR (95% CI)	*P* value
	Yes n (%)	No n (%)	Yes n (%)	No n (%)		
House and surroundings						
Water bodies on the way	73 (90)	8 (10)	18 (29)	45 (71)	22.8 (9.17–56.78)	< 0.0001***
Type of roof	49 (60)	32 (40)	43 (68)	20 (32)	0.7 (0.36–1.43)	0.3987
Rat infestation	47 (58)	34 (42)	10 (16)	53 (84)	7.3 (3.27–16.42)	0.0068**
Attached latrine	25 (31)	56 (69)	15 (24)	48 (76)	1.4 (0.68–3.01)	0.2320
Personal characteristics						
Smoke	33 (41)	48 (59)	32 (51)	31 (49)	0.7 (0.34–1.29)	0.4144
Alcohol	29 (36)	52 (64)	39 (62)	24 (38)	0.3 (0.17–0.68)	0.5580
Wearing chappals	33 (41)	48 (59)	34 (54)	29 (46)	0.6 (0.30–1.14)	0.4438
Handling animals	41 (51)	40 (49)	32 (51)	31 (49)	1.0 (0.51–1.92)	0.3190
Baths in pond/river	25 (31)	56 (69)	35 (56)	28 (44)	0.4 (0.18–0.71)	0.5501
Open defecation	65 (80)	16 (20)	24 (38)	39 (62)	6.6 (3.13–13.93)	0.0102**
Works in the field	33 (41)	48 (59)	16 (25)	47 (75)	2.0 (0.98–4.15)	0.1553
Swimming	57 (70)	24 (30)	16 (25)	47 (75)	7.0 (3.3–14.6)	0.0083**
Clinical history						
Diabetes	5 (6)	76 (94)	43 (68)	20 (32)	0.03 (0.01–0.09)	0.8611
Hypertension	13 (16)	68 (84)	39 (62)	24 (38)	0.1 (0.05–0.26)	0.7317

CI = confidence interval; OR = odds ratio.
*** $P < 0.001$; ** $P < 0.01$.

and associated risk factors of leptospirosis among mine workers from Pudukkottai and Karur districts of Tamil Nadu.

Seroprevalence was significantly (~6 times) higher among miners from Pudukkottai (65.3%) compared with workers from Karur (10.8%). This is could possibly be due to the frequent exposure of workers to wet environment particularly water-logged mining fields of Pudukkottai, which is not the case encountered in Karur. Studies conducted among high-risk population with wet environmental exposure from Andaman Islands showed a seroprevalence of 52.7% and it was only 8.3% among meat workers of New Zealand.[14,15] This shows water-logged environments increase risk of leptospire transmission to humans. This is further substantiated by evidence that demonstrated Pudukkottai mines to have an archetype favorable for leptospiral transmission. Notably, the seroprevalence of leptospirosis among control cases did not differ significantly between the two study areas (13% versus 9%). In this scenario, a surveillance system that includes systematic case reporting and monitoring of environmental and host animal populations is recommended in these mines. The predominant serogroups encountered in Pudukkottai mines included Autumnalis, Australis, Icterohaemorrhagiae, and Canicola. To source track increased prevalence of leptospirosis among workers in Pudukkottai mines, the risk factors associated with the transmission of leptospirosis were analyzed. Water-logged environments, rat infestation, and cattle rearing were found to be significantly associated with leptospirosis. This suggests that the wet environment served as an ideal niche for leptospires shed from carrier animals (cattle and rodents), playing a crucial role in the transmission of human leptospirosis.

To analyze the source of environmental contamination and human transmission, we included samples from domestic animals and field rats. The seroprevalence showed the leptospiral carrier status of animals and rats. The predominant serogroup encountered was Autumnalis. The seroprevalence among

TABLE 3
Univariate matched ORs, CIs, and *P* values of potential risk factors associated with leptospirosis among miners from Karur

Parameters	Cases (*N* = 81)		Controls (*N* = 63)		OR (95% CI)	*P* value
	Yes n (%)	No n (%)	Yes n (%)	No n (%)		
House and surroundings						
Water bodies on the way	5 (38)	8 (62)	24 (56)	19 (44)	0.4 (0.13–1.76)	0.4818
Type of roof	6 (46)	7 (54)	15 (20)	28 (80)	1.6 (0.45–5.6)	0.2059
Rat infestation	3 (23)	10 (77)	25 (58)	18 (42)	0.2 (0.05–0.89)	0.6421
Attached latrine	8 (62)	5 (38)	15 (35)	28 (65)	2.9 (0.82–10.7)	0.0840
Personal characteristics						
Smoke	7 (54)	6 (46)	26 (60)	17 (40)	0.7 (0.21–2.66)	0.3825
Alcohol	8 (62)	5 (38)	30 (70)	13 (30)	0.6 (0.19–2.52)	0.4050
Wearing chappals	8 (62)	5 (38)	26 (60)	17 (40)	1.0 (0.29–3.73)	0.3064
Handling Animals	7 (54)	5 (38)	25 (58)	18 (42)	1.0 (0.27–3.69)	0.3154
Baths in pond/river	8 (62)	5 (38)	29 (67)	14 (33)	0.7 (0.21–2.79)	0.3795
Open defecation	4 (31)	9 (69)	15 (35)	28 (65)	0.8 (0.21–3.15)	0.3624
Works in the field	7 (54)	6 (46)	24 (56)	19 (44)	0.9 (0.26–3.20)	0.3365
Swimming	8 (62)	5 (38)	29 (67)	14 (33)	0.7 (0.21–2.79)	0.3795
Clinical history						
Diabetes	6 (46)	7 (54)	27 (63)	16 (37)	0.5 (0.14–1.77)	0.4760
Hypertension	6 (46)	7 (54)	27 (63)	16 (37)	0.5 (0.14–1.77)	0.4760

CI = confidence interval; OR = odds ratio.

TABLE 4

Univariate matched ORs, CIs, and *P* values of potential risk factors associated with leptospirosis among miners from Pudukkottai compared with Karur

Parameters	Cases (*N* = 81)		Controls (*N* = 63)		OR (95% CI)	*P* value
	Yes n (%)	No n (%)	Yes n (%)	No n (%)		
Mining environment						
Water bodies on the way	73 (90)	8 (10)	6 (46)	7 (53)	10.6 (2.86–39.54)	0.0011**
Surroundings of mine						
Wet	61 (75)	20 (25)	3 (23)	10 (77)	10.6 (2.54–40.63)	0.0455**
Rat infestation	47 (58)	34 (42)	3 (23)	10 (77)	4.6 (1.17–18.01)	0.0318
Animals in mine						
Dog	37 (46)	44 (54)	3 (23)	10 (77)	2.8 (0.71–10.94)	0.0941
Goat	33 (41)	48 (59)	2 (15)	11 (85)	3.7 (0.78–18.18)	0.0518
Cattle	53 (65)	28 (35)	2 (15)	11 (85)	10.4 (2.15–50.27)	0.0013**

CI = confidence interval; OR = odds ratio.
** $P < 0.01$.

animals tracked the source of human leptospirosis to other animals, particularly rodents and cattle in urine-contaminated environments. Miners are at a high risk of acquiring skin abrasions due to their nature of work. Contact with leptospire-enriched environments facilitates bacterial entry through damaged skin and resulted in higher prevalence of leptospirosis among miners. This suggests that protective clothing and avoidance of exposure to leptospires among control groups may be the probable factors for low prevalence.

The role of rodents in the transmission of leptospires to the humans as well as to the cattle and from cattle to humans is well studied. A study from Cauvery River Valley showed that leptospires, apparently derived from rats, provided an important source of infection in dairy cattle and cattle farm workers in Tiruchirappalli, south India.[16] In this investigation, the rodent carriership was also established by isolation of serogroup Autumnalis from rodent kidney tissues providing a definite evidence on the prevalence of this serogroup in these mining environments and as a major source of infection. Further, the univariate analysis also confirmed the role of rat infestation ($P < 0.0318$) as the significant risk factor along with cattle rearing ($P < 0.0013$) from these mines. Usually, it is believed that the agricultural workers are the most exposed population to leptospires[17,18] with the highest seroprevalence of 62.5%.[16] This is due to their frequent exposure to water-logged fields, which are an ideal niche for leptospires sheded by cattle and rodents crossover in the fields during agricultural activities or feeding grains, respectively. Similarly, environmental conditions prevailed in the mines enhanced the exposure of miners from Pudukkottai to leptospiral infection.

TABLE 5

Seroprevalence of leptospirosis among carrier animals of Pudukkottai district

Serogroup	Rats (*N* = 9)	Cattle (*N* = 39)	Dogs (*N* = 4)	Goats (*N* = 7)
Australis	1 (4.3)	–	–	1 (3.4)
Autumnalis	5 (21.7)	12 (13.9)	1 (6.7)	–
Ballum	–	2 (2.3)	–	2 (6.9)
Bataviae	–	–	–	1 (3.4)
Canicola	–	7 (8.1)	2 (13.3)	–
Grippotyphosa	–	–	–	–
Icterohemorrhagiae	1 (4.3)	–	1 (6.7)	–
Javanica	2 (8.7)	–	–	3 (10.3)
Pomona	–	18 (20.9)	–	–

Numbers represent n (%).

In conclusion, leptospirosis is endemic among mine workers associated with wet environment compared with dry mine workers. Considering the associated risk factors and the higher seroprevalence among the mining groups, miners can also be considered as occupation-associated high-risk population for leptospirosis.

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