Risk factors associated with leptospirosis during an outbreak in Middle Andaman, India


Regional Medical Research Centre (ICMR), WHO Collaborating Center for Diagnosis, Research Training & Reference in Leptospirosis, Port Blair; *National Institute of Epidemiology (ICMR) Chennai & **Bharathidasan University, Trichy, India

Received September 11, 2007

Background & objectives: Leptospirosis outbreaks occur frequently in North and South Andaman Islands but not in Middle Andaman. In 2002, an outbreak appeared in Middle Andaman for the first time. Although a study on risk factors was conducted in North Andaman, it used seropositivity to define leptospirosis. Since seropositivity might not indicate current leptospiral infection and as no study on risk factors was conducted in Middle Andaman, we carried out this study to identify the risk factors during the outbreak.

Methods: A suspected outbreak of leptospirosis occurred in Rangat of Middle Andaman during October - November 2002. Suspected cases were screened for leptospirosis using microscopic agglutination test (MAT). Fifty two patients confirmed to have leptospirosis based on rising titres in MAT on paired sera, and 104 age, sex and neighbourhood seronegative matched controls, were included in the study. A conditional multiple regression by backward elimination process was carried out with acute leptospirosis as the dependent factor and various environmental, occupational and behavioural factors as independent factors. A stratified analysis was also carried out.

Results: The presence of cattle in the house, drinking stream water, contact with garbage, walking barefoot and standing in water while working were identified as significant factors associated with leptospirosis. Stratified analysis showed a dose response relationship between number of cattle in the house and the risk of leptospiral infection suggesting that cattle could be a source of infection.

Interpretation & conclusions: Identification of the potential risk factors would help understand the transmission dynamics of the disease and formulate public health interventions.

Key words Case control study - leptospirosis - logistic model - outbreak - risk factors

Leptospirosis is considered as one of the most widespread zoonotic diseases in the world1. Although the incidence of the disease seems to have decreased in developed countries, it is apparently emerging rapidly as a significant public health problem in developing countries. Some of the countries where leptospirosis is under surveillance have recorded this increase in incidence2. Most of the countries in South East Asia are
endemic to leptospirosis. A number of outbreaks have occurred during the past few years in various places such as Nicaragua\textsuperscript{3}, Salvador\textsuperscript{4} and Rio de Janeiro\textsuperscript{5} in Brazil and Orissa\textsuperscript{6,7}, Mumbai\textsuperscript{8} and Andaman Islands\textsuperscript{9,10} in India.

Leptospirosis is known to be endemic in Andaman Islands since early years of 20\textsuperscript{th} century\textsuperscript{11}. However, no information is available about the status of leptospirosis in Andamans after 1931. During late 1980\textsuperscript{s} post-monsoon outbreaks of leptospirosis, locally known as Andaman haemorrhagic fever (AHF), started occurring\textsuperscript{9}. These outbreaks occured in two foci, one in South Andaman Island and the other in North Andaman Island. Reported annual incidence ranged between 40-80 per 100,000 population\textsuperscript{12}.

Although the basic determinants of leptospiral transmission \textit{viz.}, presence of carrier animals, environment suitable for the survival of leptospires and occupational factors of people that predispose them to leptospirosis are common, the magnitude and nature of these factors vary from community to community. Therefore, the specific risk factors for acquiring leptosporal infection could be unique to each community. An understanding of the transmission cycle of the disease in the community and the modifiable risk behaviours is essential for planning strategies for prevention and control.

A study on risk factors was conducted in North Andaman in 1996\textsuperscript{13}, which used seropositivity as the outcome variable and the strength of association (odds ratio) observed between seropositivity and various factors were in the range of 1.0-2.0. Seropositivity does not necessarily indicate current leptospiral infection. This would have resulted in differential misclassification to some extent leading to underestimation of the strength of association between the potential risk factors and seropositivity.

In 2002, no significant outbreak occurred at either of the two usual foci \textit{viz.} South Andaman and North Andaman Islands, but an outbreak occurred in the Middle Andaman Island. A matched case-control study was carried out during this outbreak with the objective of identifying risk factors of acute leptospirosis among the residents of the affected villages of Middle Andaman.

Material & Methods

\textit{Study area:} The study was conducted in Rangat town and adjoining villages in Middle Andaman, Andaman & Nicobar Islands, during October – November 2002. Rangat is a small town in Middle Andaman Island and is the second most densely populated area in Andaman and Nicobar Islands after Port Blair, located in South Andaman Island. About half of the population of middle Andaman is engaged in outdoor work (cultivators, agricultural labourers, marginal workers, livestock farmers, fishermen, forestry workers, hunters, etc.)\textsuperscript{14}.

The major crop cultivated during the monsoon season is rice, harvested during October – November.

\textit{Outbreak and selection of cases and controls:} During October 24 - November 30, 2002, a total of 114 suspected cases of leptospirosis were admitted to Community Health Centre (CHC), Rangat. It was considered as a public health response and hence prior approval of the institutional ethics committee could not be sought. However, the study was later reviewed by the Scientific Advisory Committee of Regional Medical Research Centre, Port Blair, and was approved.

A suspected case of leptospirosis was defined as any patient who reported to CHC, Rangat, on or after October 1, 2002 with complaints of fever associated with severe muscle tenderness, any bleeding tendencies including sub-conjunctival haemorrhage, jaundice, cough, haemoptyis and breathlessness, oliguria or signs of mengeal irritation. Any suspected case of leptospirosis attending CHC, Rangat, was eligible to be selected into the study. A set of eligible controls for each case was selected from the neighbourhood of the cases matching for age (±5 yr) and sex. The nature of the study was described to them and their consent was obtained. Three controls were selected for each case.

\textit{Clinical specimens and laboratory tests:} Blood sample (2-3 ml) was collected during the acute phase and a second sample 10-14 days later from the suspected cases. One blood sample (2-3 ml) was collected from all the eligible controls. Samples were allowed clot for 6 h at 4°C. Test tubes were then centrifuged and the separated sera were transferred to sterile serum vials, and stored at -70°C until processed.

Lepto-dipstick test\textsuperscript{15} was done following standard procedure on the acute serum samples of the suspected cases at the CHC as an aid to rapid diagnosis for initiating specific treatment. Microscopic agglutination test (MAT) was done on all acute and convalescent serum samples from suspected cases and the samples from eligible controls following standard procedure\textsuperscript{16}. A panel of 12 leptospiral strains representing the
common circulating serogroups of leptospires in India was used as antigens in MAT. MAT was done at doubling dilutions starting from a titre of 1 in 40. Positive samples were titrated up to a titre of 1 in 2560. Isolation of leptospires was attempted from the acute blood samples of suspected patients following standard procedures. Two to three drops of blood was inoculated into tubes containing Ellinghausen-McCullough-Johnson-Harris (EMJH) medium (Difco Laboratories, USA) and incubated at 30°C. Cultures were examined under dark ground illumination weekly till six weeks.

**Diagnostic criteria:** A positive dipstick test was considered as an indication of possible current leptospiral infection for the purpose of initiating specific treatment. Isolation of leptospires from the blood was considered as a confirmatory evidence of current leptospiral infection. Seroconversion (negative to a titre of 1:160 or more) or four-fold rise in titre was considered as strong evidence in favour of current leptospiral infection in cases. A titre of 1:160 was taken as the cut-off for seroconversion as well for the diagnosis of possible current leptospiral infection in the controls as it was the closest titre to 1 in 200, which is the ideal cut-off in endemic areas. A positive reaction at any titre in controls was considered as an indication of possible current leptospiral infection among controls.

**Information about potential risk factors:** Information about potential risk factors was obtained by interviewing the cases and controls using a structured questionnaire, developed based on a validated standard questionnaire used elsewhere in the islands as well as on the information on personal and occupational behaviour and recent behavioural changes obtained through focus group discussions with the villagers. An epidemiologist/trained field worker/social worker interviewed the cases and controls. The questions were under three broad categories *i.e.* environmental (type of house, house surroundings, proximity to water bodies, ownership of house and agricultural land), contact with animals (rearing cattle, swine, cat or dogs and rat infestation of houses) and behavioural/occupational factors (participation in agricultural activities, fishing, contact with garbage and sewage, cleaning animals, direct contact with animal urine, and recreational activities such as swimming).

All the questions except that regarding source of water for drinking and washing elicited a ‘yes’ or ‘no’ response and hence the corresponding variables were dichotomous. For water source, the response was one of the four categories *i.e.* indoor tap, public tap, well or stream. Questions regarding behavioural and occupational exposure to possible sources of leptospires sought information about such exposure during a four-week period prior to onset of symptoms in the case of cases and during the corresponding period for their matched controls.

**Statistical analysis:** Univariate odds ratios (ORs) for the potential risk factors were calculated by a matched analysis and 95 per cent confidence intervals (95% CI) were calculated using maximum likelihood ratio method. McNamara’s χ² and P values were calculated to test the statistical significance. Analysis was done on EpiInfo Ver 6.04. Biologically plausible first order interaction terms were listed. A stratified analysis was done for each pair of interaction terms and χ² for evaluation of interaction was calculated. If the test suggested (at 5% level) that the ORs differed by stratum (interaction), the interaction term was used for further analysis.

Conditional multiple logistic regression was done to identify independent risk factors and interaction terms using EpiInfo 2002. A backward elimination method following the procedure described in the online manual of the software was used. In the initial step, all the variables and identified interaction terms that were associated with leptospirosis with statistical significance at 0.20 level were included. In each subsequent step, the variable/interaction term with lowest significance was removed. Individual variables of interaction terms present in the model were retained irrespective of statistical significance. The process was repeated till all the variables in the model were significant with P < 0.05.

**Results**

**Suspects and confirmed cases:** Paired blood samples were available for 79 patients and 38 of them showed a seroconversion and 17 had a four-fold rise in titre. Hence a diagnosis of leptospirosis was confirmed in 55 of the 79 patients who could be adequately followed up. Leptospires could not be isolated from the blood of any of the patients. Based on MAT titres, Icterohaemorrhagiae and Grippotyphosa were the common infecting serogroups.

**Socio-demographic characteristics:** Age of the patients ranged from 3 to 62 yr. The 55 patients included 35 (63.6%) males and 20 (36.4%) females. The mean age was 23.8 yrs (SD: 11.6 yr). Females were slightly older (mean age: 25.7 ± 13.8) than males (mean age 22.9 ± 10.3). About 50 per cent of the patients were in the age range from 3 to 15 yrs (mean age: 10.8 yrs)
group 15-34 yr and 15 per cent were children below 15 yr of age. Two confirmed cases were in preschool age group.

The households of most of the cases owned agricultural land. Except for married ladies who were not doing any work other than household duties, most adults reported that they used to work in the agricultural fields. Hence none of them were categorized as unemployed. Cultivators and agricultural labourers constituted 44.2 per cent of the patients and students 23.1 per cent.

For most of the cases, three age and sex matched eligible controls each were selected. Thus there were a total of 152 eligible controls. MAT showed positive result in 23 giving a seroprevalence rate of 15.03 per cent (95% CI: 9.96, 21.91). Finally for 52 cases, two controls each remained in the analysis.

**Prevalence of risk factors and univariate odds ratio:** Among the factors relating to house, environment and animals in house, the difference in the prevalence among cases and controls was statistically significant in the case of house with thatched roof, stream water as the source of drinking water and keeping cattle and pig in the house (data not shown). Among these factors, keeping cattle had the highest prevalence among the cases (84.6%) followed by house with thatched roof (50.0%). Among occupational behaviour factors, prevalence of walking barefoot, skin wounds, harvesting, cleaning sewage, clearing garbage and working in water were significantly different among cases and controls. Harvesting and staying in water while working had the highest prevalence among cases (71.2%).

Univariate matched analysis was performed to determine the strength of association of the potential risk factors with acute leptospirosis. Tables I and II show the univariate odds ratios with 95 per cent confidence intervals of the potential risk factors. Among the factors relating to house, environment and animals in the house, thatched roof, use of stream water for drinking and keeping cattle and pigs in house were significantly associated with leptospirosis. Among occupational and behavioural factors, walking barefoot, having wounds, harvesting, cleaning sewage, clearing garbage were significantly associated working standing in water. The strength of association was highest for keeping cattle in house (OR: 5.63) followed by harvesting (OR: 5.43) and using well water for drinking (OR: 5.0).

**Interaction terms and other independent variables:** Nineteen factors that had a P value < 0.20 were

### Table I. Univariate matched odds ratios, confidence intervals and P values of potential risk factors, outbreak of leptospirosis at Rangat, Middle Andaman, 2002. (factors related to house, environment and animals)

<table>
<thead>
<tr>
<th>Factor</th>
<th>OR</th>
<th>95% CI</th>
<th>Prevalence (%)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cases</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Own house</td>
<td>1.07</td>
<td>0.42, 2.86</td>
<td>82.7</td>
<td>81.7</td>
</tr>
<tr>
<td>Own land</td>
<td>0.89</td>
<td>0.38, 2.24</td>
<td>81.7</td>
<td>76.9</td>
</tr>
<tr>
<td>House in low-lying land</td>
<td>1.08</td>
<td>0.55, 2.14</td>
<td>61.5</td>
<td>59.6</td>
</tr>
<tr>
<td>Streams near house</td>
<td>0.79</td>
<td>0.38, 1.64</td>
<td>59.6</td>
<td>57.7</td>
</tr>
<tr>
<td>Ponds in compound</td>
<td>2.08</td>
<td>0.85, 5.27</td>
<td>34.6</td>
<td>22.1</td>
</tr>
<tr>
<td>House compound wet</td>
<td>1.07</td>
<td>0.53, 2.24</td>
<td>57.7</td>
<td>63.5</td>
</tr>
<tr>
<td><strong>Type of house:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thatched roof</td>
<td>2.85</td>
<td>1.28, 6.53</td>
<td>50.0</td>
<td>26.9</td>
</tr>
<tr>
<td>Mud wall</td>
<td>1.65</td>
<td>0.75, 4.02</td>
<td>78.8</td>
<td>68.3</td>
</tr>
<tr>
<td>Thatched walls</td>
<td>0.59</td>
<td>0.28, 1.24</td>
<td>57.7</td>
<td>70.2</td>
</tr>
<tr>
<td><strong>Source of drinking water:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stream</td>
<td>3.83</td>
<td>1.27, 11.98</td>
<td>25.0</td>
<td>8.7</td>
</tr>
<tr>
<td>Well</td>
<td>5.00</td>
<td>0.82, 52.51</td>
<td>9.6</td>
<td>1.9</td>
</tr>
<tr>
<td><strong>Source of water for washing:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stream</td>
<td>1.44</td>
<td>0.60, 3.31</td>
<td>28.8</td>
<td>22.1</td>
</tr>
<tr>
<td>Well</td>
<td>2.25</td>
<td>0.78, 7.64</td>
<td>17.3</td>
<td>7.7</td>
</tr>
<tr>
<td><strong>Animals kept in the house:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cattle</td>
<td>5.63</td>
<td>2.20, 16.41</td>
<td>84.6</td>
<td>49.0</td>
</tr>
<tr>
<td>Pigs</td>
<td>2.90</td>
<td>1.18, 7.45</td>
<td>34.6</td>
<td>16.3</td>
</tr>
<tr>
<td>Goats</td>
<td>1.29</td>
<td>0.60, 2.81</td>
<td>32.7</td>
<td>26.9</td>
</tr>
<tr>
<td>Cats</td>
<td>0.75</td>
<td>0.35, 1.58</td>
<td>42.3</td>
<td>49.0</td>
</tr>
<tr>
<td>Dogs</td>
<td>1.75</td>
<td>0.85, 3.57</td>
<td>59.6</td>
<td>45.2</td>
</tr>
<tr>
<td>Rat infestation</td>
<td>1.69</td>
<td>0.74, 3.79</td>
<td>78.8</td>
<td>68.3</td>
</tr>
</tbody>
</table>

*Factors with statistically significant association at 5% level and included in logistic regression
selected for inclusion in the multivariate model. Out of the possible 171 first order interaction terms between these 19 factors, 55 were selected based on biological plausibility. Stratified analysis of these 55 pairs of variables identified three possible interaction terms based on $\chi^2$ for evaluation of interaction terms. All these terms involved presence of cattle in house. These were barefoot walking, harvesting and contact with animal urine.

Odds ratio for presence of cattle was 9.92 for those walking barefoot whereas it was only 2.22 for those wearing footwear. Odds ratios for presence of cattle were significantly higher, though to a smaller extent than in the case of barefoot walking, for the other two factors viz., harvesting and contact with animal urine also.

**Multiple logistic regression:** All the three interaction terms were removed during the backward elimination process. Five variables remained in the model. Presence of cattle in the house had the strongest association with leptospirosis (OR: 5.1) followed by use of streams as a source of drinking water (Table III).

**Discussion**

In North and South Andaman Islands, outbreaks occur during October-November months almost every year. The seroprevalence observed among the controls in the present study was 15 per cent. This was much less than the prevalence observed among the population of North Andaman and among some of the tribes of the islands. Controls were screened immediately after the outbreak was over and hence at least some the seropositives would represent those who had an asymptomatic infection during the outbreak. The lower seroprevalence in Rangat after an outbreak indicated that leptospirosis was not highly endemic here.

Most other factors were fairly common with prevalence in the range of 25-80 per cent. A few of the factors such as use of stream water and well water were rare. The factors that had a significantly different prevalence among cases and controls were house with thatched roof and use of stream water for drinking among the factors relating to house and environment, cattle and pig among the animals in house and barefoot walking, wounds, harvesting, cleaning sewage, clearing garbage and working standing in water among behavioural and occupational factors studied.

In the present study unlike earlier studies, the only occupational factor associated with leptospirosis...
was standing in water while working. Although wearing footwear might not offer much protection against such exposures it may offer some protection when the transmission is due to exposure to contaminated environment in and around the house.

In the present study the factor with the strongest association with leptospirosis was presence of cattle in the house. Although it is often implicated as a carrier of leptospires belonging to the serovar Hadjo, infection with other serogroups particularly Grippotyphosa is also common among cattle\(^2^5\). During the present outbreak, two predominant serogroups were involved i.e., Icterohaemorrhagiae and Grippotyphosa. It is possible that infections with the two different serogroups of leptospires were spread through two separate transmission cycles during the outbreak and cattle were the source of infection in one of these. Because of the small number of cases it was not possible to study the risk factors associated with infection by individual serogroups. As it has been shown that seroprevalence is not a reliable indicator of leptospiral carrier state\(^2^6\) such studies should use bacteriological or the more sensitive molecular tools. A limited survey for leptospiral excretion was conducted among cattle and pigs in the affected area immediately after the outbreak. While no isolate of leptospires were recovered from pigs, two isolates were recovered from cattle (unpublished observation).

Use of stream water for drinking was the factor with the second strongest association with leptospirosis. Although, drinking water is occasionally implicated as the source of leptospiral infection\(^2^7\), its in leptospiral transmission is unclear. The leptospires are unlikely to survive the gastric acid barrier. During swallowing contaminated water, leptospires may however penetrate oral, oro-pharyngeal or oesophageal mucosa. Abrasions in the mucosa may also facilitate entry of leptospires into body. There has been strong evidence of leptospirosis being transmitted through ingestion as in the case of transmission from a nursing mother with leptospirosis to her baby\(^2^8\) and the observed association between swallowing water while swimming during an outbreak in Japan\(^2^9\). In the present study, the possibility of some unstudied confounding factors being responsible for the observed association between use of stream water for drinking and leptospirosis cannot be ruled out completely.

Standing in water for prolonged period of time has been identified as a risk factor in other studies also\(^2\). Although standing in water while working and walking barefoot are already identified risk factors in other areas of Andaman Islands, rearing cattle, contact with garbage and use of stream water for drinking are newly identified risk factors during the current outbreak. These pertain to one possible maintenance host that act as a source of infection (cattle), two factors relating to environmental and water sanitation (use of stream water for drinking and contact with garbage) and two pertaining to occupation and personal habits (working standing in water and walking barefoot). Control measures targeting all these factors can be planned. Our study indicated that cattle could probably be an epidemiologically important carrier host in the study area. This needs further proof. The next step should be to estimate leptospiral excretion rate among cattle population of the area.

Leptosporal contamination of water sources can be tested by culture and serotyping or using PCR specific to pathogenic leptospires. Improved water availability would reduce the risk due to use of unsafe water. Better environmental sanitation and prompt removal of garbage should also be components of control programmes as contact with garbage has been shown to be a risk factor. The habit of barefoot walking should be discouraged through health awareness programmes. Working standing in water is difficult to modify, as it is unavoidable for rice farmers. However, further studies can be conducted on the efficacy and acceptability of protective footwear.

The study was conducted in a confined area. The risk factors identified in one area might not have any role in leptosomal transmission in other areas. Hence the findings cannot be generalized to larger populations. Because the cultural habits of people and the nature of environment and animal population are not much different in different parts of Andaman Islands, these findings can be generalized to the population of the Islands and could be the basis of a comprehensive leptospirosis control strategy.

Acknowledgment

The authors acknowledge the help and guidance of Prof. K. Ramachandran, Formerly Head of the Department of Biostatistics, All India Institute of Medical Sciences, New Delhi, Drs Sunil Kumar and Sidha Raju, Senior Medical Officers, Community Health Centre, Rangat, Middle Andaman and the technical staff of the Leptospirosis Laboratory, National Leptospirosis Reference Centre, (RMRC, ICMR), Port Blair.

References


