A SEROLOGICAL SURVEY OF ARBOVIRAL DISEASES AMONG THE HUMAN POPULATION OF THE ANDAMAN AND NICOBAR ISLANDS, INDIA

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Abstract. In an attempt to determine the prevalence of certain arthropod-borne viruses of public health importance amongst the human population of the Andaman and Nicobar Islands of India, 2,401 sera were collected from six major localities. The sera were analysed by the hemagglutination inhibition (HI) and neutralization (N) tests, using Chikungunya (CHIK), Japanese encephalitis (JE), West Nile (WN), dengue (DEN-2), Langat (TP-21) and Kyasanur Forest disease (KFD) viral antigens. The highest prevalence of HI antibodies was detected against KFD virus (22.4%), followed by Langat (20.2%), JE (5.9%), DEN-2 (3.1%), CHIK (2.9%) and WN (0.8%) viruses. Cross-reactions to the viral antigens were also noted. The results of N tests indicated a high prevalence of DEN-2 (25.4%) virus, followed by Langat (17.5%), CHIK (15.3%), KFD (12%), JE (2.1%) and WN (1.8%). These results are discussed in relation to important epidemiological parameters like age, sex and geographical location. To our knowledge, this is the first report of an extensive serosurvey of arthropod-borne viruses on these islands.

INTRODUCTION

Serological surveys in different geographical areas of a country provide data for the mapping of endemic zones and susceptible populations. Geographical epidemiology plays an important role in determining the prevalence of diseases in different regions, variations in the disease pattern, and the ecological risk factors responsible for these variations. The Andaman and Nicobar group of islands in India are situated strategically from the point of view of spread of diseases. The islands lie closer to Southeast Asia and a large number of people from mainland India visit them; people from the islands also visit the mainland. Surprisingly, there is hardly any information regarding the prevalence of arthropod-borne viral diseases on these islands. A serological survey of the resident human population was therefore undertaken by the National Institute of Virology (NIV) between December 1988 and January 1989. The results of the survey are presented in this paper.

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MATERIAL AND METHODS

Study area

The study area has already been described in earlier communications (Joshi *et al*, 1991; Padbidri *et al*, 1995). For administrative purposes, these islands have been divided from north to south into six localities: North Andaman, Middle Andaman, South Andaman, Little Andaman, Great Nicobar, and Nicobar Islands. Four rare tribes, Onges, Jarwas, Sentinels and Greater Andamanis inhabit these various Islands. Besides these, settlers from various states from the Indian mainland, Burma and other refugees also inhabit these Island.

Collection and transportation of specimens

The project was approved by the Scientific Advisory Committee of the Institute, which

supervises the research activities.

Blood samples were randomly collected from children and adults of all age groups and both sexes who gave a history of having stayed on the islands for ≥5 years. Informed verbal consent was obtained before taking the sample. About 5 ml of blood were collected from each individual. The sera were separated and stored continuously on wet ice until transportation to the NIV, where they were maintained at -20ºC until testing.

Serological tests

All the sera were subjected to the hemagglutination inhibition (HI) (Clarke and Casals, 1958; Sever, 1962) and neutralization (N) (Banerjee and Desai, 1973) tests employing Chikungunya (CHIK), Japanese encephalitis (JE), West Nile (WN), dengue (DEN- 2), Kyasanur Forest disease (KFD) and Langat (TP-21) virus antigens. The relevant details of the N test are present in Table 1.

Statistical analysis

The data was analysed using *dbase* III+ and EPI-INFO 5 software. The chi-square test was used to analyze the significance of variation in the serological prevalence of the viruses. In order to analyze the independent association of factors like geographic location, age and sex on the seroprevalence, multivariate analysis using stepwise regression was conducted using BMDP software.

RESULTS

A total of 2,401 sera were collected and details of the collection, according to the six major geographical locations, age and sex of the donors are presented in Table 2. As many as 13 islands/locations were visited, depending

Table 1
Detials of the N test.

Virus	Age of the mice	Route of inoculation	Dosage (in logs)
CHIK	Infant	IP	2.21-2.23
JE	Adult	IC	2.0-2.49
WN	Adult	IC	2.16-2.53
DEN-2	Adult	IC	2.14-2.40
KFD	Adult	IP	2.14-2.40
TP-21	Adult	IC	1.92-2.45

Table 2
Collection of the survey sera by age, sex and geographic location.

Locality	Age groups (Years)											
	0 - 9		10 - 19		20 - 29		30 - 39		≥40		Total	
	M	F	M	F	M	F	M	F	M	F	M	F
North Andaman	47	39	130	126	50	79	37	39	59	72	323	355
Middle Andaman	50	48	211	197	105	93	64	46	110	50	540	434
Little Andaman	5	2	24	26	18	16	27	8	17	5	91	57
South Andaman	2	0	16	2	22	5	4	3	6	7	50	17
Great Nicobar	4	4	35	32	49	18	31	15	40	13	159	82
Nicobar Islands	8	7	49	43	35	20	35	11	67	18	194	99
Total	116	100	465	426	279	231	198	122	299	165	1,357	1,044

Table 3
Area-specific prevalence of HI antibodies (expressed as percentages).

Locality	N	JE	WN	DEN-2	CHIK	TP-21	KFD
North Andaman	678	3.69	0.59	4.72	3.03	9.88	10.93
Middle Andaman	974	5.34	0.51	2.36	4.62	16.63	17.57
Little Andaman	148	7.43	0.68	8.78	2.70	39.86	72.30
South Andaman	67	10.45	1.49	2.99	0.0	29.85	25.37
Great Nicobar	241	10.79	2.49	0.83	0.0	26.56	43.44
Nicobar Islands	293	6.83	0.68	1.02	0.34	38.49	30.03
Total	2,401	5.87	0.79	3.12	2.94	20.18	22.37

N=Total number of sera tested.

Table 4
Age-specific prevalence of HI antibodies.

Age group (years)	JE	WN	DEN-2	CHIK	TP-21	KFD
0-9	9/216[a]	-	2/216	7/184	21/216	28/215
	(4.17)	-	(0.93)	(3.80)	(9.72)	(13.02)
10-19	55/891	7/891	29/891	6/834	106/890	145/878
	(6.17)	(0.79)	(3.25)	(0.72)	(11.91)	(16.51)
20-29	26/510	8/510	14/510	13/439	112/510	110/459
	(5.10)	(1.57)	(2.75)	(2.96)	(21.96)	(23.97)
30-39	19/320	-	12/320	14/301	98/320	94/293
	(5.94)	-	(3.75)	(4.65)	(30.63)	(32.08)
>40	32/464	4/464	18/464	24/422	147/463	133/435
	(6.90)	(0.86)	(3.88)	(5.69)	(31.75)	(30.57)
Total	141/2,401	19/1,865	75/2,401	64/2,180	484/2,399	510/2,280
	(5.87)	(1.02)	(3.12)	(2.94)	(20.18)	(22.37)

Figures in parentheses are percentages; [a]Number of sera positive/Number of sera tested.

on logistics and weather conditions.

Prevalence of HI antibodies

Table 3 shows the distribution of HI antibodies against all the six viruses tested. The highest prevalence was observed for KFD (22.4%) and Langat (20.2%), while the prevalence was much lower for JE (5.9%), DEN-2 (3.1%), CHIK (2.9%) and WN (0.8%). Except for WN virus, the difference between the area-specific serological prevalence was statistically significant for all the viruses.

Age-specific prevalence increased with age for all the viruses except WN (Table 4). The prevalence of JE, DEN-2, CHIK and Langat antibodies was highest in ≥ 40 year age group. For WN, prevalence was highest in the 20-29 year group, while for KFD prevalence was highest in the 30-39 year group. The difference between age-specific prevalence was statistically significant for CHIK, Langat and KFD; it was not significant for JE and DEN-2.

Except for DEN-2, the sex-specific prevalence was higher in males for all the viruses tested. The M: F ratio of HI positivity ranged from 1:0.26 for WN to 1:1.14 for DEN-2 (data not shown).

Geometric mean HI titers (GMT)

The area-specific GMT did not differ

Table 5
Multivariate analysis of HI antibody prevalence.

Location	JE	DEN-2	CHIK	KFD	TP-21
Andaman group	1	1	1	1	1
Nicobar group	[a]1.71(<0.01)	0.24(<0.01)	*****	1.87(<0.01)	2.21(<0.01)
	[b](1.18-2.49)	(0.1-0.6)		(1.47-2.39)	(1.76-2.78)
Age group (years)					
0-29	1	1	1	1	1
30+	1.10(>0.05)	1.59(>0.05)	2.96(<0.01)	1.87(<0.01)	2.34(<0.01)
	(0.76-1.57)	(0.99-2.55)	(1.77-4.92)	(1.52-2.31)	(1.89-2.90)
Sex					
Female	1	1	1	1	1
Male	1.11(>0.05)	0.7(>0.05)	1.36(>0.05)	1.85(<0.01)	2.03(<0.01)
	(0.78-1.58)	(0.44-1.11)	(0.80-2.31)	(1.49-2.30)	(1.61-2.55)

[a]OR (p-value); [b]Confidence interval

significantly for the viruses except for CHIK. The GMT was highest for JE, WN, and KFD in Little Andaman; it was highest for TP-21 and CHIK in Middle Andaman, whereas for DEN-2 it was highest in Great Nicobar. Age-specific GMT also did not appear to differ significantly for all the viruses except for CHIK. Sex-specific GMT was higher for males in all the viruses except for WN.

Cross-reactions between the virus antigens

In the HI test, a large number of sera showed cross- reactions amongst the six viral antigens. 22.9% of the sera were positive against only one viral antigen, 20.6% were positive against two antigens, 11.8% against three, 12.8% against four and 30.2% against five antigens. The remaining 1.7% sera reacted with all the six viral antigens used in the test.

Multivariate analysis

Multivariate analysis (Table 5) carried out using stepwise regression analysis to examine the independent association of geographic location, age and sex on the seroprevalence of HI antibodies against these viruses, showed that all these three factors had a significant association with the seroprevalence for KFD and Langat. For JE and DEN-2, geographic location was the most important factor. The Andaman group (OR 1, referent) was more likely to have DEN-2 HI antibodies compared with the Nicobar group (OR 0.24, CI 0.1-0.6). For JE, KFD and TP-21, the Nicobar group was more likely to have HI antibodies (Table 5). Sex influenced the seroprevalence of CHIK, KFD and Langat in a significant way. WN was not subjected to analysis because the seroprevalence was very low.

Prevalence of N antibodies

N antibodies against JE, WN, and DEN-2 were detected in all the areas in varying percentages (Table 6). These antibodies were not detected on the Nicobar Islands for JE and on the South Andaman for WN. The highest prevalence for JE was detected in South Andaman while that for DEN-2 was detected on Nicobar Islands and Middle Andaman. N antibodies to CHIK, Langat and KFD were detected on Middle Andaman. For the other islands, the number of sera tested for N antibodies from each area was not sufficient to draw any conclusions regarding the geographic distribution of CHIK, KFD and Langat.

N antibodies against JE, WN and DEN-2 were detected in all age groups (Table 7). For JE and WN, titers were at their highest in ≥40 year age group, while for DEN-2 they were at their highest in the 0-9 year age group.

Table 6
Area-specific prevalence of N antibodies.

Locality	JE	WN	DEN-2	CHIK	TP-21	KFD
North Andaman	8/169[a]	1/179	17/99	3/18	0/3	1/10
	(4.73)	(0.56)	(17.17)	(16.67)	(0.0)	(10.00)
Middle Andaman	9/410	8/411	98/314	5/37	1/13	1/17
	(2.20)	(1.95)	(31.21)	(13.51)	(7.69)	(5.88)
Little Andaman	1/122	3/124	3/66	0/3	6/21	9/47
	(0.82)	(2.42)	(4.55)	(0.0)	(28.57)	(19.15)
South Andaman	3/40	0/31	4/22	0/0	0/0	0/0
	(7.50)	(0.00)	(18.18)	(0.0)	(0.0)	(0.0)
Great Nicobar	1/137	3/139	16/78	0/0	0/2	0/17
	(0.73)	(2.16)	(20.51)	(0.0)	(0.0)	(0.0)
Nicobar Islands	0/171	4/146	44/138	1/1	0/1	0/1
	(0.00)	(2.74)	(31.88)	(100.0)	(0.0)	(0.0)
Total	22/1,049	19/1,030	182/717	9/59	7/40	11/92
	(2.10)	(1.84)	(25.38)	(15.25)	(17.50)	(11.96)

Figures in parentheses are percentages; [a]Number of sera positive/Number of sera tested.

Table 7
Age-specific prevalence of N antibodies.

Age group (years)	JE	WN	DEN-2	CHIK	TP-21	KFD
0-9	1/48[a]	1/51	10/21	0/7	0/2	1/8
	(2.08)	(1.96)	(47.62)	(0.0)	(0.0)	(12.50)
10-19	3/309	6/305	43/179	0/8	3/15	3/42
	(0.97)	(1.97)	(24.02)	(0.0)	(20.00)	(7.14)
20-29	4/236	2/231	48/179	1/11	2/9	1/16
	(1.69)	(0.87)	(26.82)	(9.09)	(22.22)	(6.25)
30-39	3/184	4/182	33/132	0/13	2/6	3/12
	(1.63)	(2.20)	(25.00)	(0.0)	(33.33)	(25.00)
≥40	11/272	6/261	48/206	8/20	0/8	3/14
	(4.04)	(2.30)	(23.30)	(40.00)	(0.0)	(21.43)
Total	22/1,049	19/1,030	182/717	9/59	7/40	11/92
	(2.10)	(1.84)	(25.38)	(15.25)	(17.50)	(11.96)

Figures in parentheses are percentages; [a]Number of sera positive/Number of sera tested.

Sex-specific prevalence of N antibodies was significantly higher in males for CHIK, Langat and KFD viruses. It did not differ significantly for JE, WN and DEN-2. The M: F ratio ranged from 1:0.28 for CHIK to 1:0.73 for WN (data not shown).

DISCUSSION

This serological survey indicated that all the six arthropod-borne viruses studied were prevalent in the Andaman and Nicobar groups of islands, although the prevalence differed

according to age, sex, geographic location and the individual virus. The geographic location had a significant influence on the prevalence of antibodies to all the viruses. This might be explained by the possible impact of ecological characteristics of the areas on the natural cycles of the arthropod-borne viruses under consideration. While age and sex were not important in the context of JE and DEN-2, these factors influenced KFD, Langat and CHIK prevalence. While it can be said that people of all ages and sex are exposed to JE and DEN-2 viruses, even though the prevalence may vary, only people at high-risk are exposed to KFD, Langat and CHIK viruses. This is consistent with the known epidemiology of these viruses; further studies to identify the at-risk groups and the risk activities for exposure to the viruses are required.

CHIK antibodies were found to be absent in the sera collected from Great Nicobar and South Andaman. KFD and Langat virus antibodies were found to be highly prevalent in the people of Little Andaman, Great Nicobar and Nicobar Islands. On the mainland, KFD antibodies have been reported only amongst the human population of Karnataka and Saurashtra (Banerjee, 1988). Considering the present findings, the Andaman and Nicobar islands might be a major silent focus of KFD virus activity, however there is no corroborative data from case reports of KFD or the tick and monkey populations of these islands. Deforestation and urbanization, factors similar to those encountered in Karnataka, might have played a role in establishing KFD virus on these islands. The presence of KFD virus and its vectors on these islands needs to be confirmed urgently. The higher prevalence of Langat (TP-21) virus could perhaps be explained by its antigenic relationship with KFD virus (Joshi *et al*,1991). JE virus activity was also recorded on these islands, though at a low level. Great Nicobar and South Andaman had the highest JE prevalence. The results of an earlier serological survey of the domestic animals of these islands (Joshi *et al*, 1991) are consistent with the results of the present serological survey. Entomological aspects of the life cycle of these viruses need to be studied before conclusions are reached regarding the presence or absence of the diseases in these islands.

The variation in seroprevalence by age group was in keeping with the known epidemiology encountered on the mainland. Males showed a higher prevalence of all the six viruses, implying greater exposure. The GMTs did not vary much by geographic area, indicating a similar response by people from all the localities.

The overall percentage prevalence of N antibodies against DEN-2 (25.4%) was higher than that of HI antibodies (3.1%). The prevalence of N antibodies against JE was less than that of HI antibodies. The presence of N antibodies in a serum indicates definite exposure of the individual to the virus and is a reliable indicator of the presence of virus in the area. N antibodies last longer than the HI antibodies, therefore in a serological survey N antibodies might have a higher prevalence than HI antibodies (Casals, 1973).

The higher prevalence of N antibodies to DEN-2 compared with the HI antibody prevalence in the majority of the areas might be due to the neutralizing response of specific viruses. It has also been shown that in areas where several viruses of a group are active there may be a much larger discrepancy between the results of the N and HI tests (Casals, 1973). The N test is more reliable than the HI test when testing sera from localities where many flaviviruses coexist (Oda *et al*, 1996).

The islands differ from one another in terms of the tribal inhabitations, living condition, farming practices, ecology, and exposure to the outside world. The frequency of contact of aboriginal people with mainland India (via tourists or migrant people), rapid urbanization in a flourishing culture, rapidly expanding tourism industry, unexplored forest bearing silent foci that are activated by human contact, and changes in the animal population and vector distribution are some of the factors that might be responsible for the prevalence of a particular virus in these islands, which offer port facilities to ships in the region. These ships

might introduce new viral infections to these islands and, at the same time, carry local viruses to the outside world.

The lack of entomological data with reference to the viruses studied makes it difficult to explain the natural cycle of these viral infections in the Andaman and Nicobar islands. In-depth studies that correlate human, animal, entomological and ecological data over a period of time could unravel the endemicity of the arboviruses and their natural cycles on these Islands.

This is the largest serological survey ever carried out in the study area and it required a great deal of time and resources. The HI and neutralization tests for the six viruses required years of laboratory work. Long periods of observation (21 days) were needed for the neutralization test. Though the ecology and other factors might have changed slightly in subsequent years, this study remains an important key to an understanding of the interplay of the various factors in the transmission dynamics of arboviral diseases in the region. The questions raised by this study will, hopefully, stimulate further studies that will advance our knowledge of these diseases.

ACKNOWLEDGEMENTS

This project was funded by the Indian Council of Medical Research. The authors would like to thank Dr K Banerjee, the former Director of the NIV, for all his help and encouragement during this study. The authors are enormously grateful to Surg Cdr JS Nagra, the former Director of the Directorate of Health Services, Andaman and Nicobar Administration, Port Blair, and all his colleagues for the kind help and cooperation that was extended to our teams during the fieldwork phases. Laboratory facilities at the Regional Medical Research Center, Indian Council of Medical Research, Port Blair, were generously made available to us and we are thankful for this. The kind assistance rendered by Mrs V Venkatesh, and Mr Atul Walimbe in the compilation and statistical analysis of the data is gratefully acknowledged.

REFERENCES

Banerjee K, Desai PK. Survey of arbovirus antibodies in South India. *Indian J Med Res* 1973; 61: 344-51.

Banerjee K. Kyasanur Forest disease. In: Monath TP, ed. Arthropod borne viruses, Vol III. Boca Raton, USA: CRC Press 1988; 93-116.

Casals J. Arboviral infections. In: Paul JR, White C, eds. Serological epidemiology. New York: Academic Press, 1973: 102- 18.

Clarke DH, Casals J. Technique for hemagglutination and hemagglutination inhibition with arthropod borne viruses. *Am J Trop Med Hyg* 1958; 7: 561-73.

Joshi MV, Rodrigues FM, Umarani UB, *et al*. Prevalence of antibodies to certain flaviviruses in domestic animals of Andaman and Nicobar Islands. *Indian J Virol* 1991; 7: 133-7.

Oda K, Igarshi A, Kheong CT, *et al*. Cross-sectional serosurvey for Japanese encephalitis specific antibody from animal sera in Malaysia 1993. *Southeast Asian J Trop Med Public Health* 1996; 27: 463-70.

Padbidri VS, Risbud AR, Mehendale SM, *et al*. An outbreak of fever with haemorrhagic manifestations in Andaman islands. *Trop Biomed* 1995; 12: 123-7.

Sever JL. Application of a microtechnique to viral serological investigation. *J Immunol* 1962; 88: 320-9.