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Leptospiral carrier state and seroprevalence among animal population – a cross-sectional sample survey in Andaman and Nicobar Islands

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SUMMARY

A study was conducted with the objective of assessing the leptospiral carrier state and seroprevalence among animal population of Andaman and Nicobar Islands. A total of 494 sera samples from different domestic animals and 85 samples from rats (*Rattus rattus*) were tested by microscopic agglutination test using nine serogroups prevalent in these islands. Antibodies to leptospires were detected in 164 samples giving an overall seroprevalence of (33·11%). The seroprevalence was highest among cows (40·32%). Of 85 rat (*Rattus rattus*) samples tested for antileptospiral antibodies six (7·1%) were positive. Leptospires were isolated from kidney of two rats and urine of one cow. Isolate from urine of cow was heavily contaminated and was subsequently lost during further subculture. The two isolates were found to be pathogenic, belonging to serogroup Grippotyphosa. The isolates were further characterized by using a set of monoclonal antibodies. The agglutination patterns of isolates were similar to that of ratnapura and valbuzzi, however these did not completely match.

INTRODUCTION

Leptospirosis is emerging as an important public health problem in India and other developing countries [1–4]. Although it is considered as an occupational hazard of agricultural workers, sewage workers, veterinarians, etc. [5–7]. Whole communities living in tropical regions with a wet environment could be at risk [8]. The transmission cycle involves interaction between one or more species of animal hosts harbouring the organism, an environment favourable for the survival of leptospires and human beings. Human infection results from either direct contact with an infected animal or, more frequently, from indirect exposure through environment contaminated with urine of carrier animals [9, 10].

Andaman and Nicobar Islands, a union territory in India, were known to be endemic for leptospirosis during the early part of twentieth century [11]. The first confirmed report of leptospirosis from these islands dates back to 1929 when Taylor and Gayle isolated leptospires from patients with Weil's disease [11]. Since 1988, post-monsoon outbreaks of febrile illness with haemorrhagic manifestations have been occurring in North and South Andaman. These outbreaks were proved to be due to leptospires. Sero-epidemiological and follow-up studies carried out in different population groups have shown that leptospirosis is highly endemic in these islands [12–15].

In farming communities such as the rural population of Andamans, both rodents and domestic animals might act as the predominant source of infection. The rice fields attract rodents and leads to exposure of people, particularly farmers, to soil and ground water contaminated with rodent urine [16]. These communities also keep domestic animals that usually graze freely on harvested fields and house

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compounds. If there is a significant carrier rate among domestic animals, they could also act as the predominant source of infection [17, 18]. Identification of the predominant carrier animal species and reduction in the load of carrier animals is an important component in the control of leptospirosis. Information about the prevalence of leptospiral infection among the animal population is essential for planning such programmes. No study has been conducted to generate this information in Andaman and Nicobar Islands. Hence, a study was conducted with the objective of assessing the seroprevalence leptospiral carrier rate among the animal population of Andaman and Nicobar Islands.

MATERIAL AND METHODS

Survey site

Andaman and Nicobar Islands are administratively divided into two districts namely Andaman District and Nicobar District. Each district is further divided into several *tehsils*. In Andaman District, human leptospirosis is highly endemic whereas in Nicobar District the endemicity rate is low. The present study was conducted in two *tehsils* namely Diglipur in Andaman District and Car Nicobar in Nicobar district.

Blood samples

Blood samples were collected from 494 apparently healthy animals of different species. Urine samples were also collected from 150 animals. Eighty-five field rats (*Rattus rattus*) were trapped from Diglipur *tehsil*. The rats were anaesthetized using chloroform. Blood samples were collected by heart puncture and urine samples by bladder puncture. These rats were then dissected and their kidneys were removed. Serum was separated from blood samples and stored at -70 °C until processed.

Microscopic agglutination test

Sera samples were tested for the presence of antileptospiral antibodies using microscopic agglutination test (MAT) following standard procedures [19]. Reference strains belonging to nine serogroups prevalent in these islands were used as antigens in MAT. These strains belonged to nine serogroups: Australis (serovar australis, strain Balico), Autumnalis (serovar autumnalis, strain Rachmati), Bataviae (serovar bataviae, strain Swart), Canicola (serovar canicola strain H Uterecht IV), Grippotyphosa (serovar grippotyphosa, strain Moskva V), Icterohaemorrhagiae (serovar icterohaemorrhagiae, strain RGA), Javanica (serovar poi, strain poi), Pomona (serovar pomona, strain pomona) and Sejroe (serovar hardjo, strain Hardjoprajitro). The antigens used were 5–7 days old auto-agglutination free cultures of reference strains grown in Elinghausen McCullough Johnsion Harris (EMJH) medium (DIFCO) with approximately $1–2 \times 10^8$ organisms/ml. MAT was done at doubling dilutions starting from 1:20. Positive samples were titrated up to end titres. A titre of 40 or more to any of the serovars was considered as evidence of leptospiral infection. A titre of 40 was used as the cut-off because it was the closest dilution to the usual cut-off of 50 used in seroepidemiological surveys [20].

Isolation of leptospires

Urine samples from domestic animals and rat kidney samples were inoculated into EMJH semisolid medium containing 2% rabbit serum and 100 μg/ml of 5-fluorouracil [21, 22] immediately after collection. The cultures were examined every 10 days up to 6 months. Positive samples were sub-cultured into EMJH semisolid media. The ability of the isolates to grow at 13 °C and in presence of 8-azaguanine at 30 °C was tested separately to discriminate between pathogenic and saprophytic species. Serogroup status of the isolates was ascertained using group sera and serovars status using a panel of four monoclonal antibodies (71C9-4, 165C83, 71C31 and 165C34) obtained from the Royal Tropical Institute (KIT), Amsterdam.

RESULTS

Out of 494 sera samples from domestic animals tested by MAT, antileptospiral antibodies against one or more serovars of leptospires were found in 164 animals with an overall seroprevalence rate of (33·2%). Six of the rat serum samples (7·1%) also showed antileptospiral antibodies. The seroprevalence was highest among cows (40·3%) followed by buffaloes (37·0%) and goats (36·3%) (Table 1). The seroprevalence was significantly higher among Andaman district than the Nicobar district (38·0% *vs.* 13·0%, $\chi^2 = 19{\cdot}72$, $P = 0{\cdot}000089$).

The serogroup of leptospires responsible for infection among the seropositive domestic animals was determined based on the highest titre in MAT. Among the seropositives in Andaman district, the serogroup that caused the highest proportion of

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Table 1. *Seroprevalance of leptospirosis among domestic animals in Andaman and Nicobar districts*

	Andaman		Nicobar		Overall	
Animal species	No.	Positive (%)	No.	Positive (%)	No.	Positive (%)
Cow	106	43 (40·6)	18	7 (38·8)	124	50 (40·3)
Bullock	89	26 (29·2)	6	0 (0·0)	95	26 (27·4)
Buffalo	46	17 (37·0)	0	0 (0·0)	46	17 (37·0)
Goat	124	56 (45·2)	44	5 (11·4)	168	61 (36·3)
Pig	26	9 (34·6)	24	0 (0·0)	50	9 (18·0)
Dog	11	1 (9·1)	0	0 (0·0)	11	1 (9·1)
Total	402	152 (37·8)	92	12 (13·0)	494	164 (33·2)

Table 2. *Distribution of different serogroups of leptospires among seropositive animals in Andaman and Nicobar districts*

Serogroup	Andaman ($n=152$)	Nicobar ($n=12$)
Grippotyphosa	48 (31·6%)	1 (8·3%)
Australis	24 (15·8%)	7 (58·3%)
Pomona	21 (13·8%)	0 (0·0%)
Canicola	15 (9·9%)	0 (0·0%)
Autumnalis	13 (8·6%)	0 (0·0%)
Javanica	3 (2%)	0 (0·0%)
Sejroe	3 (2%)	4 (33·3%)
Bataviae	2 (1·1%)	0 (0·0%)
Mixed reaction	23 (15·1%)	0 (0·0%)

infection was Grippotyphosa (31·6%) followed by Australis (15·8%), whereas in Nicobar district Australis (58·3%) followed by Sejroe (33·3%) were the serogroups that caused the highest proportion of infection (Table 2). Among the six seropositive rats, five had titres against serogroup Grippotyphosa and one against Australis.

Of these 152 positive samples from Andaman district, 129 reacted to one serovar and 23 reacted to more than one serovar. Of these 23 samples that reacted to more than one serovar, 22 reacted to two serovars and one to three serovars. The common combinations observed among the 23 sera that reacted to more than one serovar were Australis and Grippotyphosa (5), Autumnalis and Pomona (4), Australis and Autumnalis (3), Icterohaemorrhagiae and Australis (2), and Grippotyphosa and Pomona (2). None of the seropositive animals from Nicobar district showed mixed reactions. The majority of the seropositive animals in both the districts had low titres (Table 3). The highest titre observed was 640 in Andaman district and 160 in Nicobar district.

Table 3. *Distribution of MAT titres among seropositive animals in Andaman and Nicobar districts*

Titres	Andaman (%) ($n=402$)	Nicobar (%) ($n=92$)	Overall (%) ($n=494$)
Negative	250 (62·2)	80 (87·0)	330 (66·8)
40	82 (20·4)	7 (7·6)	89 (18·0)
80	29 (7·2)	4 (4·3)	33 (6·7)
160	28 (7·0)	1 (1·1)	29 (5·9)
320	11 (2·7)	0 (0·0)	11 (2·2)
640	2 (0·5)	0 (0·0)	2 (0·4)

Leptospires were isolated from the kidney specimens of two rats and the urine of one cow. However, the isolate from the cow urine was heavily contaminated and was lost during sub-cultures. These two rat isolates did not grow at 13 °C or in the presence of 8-azaguanine indicating that they belonged to pathogenic species. The isolates agglutinated with Grippotyphosa group-specific antisera at titres of 20 480 and 40 960 respectively. They did not show any agglutination against any of the other 22 group-specific antisera tested. In an attempt to identify the serovar status of these isolates they were tested using a panel of four monoclonal antibodies. The titres obtained for reference strains of different serovars of serogroup Grippotyphosa and against the rat isolates (R41 and R42) are shown in Table 4. The agglutination pattern of both these isolates had closest similarity with that of serovars ratnapura and valbuzzi. However, they did not match exactly.

DISCUSSION

Leptospirosis is highly endemic in Andaman Islands with more than half of the population exposed to leptospires [12–15]. The disease occurs in the form of seasonal post-monsoon outbreaks with considerable

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Table 4. *Monoclonal antibody titres against rat isolates and reference strains belonging to serogroup Grippotyphosa*

Serovar/strain	71C9-4	165C83	71C31	165C3
Grippotyphosa	10 240	Neg	40 960	10 240
Muelleri	10 240	Neg	20 480	81 920
Huanuco	1280	Neg	Neg	Neg
Vanderhaedeni	80	640	2560	80
Canalzonae	Neg	Neg	2560	Neg
Ratnapura	20 480	5120	5120	40 960
Valbuzzi	20 480	320	20 480	40 960
R41	10 240	320	Neg	81 920
R42	10 240	160	Neg	81 920

mortality. The control of disease is a priority in these Islands. As the exact chain of transmission of leptospirosis is not known, no permanent measures for the control of leptospirosis could be implemented.

The findings of the present study indicate that leptospiral infection is highly prevalent among the domestic animals of Andaman islands. The rats also showed a seroprevalence of 7·1 %. The prevalence was significantly lower among the animals in Nicobar district. These prevalence rates correlate well with the seroprevalence rates in human populations in these districts. In Nicobar district the soil is sandy with lower water retaining capacity and thus there is very little stagnation of water. The chances of survival of leptospires in this dry soil are negligible. Suitability of the environment for the survival of leptospires appears to be a critical factor in maintaining the infection among animals and transmission of infection from animals to humans. As the environment in Nicobar district is not suitable for the survival of leptospires, the chances of human beings contracting the infection directly or indirectly are less. This could be a reason for the lower prevalence of leptospiral infection observed in human population in this district.

In contrast, the seroprevalence in the Andamans is a reflection of the overall problem of the leptospirosis both in animals and human beings. The Andaman islands have an undulating topography with mountains, hillocks and valleys. Human settlement areas in most of the islands are in the basins surrounded by hills. The prolonged monsoon fills the basins with water and even after the end of the monsoon the soil remains wet for most part of the year. This environment facilitates the survival of leptospires and acts as a vehicle for transmission of infection, maintaining and probably amplifying the source of infection. The importance of control of infection among animals is more pronounced in this situation.

Although rats had lower seroprevalence rates, 2 of the 85 rats studied were found to be the carriers of pathogenic leptospires. Serogroup Grippotyphosa is one of the commonest infecting serogroups in man in these islands and most of the human isolates belong to this serogroup. Several human isolates obtained from these islands have been identified as belonging to serovars valbuzzi. The panel of monoclonal antibodies used in the present study was not sufficient to identify the serovars of the isolate, as the pattern was similar to those of both valbuzi and ratnapura. A larger panel with more monoclonals would be required to identify the serovars status.

In the present study, more than one third of the cattle and goats were found to be exposed to leptospires. Animals affected with leptospires continue to excrete leptospires in their urine for several months. Although leptospiral carrier state could not be directly demonstrated by isolating the organism from the urine, the high seroprevalence is an indicator that leptospiral carrier state might be high among the domestic animals. When a large proportion of the animals are carrying leptospires in an area with an environment suitable for prolonged survival of the bacteria, the level of exposure to humans and uninfected animals would be very high. Many of the links in the transmission cycle of leptospirosis such as the exposure of humans to environment, suitability of environment for the survival of leptospires, animal activity in the environment etc. cannot easily be modified. Even factors such as the load of rodent population or carrier state among the rodents are hard to modify. However, leptospiral carrier state in domestic animals, which could be an important determinant of transmission of infection, can be modified more easily as treatment regimes for curing leptospiral carrier state are available. The possibility of vaccinating domestic animals against the common infecting serovars could be considered as a possible measure for reducing the carrier state among animals.

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REFERENCES

1. Sehgal SC. Leptospirosis in the horizon. Nat Med J India 2000; **13**: 228–230.
2. World Health Organization (WHO). Leptospirosis in India – report of the investigation of a post-cyclone outbreak in Orissa, November 1999. Wkly Epidemiol Rec 2000; **27**: 217–223.
3. Ratnam S. Leptospirosis: an Indian perspective. Indian J Med Microbiol 1994; **12**: 228–239.
4. Brandling-Bennett AD, Pinheiro F. Infectious diseases in Latin America and the Caribbean: are they really emerging and increasing? Emerg Infect Dis 1996; **2**: 59–61.
5. Levett PN. Leptospirosis: re-emerging or re-discovered disease? J Med Microbiol 1999; **48**: 417–418.
6. Levett PN. Leptospirosis. Clin Microbiol Rev 2001; **14**: 296–326.
7. Campagnolo ER, Warwick MC, Marx HL, et al. Analysis of the 1998 outbreak of leptospirosis in Missouri in humans exposed to infected swine. J Am Vet Med Assoc 2000; **216**: 676–682.
8. Chan OY, Paul DR, Sng EH. Leptospirosis among abattoir workers – a serological study. Singapore Med J 1987; **28**: 293–296.
9. Faine S. *Leptospira* and leptospirosis. Boca Raton, Fla: CRC Press, 1994.
10. Alston JM, Broom JC. Leptospirosis in man and animals. Livingstone, Edinburgh; E & S 1958.
11. Taylor J, Goyle AN. Leptospirosis in Andamans. Indian Medical Research Memoirs, Supplementary series to the Indian Journal of Medical Research. Indian J Med Res 1931; Memoir No. **20**: 55–56.
12. Sehgal SC, Murhekar MV, Sugunan AP. Leptospirosis with pulmonary involvement in North Andamans. Indian J Med Res 1995; **102**: 9–12.
13. Murhekar MV, Sugunan AP, Vijayachari P, Sharma S, Sehgal SC. Risk factors in the transmission of leptospiral infection. Indian J Med Res 1998; **107**: 218.
14. Sehgal SC, Vijayachari P, Murhekar MV, Sugunan AP, Sharma S, Singh SS. Leptospiral infection among primitive tribes of Andaman and Nicobar Islands. Epidemiol Infect 1999; **122**: 423–428.
15. Singh SS, Vijayachari P, Sinha A, Sugunan AP, Rasheed MA, Sehgal SC. Clinico-epidemiological study of severe cases of leptospirosis in Andamans. Indian J Med Res 1999; **109**: 94–99.
16. Sehgal SC, Vijayachari P, Smythe LD, et al. Lai-like leptospira from the Andaman Islands. Indian J Med Res 2000; **112**: 135–139.
17. Alston JM. Leptospiral jaundice among sewer-workers. Lancet 1935; **1**: 806–809.
18. Johnson DW, Brown HE, Derrick EH. Weil's disease in Brisbane. Med J Aust 1937; **1**: 811–818.
19. Babudieri B. Animal reservoirs of leptospirosis. Ann NY Acad Sci 1958; **70**: 393–413.
20. Everard CO, Hayes RJ, Edwards CN. Leptospirosis infection in school-children from Trinidad and Barbados. Epidemiol Infect 1989; **103**: 143–156.
21. Wolff JW. The laboratory diagnosis of leptospirosis. Illinions: Charles C. Thomas, 1954: 31–51.
22. Djordjevic S, Hornitzky M, Ross AD, Wittington RJ. Restriction endonuclease analysis of Australian isolates of *Leptospira interrogans hardjo* from cattle with agalactia and abortion. Aust Vet J 1993; **70**: 98–100.
23. Ratnam S, Subramaniam S, Adinarayanan N. Experimental study with leptospires in *Bandicota bengalensis*. Ind J Expl Biol 1987; **25**: 105–107.