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## Leptospirosis among schoolchildren of the Andaman & Nicobar Islands, India: low levels of morbidity and mortality among pre-exposed children during an epidemic

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### SUMMARY

Leptospirosis is an important public health problem in the Andaman Islands. The disease is being increasingly reported among children and adolescents in recent times. An attempt was made to find out the level of exposure to leptospire, to estimate the incidence of infection and to identify the risk factors for acquiring infection among children. A sample of 1544 schoolchildren was selected. Presence of anti-leptospiral antibodies was tested using the microscopic agglutination test (MAT). Students were interviewed for behavioural factors. In total, 341 (221 seronegative and 120 seropositive) students were followed up clinically and serologically during a subsequent outbreak. An overall seropositivity rate of 23·6% (95% CI 21·54–25·81) was observed. Infection rate was 33·5% among seronegatives whereas re-infection rate was 16·7% among seropositives during the outbreak that occurred 1 month after the first sample collection. Morbidity and mortality were found to be higher among seronegative individuals than seropositives. More than 90% of leptospiral infections were found to be subclinical or unnoticed. The high level of exposure among the children results in high infection rates and because they have less previous exposure than adults, they do not have sufficient protection to resist clinical illness during outbreaks.

### INTRODUCTION

Leptospirosis, a zoonosis of worldwide distribution, is endemic in many tropical countries including several parts of India. Usually it occurs as an occupational disease among workers exposed to infected animal urine directly or indirectly through contaminated environment [1]. Leptospirosis is endemic in the Andaman group of islands and usually occurs as post-monsoon outbreaks [2]. The case-fatality ratios in these outbreaks have been in the range of 10–50%

[2, 3]. Seroprevalance in the range of 30–60% has been observed among the settler population. However seroprevalance was comparatively low among the tribal population [4, 5].

Pulmonary haemorrhage is a common clinical presentation of leptospirosis in the Andamans [3]. The median age of patients has shown a decrease for the past 10 years. Although many social and behavioural factors have been shown to be associated with seropositivity in these islands [6] the exact reasons for this decrease in age is not understood. As a first step in understanding the reasons, it was considered worthwhile to estimate the level of exposure of the children to leptospire. Since almost all children in the 5–18 years age group attend schools, the study was conducted in a sample of school students. The study

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Table. Seroprevalence of leptospiral infection among males and females in Andaman and Nicobar districts

District	No. of students	Samples tested and positives							$\chi^2$ (males vs. females)	P		
		Male			Female			Total				
		n	+ve	(%)	n	+ve	(%)	n			+ve	% (95% CI)
Andaman	49 488	662	188	28.4	591	164	27.7	1253	352	28.1 (25.6–30.7)	0.07	0.799
Nicobar	9601	162	8	4.9	129	5	3.8	291	13	4.5 (2.4–7.5)	0.19	0.663
Overall	59 089	824	196	23.7	720	169	23.4	1544	365	23.6 (21.5–25.8)	0.02	0.885

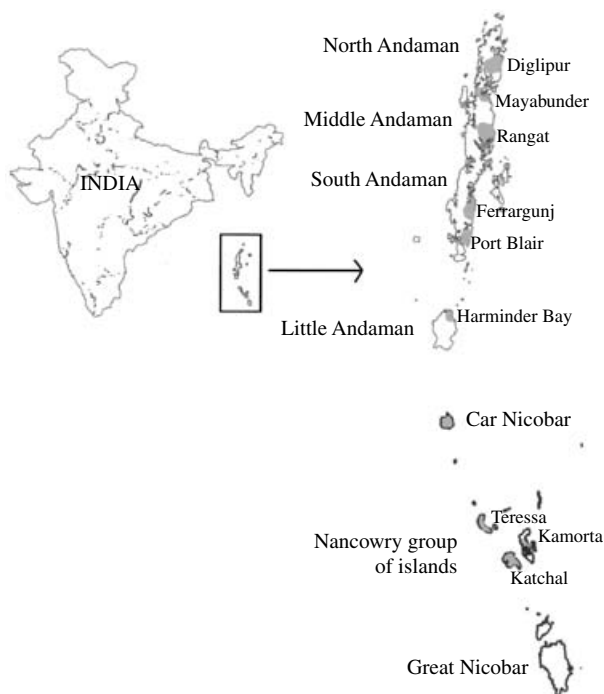


Fig. 1. Map of the Andaman & Nicobar Islands (regions shaded in grey indicate areas sampled).

included a serosurvey among a sample of schoolchildren, follow up of a subsample of students living in an epidemic-prone area to estimate the incidence of infection and a case-control study to identify risk factors associated with leptospiral infection among the students.

The present survey was carried out in rural schools of the Andaman & Nicobar Islands.

The Andaman & Nicobar Islands is an archipelago of 572 islands, islets, reefs and rocks in the Bay of Bengal situated about 1200 km east of the Indian subcontinent. Approximately 86% of the land area of the islands is covered by evergreen forest. The climate is tropical throughout the year with temperature in the range of 25–32 °C and relative humidity in the range of 70–100%. The islands receive an annual rainfall in excess of 3300 mm. The Andaman &

Nicobar Islands, which is a Union Territory of India, comprises two districts, namely the Andaman district and the Nicobar district. Each district is further divided into several administrative subunits called *tehsils*. Andaman district has five *tehsils*, i.e. Diglipur, Mayabunder, Rangat, Ferrargunj and Port Blair whereas Nicobar district has two *tehsils*, i.e. Nicobar and Nancowry (Fig. 1).

There are 301 rural schools in these islands, 244 in the Andaman district and 57 in the Nicobar district, with a student population of 59 089 [7]. Three to four schools were randomly selected from each of the seven *tehsils*. A total of 1544 sera were collected from schoolchildren studying in 26 schools. The details of sampling are given in the Table. Nicobar district is a predominantly tribal area and all the selected students from this district belonged to the Nicobarese tribe. The nature of the study was explained to the students in their classes and they were asked to obtain the consent of their parents. The students whose parents consented were selected for inclusion in the study. A total of 3–5 ml of venous blood was collected and the serum was separated. Serum samples were stored at –70 °C until examined for anti-leptospiral antibodies.

The microscopic agglutination test (MAT) was used to detect anti-leptospiral antibodies in the serum samples. The test was performed as reported earlier [8] using 12 live leptospiral strains belonging to 12 serogroups, which are prevalent in India. The strains belonged to serogroups, Australis (serovar australis, strain Ballico), Autumnalis (serovar rachmati, strain Rachmat), Ballum (serovar ballum, strain Mus 127), Canicola (serovar canicola, strain H.Utrecht IV), Grippotyphosa (serovar grippotyphosa, strain Moskva V), Hebdomadis (serovar hebdomadis strain Hebdomadis), Icterohaemorrhagiae (serovar ictero-haemorrhagiae, strain RGA), Javanica (serovar poi strain Poi), Pomona (serovar pomona, strain Pomona), Sejroe (serovar hardjo, strain Hardjoprajitno), Pyrogenes (serovar pyrogenes, strain Salinem) and

Sehgali (serovar portblairi, strain DS 2). Serovar portblairi is a new serovar recovered from the Andaman Islands and described recently [9]. MAT was performed in microtitre plates using a 5- to 7-day-old culture at a density of  $1-2 \times 10^8$  leptospire/ml without containing any 'breeding nests' (auto-agglutinations). All the serum samples were screened at double dilutions starting from 1/25 up to the end titre. A titre of  $\geq 100$  to any one of the serovars used in MAT was considered indicative of a past leptospiral infection.

An outbreak of leptospirosis occurred in one of the *tehsils* (Diglipur) of Andaman district 1 month after the collection of the blood samples for serosurvey. In total, 341 students from this *tehsil* were followed up during the outbreak. These 341 students included 120 students who were seropositive in the first sample and 221 who were seronegative. The study subjects in this *tehsil* were monitored for any acute febrile illness during the outbreak. Acute and convalescent sera were collected from all persons who had acute febrile illness and screened for anti-leptospiral antibodies using Lepto dipstick [10], IgM ELISA (Serion Immundignostica, Würzburg, Germany) and MAT. Follow-up serum samples were also collected from these 341 schoolchildren from this *tehsil* 3-4 weeks after the start of the outbreak and screened using MAT to estimate the incidence of infection.

Risk factor study was conducted among the students who were seronegative in their first samples in this *tehsil* where the epidemic occurred. Those who seroconverted from negative (titre  $< 25$ ) to a minimum titre of 100 were considered as cases and those who were still seronegative in the second sample were considered as controls. Information about factors pertaining to characteristics of the house and surroundings and activities during the follow-up period was collected using a structured questionnaire. A univariate unmatched analysis was done on these factors. Those factors with a *P* value less than 0.25 were included in a multiple logistic regression using backward conditional procedure. Analysis was done using SPSS version 10.0 (SPSS Inc., Chicago, IL, USA).

Out of 1544 sera from schoolchildren, anti-leptospiral antibodies were detected in 365 samples with an overall seropositivity rate of 23.6% (95% CI 21.54-25.81) (Table). Seroprevalence of leptospirosis was significantly higher in the Andaman district (28.1%) compared to that among the tribal students from the Nicobar district (4.5%,  $\chi^2 = 73.02$ ,  $P = -0.0000$ ). For both the districts, the seroprevalence among males was not statistically different from that of females

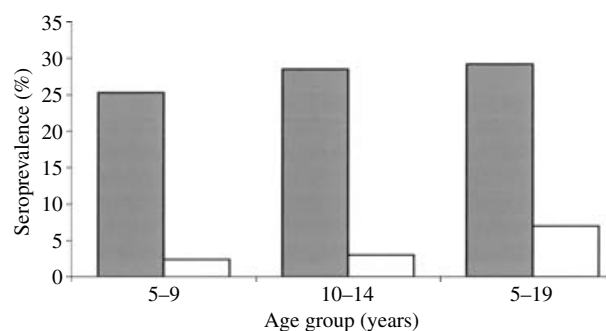


Fig. 2. Seropositivity of leptospiral infection among schoolchildren in different age groups. ■, Andaman; □, Nicobar.

(Table). In both the districts, the seroprevalence rate increased with age (Fig. 2) but did not show statistically significant linear trends.

Highest titres in MAT were found against serovars australis (37.9%), grippotyphosa (26.6%), canicola (12.3%), icterohaemorrhagiae (6.5%), pomona (1.6%) and equal titres against multiple serovars were seen in 15.0% of the serum samples. Infecting serogroups in both districts did not differ greatly. Out of 365 positive samples a titre of 100 was observed in 186 samples, 200 in 98, 400 in 45 and  $\geq 800$  in 36. The geometric mean titre for serogroups Australis, Grippotyphosa, Canicola, Icterohaemorrhagiae and Pomona were 202.35, 219.6, 144.03, 192.48 and 126.47 respectively for the Andaman district and the corresponding values for the Nicobar district were 131.63, 159.17, 100.00, 100.00 and 100.00 respectively.

Antibodies against leptospire were demonstrated in 74 (33.5%) of 221 follow-up samples from seronegatives indicating new infections during the period of 2 months. Among the 120 seropositives, a four-fold or greater rise in titre was demonstrated in 20 (16.7%), 21 (17.5%) showed a decline in titre, 13 (10.8%) became seronegative at an initial dilution of 25, and 66 (55.0%) showed the same titre in the second sample. A four-fold rise in titre in 20 followed-up individuals might be due to new or re-infections during the follow up. Out these 20 individuals, 12 were initially infected with serogroup Australis, five with Grippotyphosa, two with Canicola and the remaining one with Icterohaemorrhagiae. Out of 12 initially infected with serogroup Australis, seven were re-infected with serogroup Grippotyphosa, three with serogroup Australis, and the remaining two with serogroup Canicola. Of five individuals who were initially infected with serogroup Grippotyphosa, four were re-infected with serogroup Australis and the remaining one with serogroup Grippotyphosa. One

each was re-infected with serogroup Australis and serogroup Grippotyphosa among the two individuals initially infected with serogroup Canicola. One who was initially infected with serogroup Icterohaemorrhagiae was re-infected with serogroup Grippotyphosa.

Among the 120 individuals who were seropositive in the first sample, 67 were positive against Australis, 25 against Grippotyphosa, 8 against Icterohaemorrhagiae and 6 against Canicola. The remaining 14 gave equal titres against more than one serovar. Out of the 67 individuals seropositive against Australis, re-infection with Australis occurred in three (4.5%) whereas among the 221 seronegatives Australis infection occurred in 46 (20.8%) and the difference was statistically significant ( $\chi^2=8.60$ ,  $P=0.0034$ ). Similarly the incidence of infection with Grippotyphosa among those seropositive for Grippotyphosa (4.0%) was lower than that among seronegatives (9.05%). However the difference was statistically non-significant ( $\chi^2=0.23$ ,  $P=0.6320$ ). Out of the 67 individuals who were seropositive against Australis, seven (10.4%) were re-infected with Grippotyphosa whereas among the 221 seronegatives, 20 (9.1%) were infected with Grippotyphosa and the difference was statistically non-significant ( $\chi^2=0.89$ ,  $P=0.346$ ). Among the 25 individuals who were seropositive against Grippotyphosa, four (16%) were infected with Australis whereas among the 221 seronegatives, 46 (20.8%) were infected with Australis. Although incidence of infection with Australis was 4% lower among those who were seropositive against Australis than among seronegatives, it was statistically non-significant ( $\chi^2=0.09$ ,  $P=0.761$ ).

Eighteen students had febrile illness during the outbreak period and nine of them showed seroconversion or a four-fold rise in titre in their convalescent sample. These nine students gave positive reactions in Lepto dipstick and IgM ELISA (titre >160). Six of them were seronegative initially and three were seropositive (the initial MAT titres of these three patients at the time of the serosurvey were 200, 200 and 400, whereas during febrile illness the titres on acute samples were 200, 100 and 400 respectively). All these patients' convalescent sera showed a titre of >3200. The common clinical features of these patients included fever, generalized body ache, headache and cough. None of the patients had either hepatic or renal dysfunctions. However two of the children among the seronegatives (at the time of serosurvey) had haemoptysis and were admitted to

hospital. One of them soon died due to massive haemoptysis and respiratory distress. Out of nine patients with evidence of leptospirosis, six were infected with serogroup Australis and three with serogroup Grippotyphosa. Both the patients who had haemoptysis were infected with serogroup Grippotyphosa.

In the Andaman district some of the risk factors studied were found to have a significant association with presence of leptospiral antibodies. These factors were mud flooring in the house (OR 1.52), presence of domestic animals, i.e. cattle and goats, in the house (OR 1.4), bathing in ponds (OR 1.8), recent agricultural work (OR 1.42) and recent outdoor activity (OR 1.58).

Leptospirosis occurs frequently in the Andaman district with regular post-monsoon outbreaks. In contrast, the disease is very rare in the Nicobar district. This tendency correlates well with the seroprevalence rates seen in students from the two districts. The distribution of the anti-leptospiral antibody titres also shows great variations between the two districts. In the Nicobar district none had titres of >200, whereas in the Andaman district 4.3% of the asymptomatic children had a titre of  $\geq 400$ , 12.4% had  $\geq 200$  and 27.2% had titres of  $\geq 100$ .

The geometric mean titres against common infecting serogroups in the Andaman district were high when compared to the Nicobar district. The population of the Nicobar district consists mainly of the Nicobarese tribe. The low seroprevalence rate observed among the students of the Nicobar district is a reflection of the low level of exposure of the Nicobarese tribe as a result of the dry environment they live in. In an earlier study, a seroprevalence of 53.5% was observed among the Shompens tribe who also live in one of the islands of the Nicobar district [5]. The Shompens are a nomadic tribe living in the jungles of Great Nicobar. The jungles of Great Nicobar have a wet and muddy environment and there are several streams. The Shompens are a primitive tribe and they live in close association with animals. Because of this they have a higher chance of acquiring leptospiral infection. The school students included in the present study are from the islands of Car Nicobar and the Nancowry group of islands. These islands are sandy and the Nicobarese, although considered as a tribe, lead a modern life-style and have better hygienic practices. This might be the reason for the lower seroprevalence among them. Such differences in leptospiral incidence and prevalence between closely located islands are also seen in other places. The difference in the incidence of leptospirosis between Sumatra and Java

islands of Indonesia [11] and Trinidad and Barbados in the West Indies [12] are some examples.

Although males usually form the highest risk group, as they are more active in outdoor activities like agricultural work, in the case of both Andaman and Nicobar districts, no significant difference in the prevalence rates between males and females has been observed. Although some degree of male predominance has been observed among hospitalized cases of severe leptospirosis [3], during outbreaks males and females are almost equally affected [2]. The equal seroprevalence in males and females and the young age at which the disease is acquired indicate that leptospiral infection probably occurs in the living environment. In both Andaman and Nicobar districts, the seroprevalence rate increases with age. This increase might be the result of cumulative exposure over the years, and is expected in endemic areas in the case of most communicable diseases.

More than 30% of the seronegatives that were followed up, seroconverted during a period of 2 months, when there was an outbreak in the area. In contrast 16.7% of the seropositives showed a four-fold or higher rise in titre in the follow-up sample. This rise in titre might be due to either new or re-infections during follow up. The difference in the incidence of infection between seropositives and seronegatives is significant ( $\chi^2 = 11.02$ ,  $P = 0.0009033$ ) indicating increased protection after past infection. The severity of illness and case-fatality ratios were observed to be more among seronegative individuals than seropositives during an epidemic. Moreover, out of 94 children who showed evidence of new infections or re-infections only nine (9.6%) developed clinical illness indicating that more than 90% leptospiral infections were subclinical or unnoticed.

The protection against leptospiral infection is generally serovar-specific rather than genus-specific. A statistically significant reduction in the incidence of infection with Australis was observed among those who were initially seropositive for Australis. A similar reduction was also observed for infection with Grippityphosa. Although a reduction in the incidence of infection with Australis was observed in those who were seropositive for Grippityphosa, it was not statistically significant. Thus, the present study shows some evidence of protective effect of microscopic agglutinating antibodies against re-infection with same serogroup, however, no conclusive evidence could be obtained for the existence of cross-protection against heterologous serogroups.

Although the proportion of re-infection with homologous serogroups (20%) was lower than that with heterologous serogroups (80%), it is difficult to explain why past infection failed to protect against re-infection with the same serogroup. However, there is a possibility that these re-infections either might be due to the same serovar or due to a different serovar of the same serogroup as only one representative serovar of a particular serogroup is included in the MAT.

The risk factors found associated with leptospiral infection among schoolchildren were the known factors, which predispose the infection and the same association has also been obtained in the general population [6]. A new observation was the association of mud flooring of the house with infection, which needs further studies to find out how it facilitates transmission of infection.

The environment and life-style of the people of the Andaman Islands are highly conducive for the spread of leptospirosis. The population is exposed to the infective agent from childhood and the level of exposure increases with age, as their outdoor activities become more frequent. The repeated exposures result in the build up of partial immunity. Thus, children and adolescents who become newly infected without the partial protection conferred by prior exposures, become the victims during outbreaks. The relatively lower level of prior exposure and the resulting low partial protection they have explains the higher severity and frequent fatal complications seen among the children.

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