

EMERGING AND RE-EMERGING BACTERIAL ZONOOSES SCREENING OF HIGH RISK GROUPS FOR LEPTOSPIRAL SEROVARs

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ABSTRACT

Objective: To collect information related to leptospirosis among high-risk groups: agricultural workers, butchers, construction workers, and hospital sanitary workers through questionnaires and to determine the presence of leptospire among these high-risk groups (both genus and serovar-specific).

Methods: A total of 367 eligible subjects were included (112 agricultural workers, 108 construction site workers, 89 sanitary workers, and 58 butchers).

Results: The genus-specific enzyme-linked immunosorbent assay was performed for all the serum samples (n=367) and showed reactivity among 149 subjects. The results were compared with the serovar-specific microscopic agglutination test, which showed positivity in 158 subjects.

Conclusion: Screening the occupational risk group for the presence of leptospiral prints in order to provide appropriate medical check-ups and the earliest treatment. An educational program for rodent control and infection control where the seroprevalence is high should be organized.

Keywords: Bacterial zoonoses, Leptospirosis, Seroprevalence.

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INTRODUCTION

Zoonotic diseases are infections that can be transmitted between animals and humans, with or without vectors. Leptospirosis, the most widespread zoonosis infecting both humans and animals, is emerging as a major public health problem. Leptospirosis is primarily an occupational disease, but a contaminated environment makes any person vulnerable to infection. The major risk groups identified include agricultural workers, veterinarians, fishermen, workers in animal farms and poultry, butchers, conservancy staff, laboratory staff, construction workers, miners, soldiers, children, housewives, and also those who participate in leisure and recreational activities [1].

Leptospirosis may be underdiagnosed because of the presence of both pathogenic and saprophytic species and multiple serovars; the diagnosis is difficult to confirm; the clinical presentation may mimic other acute febrile illness; and the disease may be mild and not be investigated in the laboratory [2]. Once diagnosed, the disease responds to a course of doxycycline, thereby reducing the mortality and morbidity related to leptospirosis. The aim of this study was to screen high-risk groups like agricultural workers, butchers, construction site workers, and hospital sanitary workers for leptospiral antibodies and the distribution of *Leptospira* serovars among these high-risk occupational groups.

METHODS

This cross-sectional observational study was conducted among high-risk groups for leptospirosis in the study area. It includes agricultural workers, butchers, construction site workers, and hospital sanitary workers. Four hundred (400) risk group individuals were planned to include in this study, with a 38% prevalence rate from a study recorded in the previous literature [3]. High-risk groups like agricultural workers, butchers, construction site workers, and hospital sanitary workers with a minimum of 6 months of work experience were included. The study was carried out after the approval of the institutional ethics committee. 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.

A structured proforma was utilized for the current study. Details like age, gender, place, occupation of the participant, nature of work, socio-economic status, exposure to rodents and other pet animals like dogs and cats, source of drinking water, and history of any vector bites were obtained. Blood samples (approximately 5 mL) were collected aseptically from all the participants. The blood samples (0.5 mL) were inoculated in the EMJH semisolid medium-impregnated McCartney bottles in the collecting site itself aseptically. An enzyme-linked immunosorbent assay (ELISA) was done using the Panbio *Leptospira* IgG ELISA test.

Table 1: Age-wise distribution of study population

Age group in years	Agricultural workers (n=112)	Butchers (n=58)	Construction site workers (n=108)	Hospital sanitary workers (n=89)
21-30	20 (17.8)	9 (15.5)	30 (27.7)	6 (6.7)
31-40	33 (29.5)	19 (32.7)	19 (17.6)	13 (14.6)
41-50	30 (26.8)	14 (24.1)	39 (37.1)	37 (41.6)
51-60	18 (16.1)	8 (13.8)	15 (13.8)	25 (28.1)
↑ 60	11 (9.8)	8 (13.8)	5 (4.6)	8 (8.9)

[Figure in parentheses denoted percentages]

Table 2: Gender wise distribution of study population

High risk groups	Male	Female
Agricultural workers (n=112)	76 (67.8)	36 (32.2)
Butchers (n=58)	53 (91.3)	5 (8.7)
Construction site workers (n=108)	60 (55.5)	48 (44.5)
Hospital sanitary workers (n=89)	56 (62.9)	33 (37.1)

[Figure in parentheses denoted percentages]

Table 3: Major Epidemiological factors associated with leptospirosis

Major epidemiological factors	Agricultural workers (%)	Butchers (%)	Construction site workers (%)	Hospital sanitary workers (%)
Contaminated environment	95.2	33.4	96.7	93
Rainfall	50.7	23.6	44	55
Animal contact	94	95.4	91.3	74

The leptospiral antibodies can be demonstrated in the serum by a microscopic agglutination test (MAT). The MAT was performed strictly by following the standardized protocol by using a panel of antigens representing both ubiquitous and locally prevalent serovars. A titer value of ≥ 80 is considered as positive. The duplicate MAT procedure was followed 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 [4].

RESULTS

In this study, a total of 367 eligible subjects were included; thereby, they are named as high-risk groups based on their exposure rate to infectious materials in the environment. Accordingly, there are 112 agricultural workers, 108 construction site workers, 89 sanitary workers, and 58 butchers.

DISCUSSION

This analytical study provided complete details related to the socio-demographic analysis of the high-risk groups who are exposed to leptospiral strains. As per the results obtained, most of the subjects included in this investigation are frequently exposed to rats and other rodents; even some of them were directly exposed to their excreta while involved in the cleaning work. This disease has been recognized as an important high-risk occupational hazard for municipal workers, agricultural workers, miners, butchers, and other related manual laborers.

Leptospiral serology and culturing are tedious [5]. The genus-specific ELISA was performed, and the results were compared with serovar-specific MAT by using serial serum specimens collected from the suspected patients with leptospiral infection. This serological analysis showed higher sensitivity in identifying acute-phase leptospirosis [6].

The comparative analysis of the diagnostic data of ELISA and MAT demonstrated the rapid confirmation of results for patients with symptoms suspicious for leptospirosis, even when samples with a high proportion of borderline or low titers are tested. It was also suggested to start broad-spectrum antibiotic therapy immediately after collecting the blood and urine samples. As a result, these techniques can be obtained shortly after the drawing of the blood sample. The application of the assay will likely improve the treatment of the patients by allowing a better diagnosis to be made, treatment to be started promptly, and response to antibiotics also increased, thereby antibiotic failure and resistance get diminished [6].

Microagglutination tests for the leptospiral antibody were developed soon after the first isolation of leptospires, which occurred >80 years ago. In the early years of the diagnosis of leptospiral infection, when few serovars were known, it was customary to include all those serovars known to occur within a region, zone, state, and country in the antigen panel and to interpret the results of serological testing as being serovar-specific [7,8]. Cross-reactions serogroups are common [5,9], as a paradoxical reaction in which the initial immune response is directed to a heterologous serovar or serogroup.

The 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

Table 4: Seropositivity in ELISA among study population

ELISA IgG	Agricultural workers (n=112)	Butchers (n=58)	Construction site workers (n=108)	Hospital sanitary workers (n=89)
Positive	48	37	49	15
Negative	64	21	59	74

ELISA: Enzyme-linked immunosorbent assay

Table 5: MAT positivity among study population

MAT positivity	Agricultural workers (n=112)	Butchers (n=58)	Construction site workers (n=108)	Hospital sanitary workers (n=89)
Positive	50	37	52	19
Negative	62	21	56	70

Table 6: Serovars involved among high risk groups

Serovars	No. of positive cases (n=158)	Highest titer values
<i>L. grippityphosa</i>	44	1:320
<i>L. icterohaemorrhagiae</i>	29	1:160
<i>L. autumnalis</i>	11	1:160
<i>L. sejiro</i>	09	1:80
<i>L. pomona</i>	08	1:160
<i>L. australis</i>	21	1:320
<i>L. canicola</i>	08	1:160
<i>L. javanica</i>	06	1:160
<i>L. patoc</i>	7	1:160
<i>L. shermani</i>	4	1:80
<i>L. hebdomadis</i>	11	1:80

previously known serovar has been introduced into the population [10]. An additional confounding factor in areas of high endemicity is the possibility of co-infection with multiple serovars. The potential for over-interpretation of serologic data are thus much greater if only acute-phase or early convalescent-phase serum samples are available for testing [11], which was largely observed in this present study.

In the current study, serogroup Grippityphosa was dominated with the highest titer of 1:320 among 44 patients, which is observed largely in workers who are exposed to various rodents and their excreta with maximum exposure to the contaminated environment. The 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 leptospires than other high-risk groups [12].

CONCLUSION

In this study, high-risk groups like farmers, butchers, and sanitary workers were made aware that their workplaces have a high risk of leptospirosis. Screening the occupational risk group for the presence of leptospiral prints in order to provide appropriate medical check-ups and the earliest treatment. Significantly higher prevalence rates in rice

mill workers compared to the control group are identified, indicating that working in the rice mill is a significant risk factor for leptospiral infection. An educational program for rodent control and infection control where the seroprevalence is high should be organized.

AUTHORS CONTRIBUTIONS

Clitus-study conception and design, data collection; Anupriya-study conception, analysis, and interpretation of results; and Prabhusaran-data analysis and manuscript preparation.

CONFLICTS OF INTERESTS

Nil.

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