SEROPREVALENCE OF LEPTOSPIROSIS AMONG HIGH-RISK POPULATION OF ANDAMAN ISLANDS, INDIA

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Abstract. Leptospirosis is a severe spirchetal zoonosis in the world. It is considered an occupational disease of persons engaged in agriculture, sewage works, forestry, and animal slaughtering. A study was conducted with an objective of assessing the seroprevalence of leptospirosis among the high-risk groups of Andaman Islands. A total of 611 sera samples from different high-risk populations were collected and tested by microscopic agglutination test (MAT). Genetic characterization of the isolate was done by randomly amplified polymorphic DNA (RAPD) fingerprinting, and serological characterization was done using monoclonal antibody technique. Antibodies to leptospires were detected in 322 samples giving an overall seroprevalence of 52.7%. The seroprevalence was highest among agriculture workers (62.5%) followed by sewage workers (39.4%), animal handlers (37.5%), forest workers (27.3%), and butchers (30.0%). Seroprevalence among control population was 14.7%, which was comparatively less than that of the high-risk population groups. Subject sera were most commonly reacted with organisms of the serogroup Grippotyphosa followed by Australis, and the pattern was similar in control group. Four leptospires were isolated from agriculture workers who were admitted to the public health center (PHC) with complaints of fever and body ache. Human isolates were compared with two rodent (Rattus norvegicus) isolates from the same area of agriculture workers to get initial information about the transmission cycle of leptospirosis in the study community. Randomly amplified polymorphic DNA fingerprinting pattern of the strains recovered from the rodents and human patients were identified as belonged to genomo-species Leptospira interrogans. The antigenic characterization of the strains recovered from them belonged to serovar Valbuzzi of serogroup Grippotyphosa. The study showed that people engaged in high-risk activities such as agriculture, sewage cleaning, animal handling, animal slaughtering, and forestry are frequently exposed to leptospirosis, and hence control strategies targeting these populations could be more effective.

INTRODUCTION

Leptospirosis is a zoonotic disease of worldwide distribution. It affects both humans and animals and it is emerging as an important public health problem in India and other developing countries.1–5 Leptospirosis has been recognized as an important occupational hazard of agriculture manual laborers, sewage workers, animal handlers, forestry workers and other outdoor workers who work in wet conditions, and butchers. The transmission cycle involves interaction between one or more animal hosts harboring leptospires, an environment favorable for its survival, and human beings.

The Andaman Islands were known to be endemic for leptospirosis since the early part of the 20th century. The first confirmed report of leptospirosis from these islands dates back to 1929 when Taylor and Goyle isolated leptospires from patients with Weil disease.4 After that for 60 long years there was no report of leptospirosis. There was an out break of leptospirosis in Andaman Islands during the year 1988, which occurred in the form of severe pulmonary complication. Subsequent studies proved it to be leptospirosis. Later, while investigating different outbreaks, the patient’s history was asked and it was quite interesting to know that most affected person admitted to the public health center (PHC) in these Andaman Islands belonged to either the agriculture community or those who work in forestry or people with domestic animals. So a study was designed targeting the occupational groups in these islands. Recent sero-surveys have shown that Andaman Islands are endemic to leptospirosis with seroprevalence up to 55% among general population. Sero-epidemiologic and follow-up studies carried out in different population groups further supported that these islands are endemic for leptospiral infection.5,6

As leptospirosis is highly endemic in these islands, the current study was carried out to find out the seroprevalence among high-risk population groups such as agricultural manual laborers, sewage workers, animal handlers, forestry workers, and butchers, and white-collar workers as control population. Isolates of leptospires from human agricultural workers and rodent populations were compared with get initial information about the transmission dynamics of leptospirosis in the study area. This would be the first step in understanding the larger population of Andaman Islands, and a larger study can be planned in the future to know the transmission cycle of leptospirosis and which can represent the islands as a whole.

MATERIALS AND METHODS

Study area and study subjects. The Andaman Islands are an archipelago of several hundred islands situated in the Bay of Bengal with tropical climate and about 86% forest cover. The islands receive a high rainfall of about 3,000 mm and most of the rainfall occurs during the southwest monsoon, which starts by the end of May.

All the high-risk groups were selected for this study based on the previous serological and epidemiologic study conducted by this center and the reports of the human cases from public health centers. Individual consents were taken prior to blood collection. All subjects from each group lived in the same surrounding in which they worked, but a few lived out side, except for sewage workers. Health camps were orga-
nized with the help of concerned departments, and blood samples were collected for serology.

A total of 611 apparently healthy persons engaged in high-risk occupations were included in the study, and individual consent was obtained prior to the study. Among these, 104 persons were sewage workers of Andaman Public Works Department (APWD), in Port Blair Tehsil (administrative subunit of a district), and their work consists of cleaning the drainage situated with in the city Port Blair; 55 were employees of the Forest Department engaged as manual labors in the jungles situated 45 km away from Port Blair; and 392 were agricultural workers from a area called bangaloo situated 20 km from the city Port Blair. Most of the people in this area have their own rice fields and their occupation is agriculture. Their work includes everything from ploughing and harvesting to maintaining and handling working animals. Twenty were butchers working at a slaughterhouse in Port Blair, and their work includes slaughtering animals for sale purposes and cleaning animal waste. Forty were animal handlers working in a cattle farm run by the Department of Animal Husbandry, Andaman and Nicobar Administration. Complete coverage was attempted for sewage workers, forestry workers, and butchers. Agriculture workers in one area were fully covered. All these workers of different high-risk groups at work were not wearing protective clothing like gloves or boots. Apart from the different high-risk occupational group, 150 apparently healthy white-collar workers in Port Blair town were also included in the study.

**Laboratory procedure.** Serum was separated from blood samples and stored at −70°C until processed. Sera samples were tested for the presence of antileptospiral antibodies using microscopic agglutination test (MAT) following standard procedures. Reference strains belonging to 12 serogroups common in the Andaman Islands and country were included in MAT panel as antigens. These includes Australis (serovar Australis, strain Ballico), Autumnalis (serovar Autumnalis, strain Rachmati), Bataviae (serovar Bataviae, strain Swart), Canicola (serovar Canicola, strain Hond Utrecht IV), Grippotyphosa (serovar Grippotyphosa, strain Moskova V), Icterohaemorrhagiae (serovar Icterohaemorrhagiae, strain RGA), Javanica (serovar Poi, strain Poi), Pomona (serovar Pomona, strain Pomona), Sejroe (serovar Hardjo, strain Hardjoprajitno), Ballum (serovar Ballum, strain Mus 127), Cynopteri (serovar Cynopteri, strain 3522C), and Pyrogenes (serovar Pyrogenes, strain Salinem). The antigens used were 5–7 days old autoagglutination-free cultures grown in Elinghausen McCullough Johnsin Harris (EMJH) medium (Difco, Sparks, MD) with approximately 1 × 10⁸ to 2 × 10⁸ organisms/mL.

**Isolation of leptospirales from rats.** A total of 17 rats were trapped from the study area of agriculture workers. The rats were anesthetized using chloroform. These rats were then dissected and their kidneys were removed and inoculated in EMJH semisolid medium containing 2% rabbit serum and 100 μg/mL of 5-fluorouracil. These cultures were examined at every 10th day up to 6 months.

**Isolation of leptospirales from human patients.** Blood from humans was collected when they were hospitalized at the nearest PHC. Two to 3 drops of blood was inoculated in duplicate EMJH semisolid medium containing 2% rabbit serum immediately after collection. These cultures were examined at every 10th day up to 6 months.

**Serological characterization of the isolates.** Serogroup status of the isolates were ascertained by MAT using group sera and serovar status by MAT using a panel of monoclonal antibodies (71 9-4, 71 16-4, 71 17-5, 71 17-7, 165 1-4, 165 2-1, and 165 3-4) obtained from Royal Tropical Institute (KIT), Amsterdam, The Netherlands.

**Typing with mAbs.** Serological typing with mAbs was done by MAT using panels of mouse mAbs belonging to serogroups Canicola, Grippotyphosa, Hebdomadis, Icterohaemorrhagiae, Javanica, Mini, Pyrogenes, Shermanni, and Sarmin following the procedure described earlier.

**Genomic DNA isolation.** DNA was prepared as per Boom and others from the leptospiral strains representing 5 genospecies: serovar Australis, strain Ballico; serovar Rachmati, strain Rachmat; serovar Icterohaemorrhagiae, strain RGA (Leptospira interrogans); serovar Ratnapura, strain Wumalasena; serovar Vanderhoejeni, strain Kipold79; serovar Cynopteri, strain 3522C (Leptospira kirschneri); serovar Tarassovi, strain Perepelitisi; serovar Mini, strain Sari (Leptospira borgpetersenii); serovar Canalzonae, strain CZ188; serovar Weaveri, strain CZ390 (Leptospira santarosai); serovar Panama, strain CZ214K, serovar Louisiana, strain LSU1945 (Leptospira noguchii). Apart from the reference strains, DNA was also prepared from the strains recovered from the rodents (RA, RB) as well as human patients (RMRC1, RMRC2, RMRC3, RMRC4).

**Randomly amplified polymorphic DNA.** The randomly amplified polymorphic DNA (RAPD) fingerprinting was followed as per Ramadass and others. The reaction was carried out in a total volume of 50 μL consisting of 50 ng of leptospiral chromosomal DNA, 10 mM Tris-HCl (pH 9.0), 50 mM KCl, 4 mM MgCl₂, each of the four deoxynucleoside triphosphates at a concentration of 0.1 mM, each primer at a concentration of 300 pM, and 0.5 U of Taq DNA polymerase, and the PCR was carried out in a DNA Engine (MJ Research PTC 200, MA) thermal cycler. The first two cycles consisted of a denaturation at 95°C for 5 minutes, annealing of primers for 5 minutes at 40°C, and extension for 5 minutes at 72°C. The subsequent 35 cycles consisted of denaturation for 1 minute at 95°C, annealing of primers for 1 minute at 60°C, and extension for 3 minutes at 72°C, with a final extension step for 10 minutes during the last cycle. PCR products were observed in 1% agarose gels in TAE containing ethidium bromide 0.5 μg/mL and viewed under UV illumination and documented using gel documentation system. Phylogenetic tree (UPGAMA with 2.0% tolerance) was constructed using BioGene analysis software (Vilber Lourmat, France). The primers used were B11 (CCG GAA GAA GGG GCG CCA T) and B12 (CGA TTT AGA AGG ACT TGC ACA C).
no difference in seropositivity between the sexes because samples were collected mainly from male forestry workers. The difference in the seropositivity between high-risk groups and controls were statistically significant ($\chi^2 = 70.24$, $P < 0.001$). Table 1 shows the seroprevalence among different occupational groups. The seroprevalence was highest among agriculture workers (62.5%) followed by sewage workers (39.4%) and animal handlers (37.5%). Test of significance of the individual high-risk groups is mentioned in Table 2.

The serogroup of leptospires responsible for infection, as determined by the highest MAT titers among different risk groups and control population, is summarized in Table 3. Among all the risk groups, Grippotyphosa was the commonest infecting serogroup followed by Australis, and similar pattern was observed among control group. Among control population, about 23% of the infections were due to serogroups Canicola and Icterohaemorrhagiae. However, infection with these serogroups was not observed among the high-risk groups except for sewage workers; 10% of the infection was due to serogroup Canicola among sewage workers. Mixed equals were seen in 32 (9.9%) of the seropositive high-risk group individuals. Most of the mixed equals found were with the combination of Grippotyphosa and Australis. Three samples reacted to more than two serogroups, with the combination of Australis, Grippotyphosa, and Canicola.

Figure 1 shows the titer distribution among seropositive high-risk group individuals and controls. The highest titer observed among control population was 1 in 100 in two individuals, whereas among high-risk group persons titers up to 1 in 1,600 were observed. The highest MAT titer of 1 in 1,600 was observed against Grippotyphosa for the sera samples collected from sewage workers.

All 4 human isolates recovered were from the agriculture worker group. After 2 months of sample collection, 20 people from agriculture workers were admitted to the PHC with complaints of fever, body ache, and headache at different intervals. Blood samples were collected and cultured. The RAPD fingerprinting pattern of the four human and 2 rat isolates exhibited a maximum of 7 bands in the range 300–2,000 bp. The banding pattern obtained for all the six isolates was quite similar and found to be clonal in nature with the similarity pattern of 90–100% as per the phylogenetic tree (Figure 2). Moreover, all the isolates were identified as *L. interrogans*, because they showed 100% similarity with the leptospiral reference strains of *L. interrogans*. Apart from the genetic characterization, all the isolates were identified up to serovar level by monoclonal antibody techniques, and it was shown that the isolates belonged to serovar Valbuzzi of serogroup Grippotyphosa (Figure 3).

### DISCUSSION

Andaman Islands are known to be endemic for leptospirosis with the majority of the population exposed to the disease.4,6 Leptospirosis occurs in the form of seasonal post-monsoon outbreaks with considerable mortality. Suitability of the environment for the survival of leptospires appears to be a critical factor in maintaining the infection and transmission to humans. Leptospires have good affinity to areas where heavy rainfall results in waterlogging of the land. Human populations residing in such environment are at higher risk of acquiring leptospiral infection. The majority of the affected population either belong to the agriculture community or work in slaughterhouses or animal farms or live in the forest. The chances of human beings contracting the infection directly or indirectly are more in these high-risk communities.

The current study showed that the seroprevalence of leptospirosis among the high-risk population of Andaman Is-

<table>
<thead>
<tr>
<th>Serogroups</th>
<th>Butchers (%)</th>
<th>Forestry workers (%)</th>
<th>Agriculture workers (%)</th>
<th>Animal handlers (%)</th>
<th>Sewage workers (%)</th>
<th>Control population (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Australis</td>
<td>29 (33.3)</td>
<td>59 (33.3)</td>
<td>90 (33.3)</td>
<td>6 (40.0)</td>
<td>10 (24.4)</td>
<td>7 (31.8)</td>
</tr>
<tr>
<td>Grippotyphosa</td>
<td>4 (66.7)</td>
<td>9 (60.0)</td>
<td>135 (47.6)</td>
<td>7 (46.7)</td>
<td>18 (43.9)</td>
<td>10 (45.5)</td>
</tr>
<tr>
<td>Canicola</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>2 (9.1)</td>
</tr>
<tr>
<td>Icterohaemorrhagiae</td>
<td>0 (0.0)</td>
<td>1 (6.7)</td>
<td>20 (19.1)</td>
<td>2 (13.3)</td>
<td>9 (22.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Mixed reaction</td>
<td>0 (0.0)</td>
<td>1 (6.7)</td>
<td>20 (19.1)</td>
<td>2 (13.3)</td>
<td>9 (22.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Total (N = 322)</td>
<td>6 (100.0)</td>
<td>15 (100.0)</td>
<td>245 (100.0)</td>
<td>15 (100.0)</td>
<td>41 (100.0)</td>
<td>22 (100.0)</td>
</tr>
</tbody>
</table>
lands is high (52.7%). Similar studies conducted in Madras and Bangalore in mainland India showed seroprevalence of 19.8% and 9.3%, respectively. Studies conducted among high-risk populations of Chile, Trinidad, and Barbados showed seroprevalence of 22%, 49%, and 39%, respectively. It is believed that the agricultural workers are the most exposed population to leptospirosis. Our study also showed that the agriculture workers had the highest seropositivity (62.5%) when compared with other high-risk communities. This could be due to their frequent exposure to rice fields that are often waterlogged, which is the major reason for the survival of leptospires. Domestic animals such as bullocks and buffaloes are used for agricultural activities and during harvest season; when the rice is ripe, rodents visit the fields in search of food grain. So the rodents and the bullocks and buffaloes and the favorable environment could play a major role in agriculture-based communities for leptospirosis. High

Figure 1. Distribution of MAT titers among high-risk groups and controls.

Seroprevalence of leptospirosis has been observed among cattle and other domestic animals in these islands.\textsuperscript{15} Sewage workers showed 39.4% seropositivity followed by animal handlers 37.5%, butchers 30.0%, and forestry worker 27.3% in the current study. Samples collected from butchers were too low. All the different high-risk population also showed a high percentage of seropositivity when compare with the control population.

Among the control group the seroprevalence was 14.7%, which is lower when compared with a study conducted in Tamil Nadu (20.6%).\textsuperscript{16} Lower prevalence can be explained due to the urbanized surroundings and lives in an environment that doesn’t allow any favorable conditions for the survival of leptospires to cause human infection.

The commonest serogroup among the control group and high-risk population was the same. This probably indicates the same circulating serovars and same transmission cycles of infection among the high-risk populations and among urban white-collar workers.

Among the 12 leptospiral antigens belonging to 12 different serogroups used for MAT, only three serogroups, Grippotyphosa, Australis, and Canicola, reacted. This further proves that the common circulating serogroups in these islands are Grippotyphosa, Australis, and Canicola.\textsuperscript{7} Serogroup Canicola reacted only to samples collected from sewage workers and not with any other high-risk groups. But Australis reacted to samples collected from all the high-risk groups.

In the current study, serogroup Grippotyphosa showed the maximum titer of 1 in 1,600, which is observed only in sewage workers, and the rest of the high-risk population showed a maximum titer of 1 in 100 and 1 in 200 and very few with a titer of 1 in 400. This could be an indication that sewage workers are more frequently exposed to leptospires than other high-risk groups.

The RAPD pattern of the strains recovered from the rodents (\textit{Rattus norvegicus}) and humans were identified as clonal in nature and belongs to \textit{L. interrogans}. The antigenic characterization of the isolates was serovar Valbuzzi of serogroup Grippotyphosa. The commonest serovar found among the different high-risk population was also the serogroup Grippotyphosa. During all the outbreaks from 1995, the commonest serogroup involved was Grippotyphosa, and most of the patients had the occupation of agriculture, forestry, butchers, and so forth. The molecular characterization of the isolates recovered from the humans showing close similarity with the isolates recovered from the rodents indicating the role of the rodents for the transmission of leptospirosis. The rodents may be the culprits for transmitting the infection among different high-risk populations.

Leptospirosis is endemic in all population groups of the Andaman Islands. However, the endemicity, as proved by seroprevalence, is higher among the high-risk groups. The genomo-species of leptospires involved in human and animal infection are the same. The current study gives an idea about the transmission dynamics of leptospirosis in these high-risk populations. In the future, a bigger study with large number of human population from different areas and large number of different animal species can be planned to understand the exact transmission dynamics representing the Andaman Islands. Programs for control of leptospirosis in these islands can target these high-risk populations by educating them about the disease and advising them to wear protective clothing, which would make such programs more effective.

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\textbf{Figure 3.} Monoclonal antibody pattern of isolates RMRC1, RMRC2, RMRC3, RMRC4, RA, and RB. x-axis, monoclonal antibodies; y-axis, MAT titers.
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