# LEPTOSPIROSIS

**Evaluation of indigenously developed leptospira latex agglutination test for diagnosis of Leptospirosis**

A latex agglutination assay was developed for the detection of antibodies to leptospires using a mixture of proteins with molecular mass ranging from 28 kDa to 58 kDa (fig 1.1). The test was evaluated internally using serum samples from the serum bank as well as in field in South Andaman. 236 patients with suspected leptospirosis, 99 patients having diseases other than leptospirosis and 37 healthy individuals were included in the evaluation. Patients with other diseases included 42 typhoid cases, 30 hepatitis B cases, 23 malaria cases and 4 cases with dengue fever.

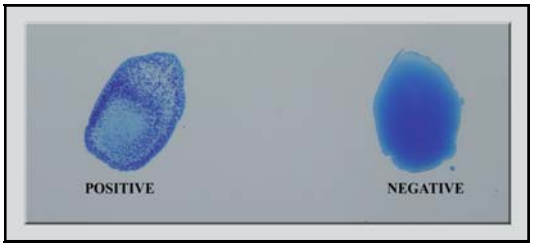



*Fig. 1.1. Positive and negative agglutination patterns in indigenously developed Latex Agglutination Test*

Out of 236 patients, isolation of leptospires was successful in 16 patients, in the remaining 220 patients, convalescent serum sample could be collected from 147 patients. Out of 147 patients who had paired sera the disease was confirmed in 82 as they had seroconversion or a four-fold rise in titre and the disease was ruled out in 66. Thus a total of 98 patients were confirmed as having leptospirosis and the disease was ruled out in 66 patients. During the first week of illness, the test had a sensitivity of 73.5%, specificity of 81.8%, positive predictive value of 85.7% and a negative predictive value of 67.5%. The overall agreement between the test results and the standard diagnostic criteria was 76.8% and the κ value of agreement was 0.5023 indicating reasonable agreement (table 1.1). The sensitivity, specificity, PPV and NPV during the second week were 89.8%. 78.8%, 86.3% and 83.9% respectively. The overall agreement with the standard diagnostic criteria and κ value of agreement were better at 85.4% and 0.693 respectively (table 1.2). The test gave positive results for 11%, 10% and 7% against sera collected from patients having enteric fever, hepatitis B and malaria. The latex particles stored at 40C and at RT ($28^oC$-$32^0C$) were found to be stable up to 1 y but lost their stability when stored at $37^o$ C for more than thee months.

*Table 1.1. Sensitivity, specificity and predictive values of latex agglutination test during the first week of illness*

| | | Std. Criteria (MAT & Isolation) | | Total |
|---|---|---|---|---|
| **Dipstick** | | **Positive** | **Negative** | |
| | **Positive** | 72 | 12 | 84 |
| | **Negative** | 26 | 54 | 80 |
| | **Total** | 98 | 66 | 164 |
| **Index** | | | **Value** | **95% CI** |
| Sensitivity | | | 73.5 | 63.4, 81.6 |
| Specificity | | | 81.8 | 70.0, 89.9 |
| Predictive Value Positive | | | 85.7 | 76.0, 92.1 |
| Predictive Value Negative | | | 67.5 | 56.0, 77.3 |
| Percent Agreement | | | 76.8 | |
| Kappa | | | 0.5023 | |

*Table 1.2. Sensitivity, specificity and predictive values of latex agglutination test during 2—4 weeks of illness*

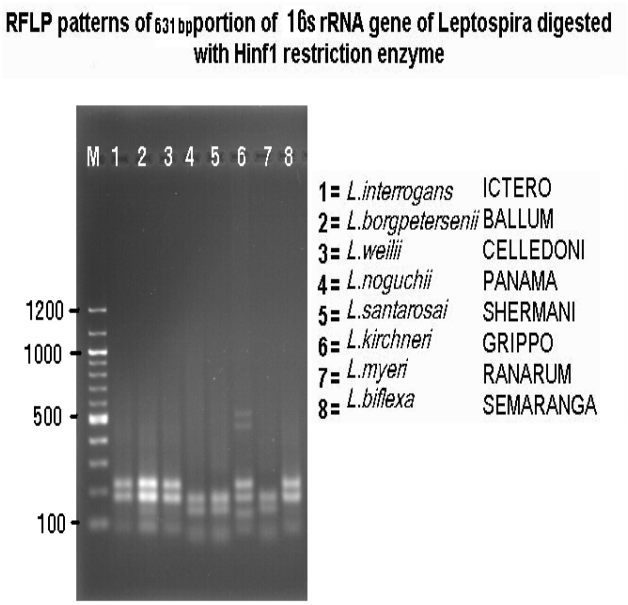
| | | Std. Criteria (MAT & Isolation) | | Total |
|---|---|---|---|---|
| **Dipstick** | | **Positive** | **Negative** | |
| | **Positive** | 88 | 14 | 102 |
| | **Negative** | 10 | 52 | 62 |
| | **Total** | 98 | 66 | 164 |
| **Index** | | | **Value** | **95% CI** |
| Sensitivity | | | 89.8 | 81.6, 94.7 |
| Specificity | | | 78.8 | 66.7, 87.5 |
| Predictive Value Positive | | | 86.3 | 77.7, 92.0 |
| Predictive Value Negative | | | 83.9 | 71.9, 91.6 |
| Percent Agreement | | | 85.4% | |
| Kappa | | | 0.693 | |

**Leptospiral carrier rate among animals slaughtered at Port Blair**

Animals brought to slaughter houses were screened for leptospiral carrier state. Kidney samples and blood samples were collected from cattle and goats slaughtered at an abattoir near Port Blair. Kidney samples were inoculated into EMJH medium. Serum was separated from blood samples and screened for anti-leptospiral antibodies by microscopic agglutination test (MAT). Till date kidney samples from 157 cattle and 109 goats have been obtained. Blood samples were collected from 135 cattle and 90 goats and were tested for anti-leptospiral antibodies using MAT. A total of 4 isolates of leptospires were obtained from kidney samples of cattle. Two of the isolates were identified as belonging to serogroup Icterohaemorrhagiae. Seropositivity was 27% in cattle 21% in goats.

**Analysis of *OmpL1* and *LipL41* genes among *Leptospira interrogans* serovars and development of systems for the expression of recombinant *OmpL1* and *LipL41* of *L. Autumnalis***

Available serodiagnostic methods for detection of leptospirosis use crude antigens from a single serovar. LPS, the major antigenic component, is serovar specific and hence may not cross react with patients infected with other serovars. There is a need to employ purified proteins from commonly circulating serovars as antigens in diagnostics. In an effort to develop diagnostic test with recombinant fusion proteins for rapid diagnosis, a project to clone the genes of leptospiral proteins OmpL1 and LipL41 in an expression system was undertaken. These proteins have been found to be immunogenic and specific to pathogenic leptospires.

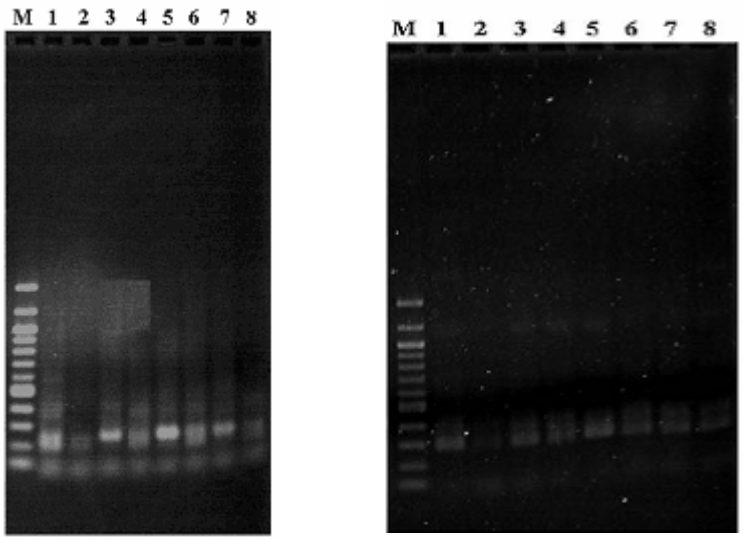



***Polymorphisms in 631-bp segment of 16 S rRNA gene:***

| Sl.No | Genospecies | Hinf1 |
|---|---|---|
| 1 | ***L. interrogans*** | A |
| 2 | *L.borgpetersenii* | A |
| 3 | *L.welii* | A |
| 4 | *L. noguchii* | B |
| 5 | *L.santarosai* | B |
| 6 | *L. kirschneri* | C |
| 7 | *L.meyeri* | B |
| 8 | *L.biflexa* | A |

Recognition of OmpL1 and LipL41 by patients' sera was analysed by immunoblotting. Specificity of these antigens was analysed by immunoblotting with control sera. OmpL1 and LipL41 genes from leptospiral serovars Autumnalis and Grippotyphosa were isolated by using specific primers and the genes were compared with other leptospiral serovars like Australis, Icterohaemorrhagiae, Canicola, Pomona, Pyrogenes, Sejore and Tarassovi. The leptospiral isolates of the corresponding reference strains were also compared in parallel. Isolates utilized belonged to serovars Autumnalis, Australis, Canicola,

Icterohaemorrhagiae, Grippotyphosa, Pomona, Pyrogenes, Javanica and Sejroe. Genomic DNA of the leptospiral isolates was extracted and purified. PCR was carried out using specific primers for OMPL1 and LipL41. Sequencing of OmpL1 and LipL41 genes of the leptospiral isolates N2 (Autumnalis) and CH31 (Grippotyphosa) was performed using Applied Biosystems automated sequencer. The sequenced results of OmpL1 and LipL41 was BLAST with the leptospiral whole genome sequence for comparison. The 1077 bp NcoI-XhoI fragment of the amplified LipL41 gene was ligated to pET-15b digested with NcoI and XhoI. The resulting construct pET-15b-LipL41 was transformed in to *E.coli* JM 109 (DE3).




23S small region digested with Hinf1 (A) and EcoR1 (B)

In confirmed cases the IgG recognition of LipL41 and OmpL1 in acute phase sera was 39.5% and 30.2% respectively. This increased to 65.1% and 55.8% in convalescent phase. Among other controls, which includes normal healthy individuals, and patients with other diseases like hepatitis, typhoid and malaria IgG recognition ranged between 0% and 10%. The PCR based amplified fragments of LipL41and OmpL1 were analyzed by using 1.5% agarose gel electrophoresis and a 1065 bp product of LipL41 and a 960bp product of OmpL1 were found for all the reference strains and for the isolates except for the nonpathogenic strain Patoc I. It reveals that the genes are conserved among the pathogenic leptospires. BLAST analysis of the sequenced OmpL1 and LipL41 of the leptospiral strains N2 (Autumnalis) and CH31 (Grippotyphosa) gave a high score value with whole genome of leptospira interrogans serovar Lai further supported the identity and conservation. The construct pET-15b-LipL41 was transformed in to E.coli JM 109

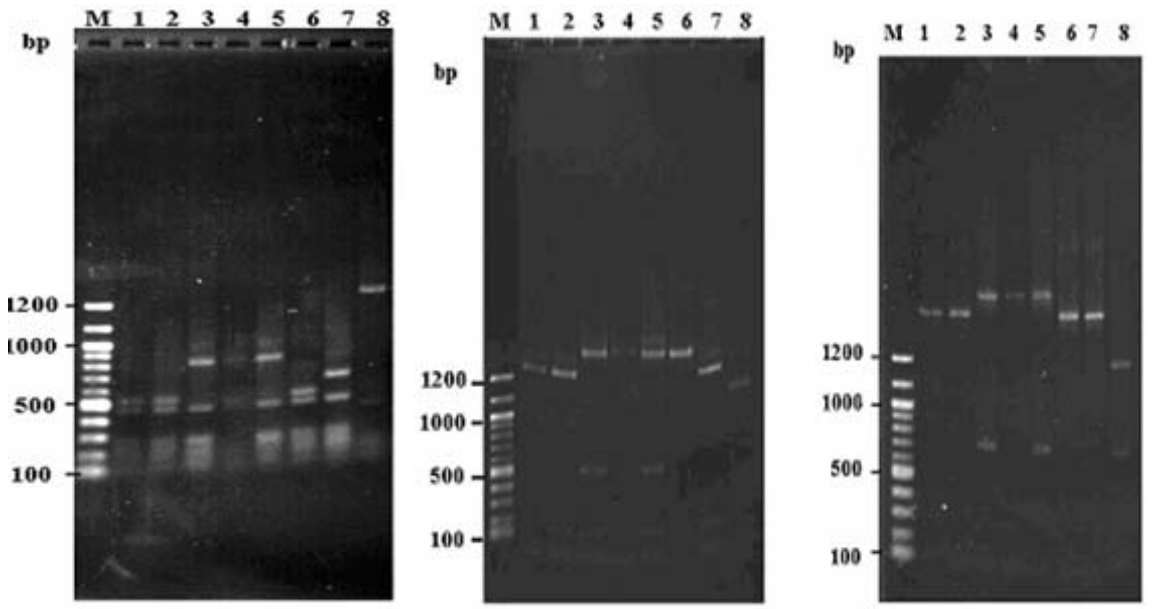
(DE3). The clones were confirmed with appropriate restriction enzyme analysis to recover the cloned fragment. Further the identity of the clone was confirmed based on PCR amplification. The project is in progress.

**Molecular characterization of leptospiral isolates using restriction fragment length polymorphism (RFLP) technique.**

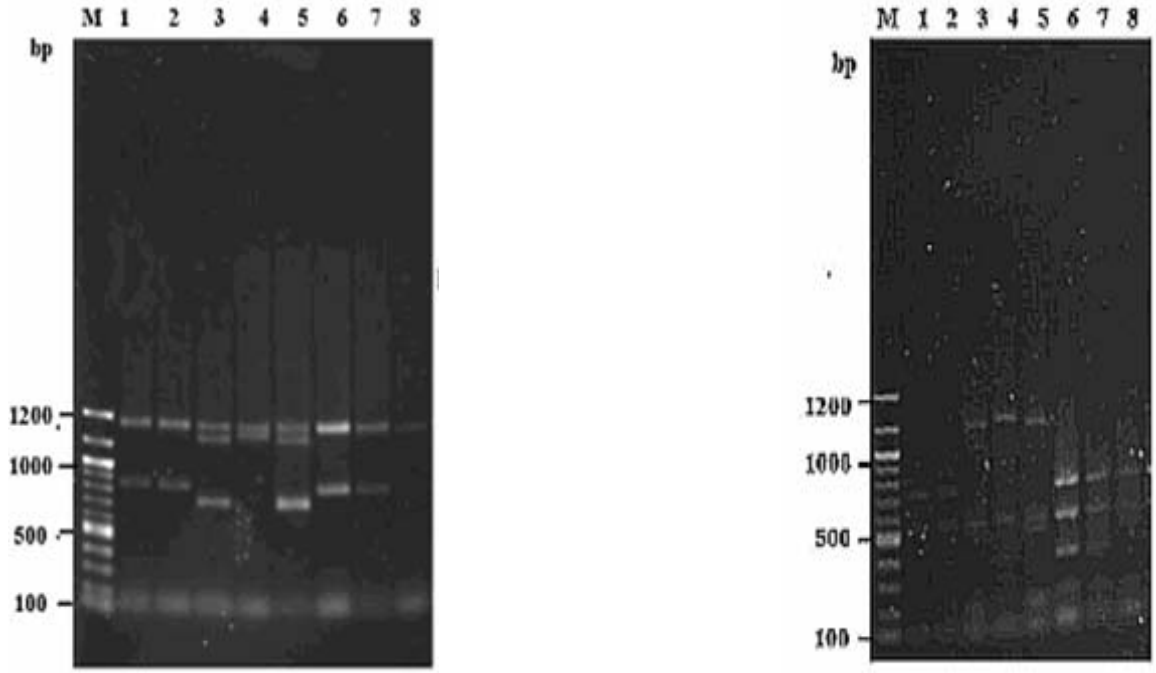
Leptospiral reference strains and clinical isolates were characterized using PCR based RFLP in the 23s and 16s rRNA regions. Attempts were made to find these polymorphisms through the selective amplification and subsequent restriction digestion of these polymorphic regions. Fifteen reference strains of *Leptospira* representing 8 different genospecies were employed for characterization. Specific primers were used to amplify a 2300 bp and a 367 bp part of 23s rRNA and a 631 bp region of 16s rRNA . The amplified fragments were digested using 11 different restriction enzymes for studying the polymorphisms. Several types of restriction patterns were obtained with the PCR-amplified regions of the ribosomal genes, indicating the presence of RFLPs within these regions (fig. , table).

The 631 bp amplified product of 16 s rRNA region showed only 3 types of polymorphism with Hin f1 restriction enzyme among the 8 genospecies studied. Among these *L. interrogans, L. borgpetersenii, L.welii* and *L.biflexa* showed similar type of RFLP pattern and cannot be distinguished from each other, where as *L. noguchii* and *L. meyeri* showed another type of pattern. However, *L. kirschneri* showed a unique pattern that did not match with the other genospecies and could be easily differentiated with this enzyme. However, with other enzymes, no further discrimination was found within this 631bp region.

The polymorphisms in the 367 bp segment of the 23 s rRNA region were also studied. PCR-RFLP analyses with the enzymes *Hin f1* and *Eco R1* showed two types polymorphisms in the 367 bp segment of the 23 s rRNA region. *L interrogans, L. borgopetersenii, L. noguchii , L kirschneri* and *L. biflexa* showed similar type of pattern forming one group which cannot be differentiated from each other (Fig) while *L. welii, L. santarosai* and *L. myeri* formed the other (Table2).




A B C



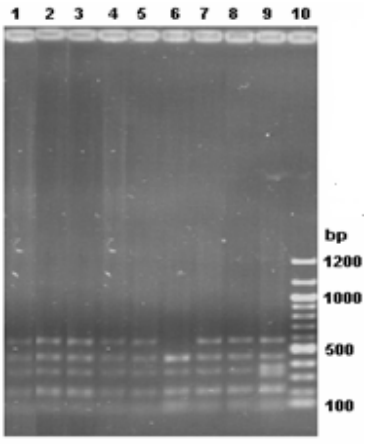

D E

2300 bp 23SrRNA digested with Hinf1 (A), Hind III (B), Pst I (C), Sma I and Hpa I

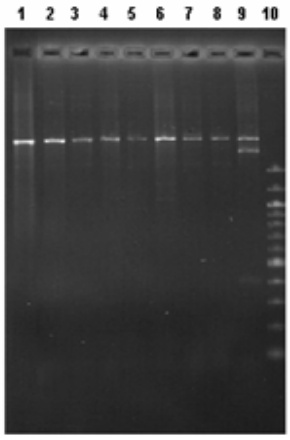
1-L. interrogans (Australis, Ballico), 2-L. borgpeterseni (Mini, Sari), 3-L. welii (Manhao, L60), 4-L. naguchi (Lousiana, LSU1945), 5-L. santarosai (Canalzonae, CZ188), 6-L. krischneri (Cynopteri, Cynopteri), 7-L. myeri (Ranarum, ICF), 8-L. boflexa (Andamana, CH11)

| Sl | Genospecies | Hinf1 | Hind III | Pst I | Sma I | EcoR1 | Hpa I |
|---|---|---|---|---|---|---|---|

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 1 | L. interrogans | A | A | A | A | A | A |
| 2 | *L.borgpetersenii* | A | A | A | A | A | A |
| 3 | *L.welii* | B | B | B | B | B | B |
| 4 | *L. noguchii* | C | C | C | C | B | B |
| 5 | *L.santarosai* | B | B | B | B | B | C |
| 6 | *L. kirschneri* | A | C | A | A | C | D |
| 7 | *L.meyeri* | D | A | A | A | C | D |
| 8 | *L.biflexa* | E | D | D | A | D | -- |




A




B

1. Ballico
2. Rachmat
3. Swart
4. Canicola
5. Hebdomedis
6. RGA
7. Pomona
8. Salinem
9. Hardjoprajitno
10. Molecular marker

RFLP pattern of 23 rRNA (2300 bp) of reference strains of different serogroups of L. interrogans sensu stricto digested with Mse I (A) and EcoR1 (B)

Different restriction sites (*Hin f1, Hind III, Pst I, Sma I, Eco R1 and Hpa 1*) were looked for so far in the PCR amplified 2300 bp region of the 23 s rRNA for 8 genospecies. A number of polymorphisms were detected in this segment (Table3). *Hin f1* digested pattern of 2300bp segment of 23S r RNA gene showed one similar pattern for *L. interrogans, L. borgpetersenii* and *L. kirschneri* while *L. welii* and *L. sanarosai* showed a second pattern. However, as these genospecies showed the third, fourth and fifth types of patterns and these three were unique for these genospecies. *Hind III* digest of 2300bp showed four types of banding patterns and did not differentiate between any of the genospecies except *L. biflexa*. *Pst1* digest of 2300 bp could differentiate *L. noguchii* and *L. biflexa* among the eight genospecies studied. *Sma 1* could differentiate *L. noguchii*

and *Eco R1* could differentiate *L. biflexa* (Fig 8). However, *Hpa I* digest of 2300 bp product did not differentiate between any of the genospecies so far studied.

**Investigation of polymorphisms within a particular genospecies (L. interrogans)**

To find out the polymorphisms among the different strains (serogroups/serovars) belonging to the same genospecies, we looked for RFLP patterns of 2300 bp region of 23s rRNA within 9 different strains (serogroups/serovars) of *Leptospira* belonging to genospecies *L. interrogans* that is the commonest genospecies worldwide.

In our study we employed the enzymes Mse I, Eco RI, Hin fI, Hind III and Hae III to look for RFLPs in the 2300 bp region of the 23 s rRNA. Among the 9 *L. interrogans* strains used in the study, the strain Hardjoprajitno showed unique banding patterns with all the five enzymes used. Also, the strain RGA showed some differences with other *L. interrogans* strains in banding pattern with the enzyme *Mse 1* while the strain Ballico showed some difference with the enzyme *Hae III*. The results of RFLP investigations in these strains are summarized in table 5. In the spacer region of the 9 different strains of *L. interrogans* digested with the enzyme Eco R1, certain polymorphisms were noted and this could be used in further differentiating leptospires at the intra-specific level.

2300 bp sequence of 23s rRNA region and RFLPs were looked into. Results obtained so far showed that 12 of the 15 isolates of *Leptospira* showed identical *Hin f1*- RFLP patterns identical with reference strains of *L. interrogans*. The banding pattern of the other three strains could not be matched with those of the 8 reference strains used in the study. Other enzymes (Dde1, EcoR1, Hind III, Pst1, Mse1, BamH1 and Hae III) also indicate that these 12 strains share the same RFLP pattern while the other three differ from these 12 strains.

**Clinical, bacteriological, serological and epidemiological follow-up of leptospirosis at PHC, Manglutan.**

A study was undertaken to gain insights into the clinical spectrum, the clinical course, prognosis and epidemiological factors associated with leptospirosis and to study the level and duration of leptospiral antibodies (IgM & microscopic agglutinating) in whom the isolation of leptospira was successful. Patients reporting to the Manglutan PHC who fulfilled the criteria for suspicion of leptospirosis were included in the study. Clinical

information was collected from patients and acute serum samples were collected on the day reporting to the hospital and convalescent samples were collected 7 to 10 days after the first. Isolation was attempted on all the patients. During the present study period 55 patients were suspected and 14 were confirmed as leptospirosis patients. Isolation of *Leptospira* was successful in 6 patients, Common clinical fetures included fever, body ache, headache, cough and vomiting. Out of 6 isolates, 3 belonged to Icterohaemorrhagiae, two to Australis and one to Grippotyphosa.

**Immune response to leptospiral infection – a follow up study of four years duration in an endemic area**

A follow up study is being conducted based at a primary health centre in South Andaman. Culture positive cases of leptospirosis detected at the PHC are being followed up to study their immune response. Patients were followed up for a period of 4 years. Blood samples were collected during the 1st week, 4th week and after 3 m, 6 m, 9 m, 1 y, 2 y, 3 y and 4 y. IgM ELISA and microscopic agglutination test (MAT) were done on the samples. The isolate from the patient and representative strains of the infecting serogroup and other serogroups were used as antigens in MAT. Geometric mean titre and seropositivity (%) were calculated for IgM antibodies and microscopic agglutinating antibodies against the infecting strain, representative strain of the infecting serogroup and heterologous serogroups. In order to understand the type of antigens responsible for persistent antibody response, individual Immunoblots were carried out using culture positive patients' sera collected at different intervals against whole cell lysate of isolates from the respective patient

During the first week 50% of patients gave positive results in IgM ELISA. However IgM positivity reached to 100% during 2nd – 4th week of illness. seropositivity declined to 50% by 3 months and all patients were seronegative at 6 months. Microscopic agglutinating antibodies against heterologous serogroups were present in 30% of patients during the first week. Seropositivity became 75% by $4^{th}$ week and remained same at 3 months. At the end of $2^{nd}$ year seropositivity was less than 10%. During the first week none of the patients were seropositive by MAT against representative strain of infecting serogroups . By 4th week more than 90% were seropositive and at 3 months 100% were seropositive. Seropositivity declined to 57% by the end of one year and to 25% at two years and remained at that level. During first week about 15% were seropositive by MAT against circulating strain of infecting serogroups. By $4^{th}$ week all were seropositive. At one year, 78% were still seropositive and at four years 40% were still seropositive. GM

titre peaked to around 1 in 640 at 4 weeks and declined. During first week about 25% were seropositive by MAT against infecting strain. By 4th week all were seropositive. At one year, 93% were still seropositive and at four years 50% were still seropositive.GM Titre peaked to around 1 in 1000 at 4 weeks and declined. Individual Immunoblotts carried out using patient's sera collected at different intervals against whole cells lysates isolates from the respective patients detected several proteins. Proteins P41/42, p37, p32 and p14 were the predominantly and persistently detected up to 4 years

Since IgM sero-positivity observed up 50% among the patients even beyond three months of illness, calibration of diagnostic titere for IgM ELISA is needed. Although MAT is still the corner stone of serological diagnosis of leptospitosis, if the local circulating strains are not included in the panel of antigens, the test may fail to detect antibodies in large proportion of patients. Several proteins were found to be evoke long lasting immune response. However further studies are needed to find out the protective nature of these proteins.

**Application of Polymerase Chain Reaction (PCR) as an early diagnostic tool for leptospirosis**

An attempt has been made to find the correlation between PCR, MAT, isolation and Lepto-Dipstick as diagnostic tools during the acute stage of the disease.

A total of 181 consecutive patients with suspected leptospirosis attending a PHC at South Andaman were included in the study. Acute and convalescent blood samples were collected from the suspects, serum separated and stored in aliquots at -20$^{o}$ C prior to use. MAT was performed on all the serum samples following standard procedure using 12 live leptospiral strains (1 – 2 x $10^{8}$ organism/ml) as antigens (Table 1). MAT was performed on all serum samples at dilution 1 in 40, 1 in 80, 1 in 160, and 1 in 320. Those found positive at 1 in 320 were titrated up to end titres.

Lepto-Dipstick test was performed on all the serum samples following standard procedure. Isolation of the organism from all the serum samples was performed as standard method in EMJH media. Isolation of leptospires from blood, seroconversion in MAT with a titre of at least 1 in 160 or four-fold rise in titre in MAT was considered as definite evidence of leptospiral infection. DNA was extracted from the acute serum samples and used for amplification in PCR. Multiplex PCR was performed for the diagnosis of leptospiral infection using two sets of *Leptospira*-specific primer pairs G1 & G2 and B64I & B64 II, in all 181samples.

In multiplex PCR using two sets of primer pairs G1 & G2 and B64I & B64II we got a 285 bp segment of DNA amplified by G1 &G2 primers from all 57 patients except two. These two patient sera showed the presence of 563 bp segment of DNA amplified by B64I & B64II primers (Fig 1). However, all the 17 isolates were later identified as members of the genospecies *L. interrogans* and so far no strain belonging to *L. kirschneri* was isolated.

Table. PCR Vs Standard diagnostic criteria (four fold rise in titre of seroconversion in MAT and/or isolation of leptospires from blood samples)

| | | **Std. Criteria (MAT)** | | **Total** |
|---|---|---|---|---|
| **PCR** | | **Positive** | **Negative** | |
| | **Positive** | 45 | 12 | 57 |
| | **Negative** | 3 | 121 | 124 |
| | **Total** | 48 | 133 | 181 |
| **Index** | | | **Value** | **95% CI** |
| Sensitivity | | | 93.8 | 81.8, 98.4 |
| Specificity | | | 91.0 | 84.4, 95.0 |
| Predictive Value Positive | | | 78.9 | 65.8, 88.2 |
| Predictive Value Negative | | | 97.6 | 92.6, 99.4 |
| Percent Agreement | | | 91.7 | |
| Kappa | | | 0.799 | |

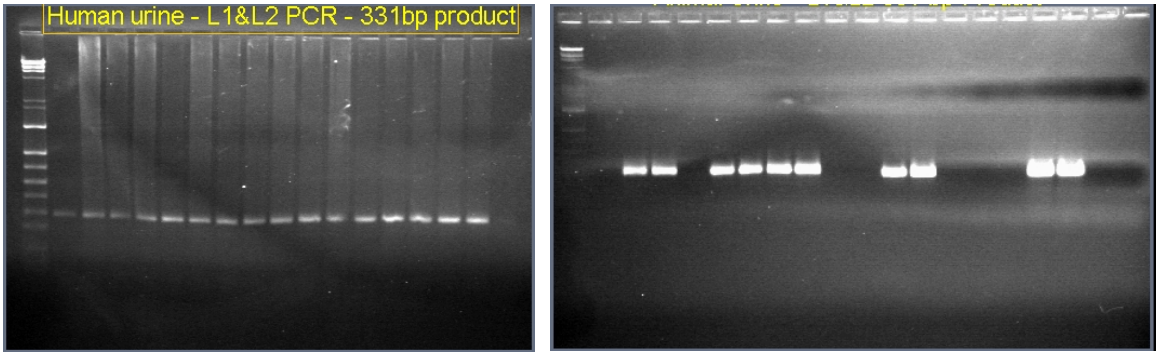
Table. Dipstick Vs Standard diagnostic criteria (four fold rise in titre of seroconversion in MAT and/or isolation of leptospires from blood samples)

| | | **Std. Criteria (MAT & Isolation)** | | **Total** |
|---|---|---|---|---|
| **Dipstick** | | **Positive** | **Negative** | |
| | **Positive** | 21 | 6 | 27 |
| | **Negative** | 27 | 127 | 154 |
| | **Total** | 48 | 133 | 181 |
| **Index** | | | **Value** | **95% CI** |

| | | |
|---|---|---|
| Sensitivity | 43.8 | 29.8, 58.7 |
| Specificity | 95.5 | 90.0, 98.2 |
| Predictive Value Positive | 77.8 | 57.3, 90.6 |
| Predictive Value Negative | 82.5 | 75.3, 87.9 |
| Percent Agreement | 81.7 | |
| Kappa | 0.456 | |

Out of the 181 suspected cases, 48 patients were diagnosed to have leptospiral infection based on the diagnostic criteria. PCR was positive in 57 patients. PCR and standard criteria was positive in 45 cases and in 121 patients both were negative. Thus PCR had a sensitivity of 93.8% and a specificity of 91%. The overall agreement between PCR and the standard criteria was 91.7% with a k statistic of 0.799 (Table 1).

Three patients had a confirmed diagnosis of leptospirosis as per the standard criteria but with negative PCR result whereas in 12 patients PCR was positive while the diagnosis of leptospirosis was ruled out by the diagnostic criteria. Out of the 3 patients who apparently had false negative results in PCR, isolation was positive in two.




PCR amplification of leptospiral gene fragment from patients sera

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Out of the 48 patients diagnosed to have leptospiral infection based on standard criteria, isolation of leptospires was positive in 17. Among these seventeen, three patients did not have any MAT titres in both the samples and in 12 of them Dipstick was negative. Thus the sensitivity of paired MAT was only 82% and that of Dipstick on acute samples was only 29.4%. If PCR is taken as the gold standard then the standard criteria had a sensitivity of 78.9% (45/57) and a specificity of 91.0% (121/133) (Table 1).

Out of the 48 patients diagnosed to have leptospirosis, dipstick was positive in 21 patients and out of the 133 patients in whom a diagnosis of leptospirosis was ruled out by the standard criteria, dipstick was negative in 127 giving a sensitivity of 43.8% and a specificity of 95.5%(Table 2).

In the present study, PCR showed high sensitivity and specificity compared to a standard criteria for diagnosis based on isolation and MAT on limited number of paired samples. Further study is going on using more number of samples to evaluate the performance of PCR in diagnosis of *Leptospira* infection.

**Polymerase chain reaction for detection of leptospires in urine samples of human and animal patients**

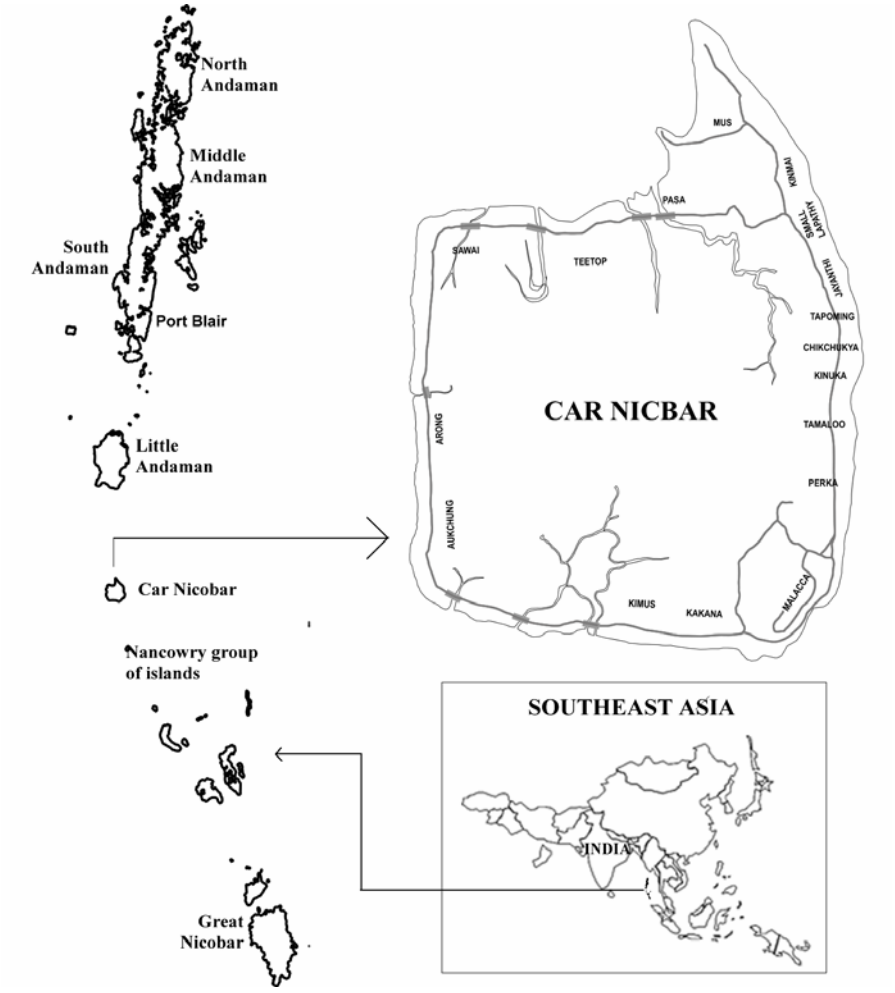
Methods currently used for the diagnosis of leptospirosis are based on serological response of the host to the infecting organisms or by culture and isolation but they are time consuming and laborious. Polymerase chain reaction, that amplifies specific target DNA from urine sample of human and animal patients of leptospirosis was standardized and attempt made to correlate urine PCR with MAT and isolation, taken as gold standard. A total of 27 paired sera and urine samples from human patients and 30 single samples suspected of leptospirosis at the nearest PHC were collected. 37 sera samples and urine samples from cattle suspected of leptospirosis were also collected from the veterinary hospital, Port Blair. PCR was done in all the urine samples and MAT was done in all the paired sera samples. PCR was positive in 8 of the paired sample collected and MAT was positive in 4 the paired samples either by seroconversion or four fold rise. There were 3 leptspires isolated from 27-paired samples. Of these 2 samples MAT was also positive and in 1 sample MAT was negative. PCR was positive in all the culture positive urine samples.

Of the 30 single sera samples and urine sample collected from the patients, PCR was positive in 9 samples and MAT was positive in 8 sera samples. Of 37 animal urine and sera samples collected, PCR was positive in 13 samples and MAT was positive in 8 samples.

# DIARRHOEAL DISEASES

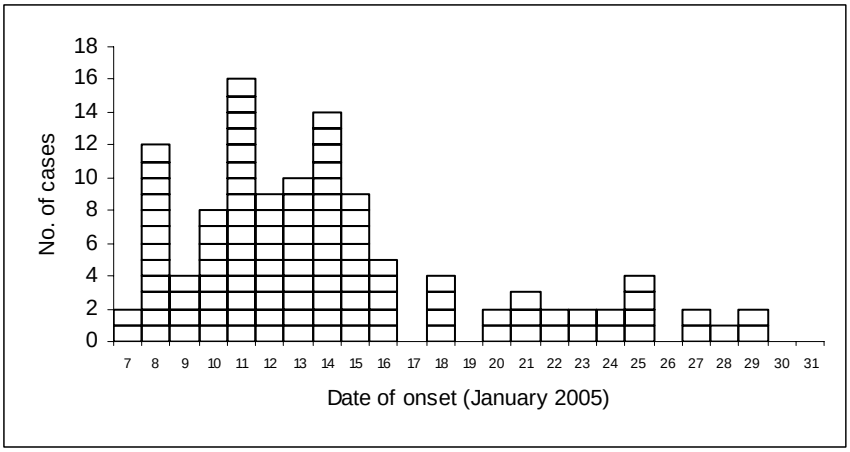
**Outbreak of rotaviral diarrhoea in a relief camp for tsunami victims at Car Nicobar**

Andaman and Nicobar Islands, a Union Territory in India, is an archipelago of more than 300 islands and islets situated in the Bay of Bengal about 1,200 km east of Indian subcontinent (Fig. 1). The earthquake and tsunami wave that occurred in 26 December 2004 severely affected the islands, particularly the southern group of islands constituting the Nicobar District. The Nicobar group of islands are inhabited predominantly by the Nicobarese tribe. Car Nicobar is the headquarters of Nicobar District and is the most populated island of the District. The Nicobarese of Car Nicobar live in 17 villages situated along the shore and all these villages were connected by the Circular Road that runs around the island along the coast.

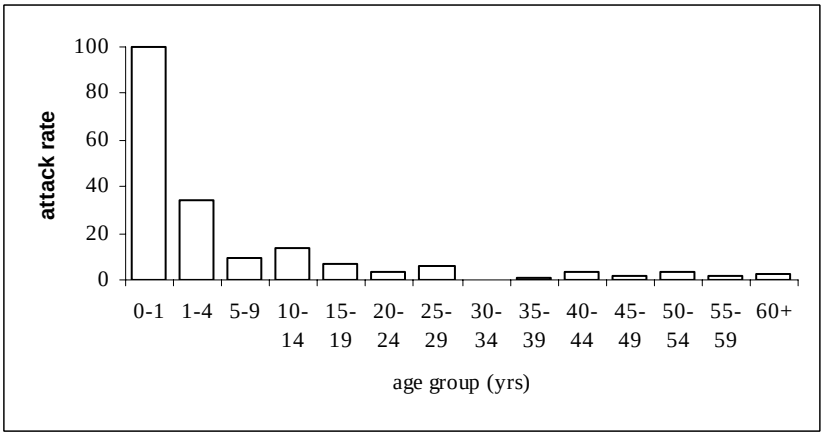



Most of the villages suffered losses in the tsunami. Several hundred people died and thousands are still missing. A large number of houses, constructed with timber and corrugated iron sheets,

were destroyed. As the tsunami waves started hitting the villages, the people fled interior into the jungles. Within a couple of days temporary camps were set up by the survivors of each village. Each village had several camps that shelter 100 – 200 inmates. Within each camp, each family built separate tents. The camp of Mus village, situated on the northern tip of the island (fig. 1) was the largest one. It was a large conglomeration of tents built on a football ground, in which more than 1,500 people were staying.




Distribution of cases of diarrhea at Mus Village Camp, Car Nicobar by date of onset




Age specific attack rates during the outbreak of diarrhea at Mus Village Camp, Car Nicobar, January 2005

The Circular Road was washed away in various places and there was no access to Mus village for several days. A jungle track was cleared later that gave access to the village. A temporary medical tent manned by paramedics, who were residents of the villages, was functioning at the camp right from the day of the disaster itself. Our team stationed at Car Nicobar was working at camps of other villages. On 11 January 2005, we visited the camp of Mus village. It was observed that there were a large number of cases of diarrhea.

From the register kept at the medical tent, it was found that this increase in the number of diarrhea had started on 7 January. Stool samples were collected from patients with diarrhea. These samples were processed for bacterial enteric pathogens at the temporary laboratory set up at the District Hospital, Car Nicobar. Samples were also sent to National Institute of Cholera and Enteric Diseases, Kolkata for testing for rotavirus. The drinking water source for the inhabitants of the camp was six wells, *viz*. ALHW, Roch, Hoy, Otak, Temple and Viper, situated at different places behind the football ground. Samples were collected from all these wells and tested for presence of coliforms using $H_2S$ coliform test kits. All the wells were super-cholrinated. We visited the camp daily since 11 January and recorded new cases. Population of the camp was enumerated and all the recorded patients were interviewed for the source of drinking water. Attack rates among people using different well water were calculated. Well water samples were daily tested for residual chlorine and daily chlorination was done. Besides halogen tablets were distributed and the people were motivated to use boiled water for drinking. Later, with the help of other aid agencies and the local Administration pit latrines were constructed.

There were a total of 1,346 people (654 males and 692 females) staying at the relief camp of Mus village. During the period 7 January to 29 January 2005, a total of 113 cases of diarrhea were treated at the medical tent giving an overall attack rate of 8.4%. There was one death giving a case fatality ratio of 0.88%. The epidemic curve (Fig. 2) showed a sharp increase from 7 January and a slow decline after 15 January. There were multiple peaks indicating secondary cases. Stool samples were collected from 25 patients. No bacterial enteric pathogen was isolated from any of the samples. RNA electrophoresis was done on 20 samples and 19 of these showed presence of Group A rotavirus. Attack rate was 100% among infants (Fig. 3) and it showed a decline with increasing age, the only exception being a higher attack rate among those aged 10-14 years compared to those in the age group of 5-9 years.

Attack rates among people using different wells ranged between 0% to 15%. There were no case of diarrhea among the eight people using the water of the well named Viper. People using the water of all other five wells were affected indicating that either all these wells were contaminated, or the source of infection was not well water. However, once daily chlorination of wells was started, number of cases started decreasing. No other specific cause could be identified as the source of infection.

**Hospital based surveillance of childhood diarrhoeas in South Andaman**

Hospital based surveillance of childhood diarrhea cases was continued during the reporting period 2003-2004. Paediatric diarrhoeaic patients in G.B.Pant Hospital, Port

Blair, were investigated for bacterial enteric pathogens. Stool samples/rectal swabs are collected from the patients and are processed to isolate bacterial pathogens. Isolated organisms were serotyped using commercially available antisera. Antibiotic sensitivity of the isolates is tested using disc diffusion technique.

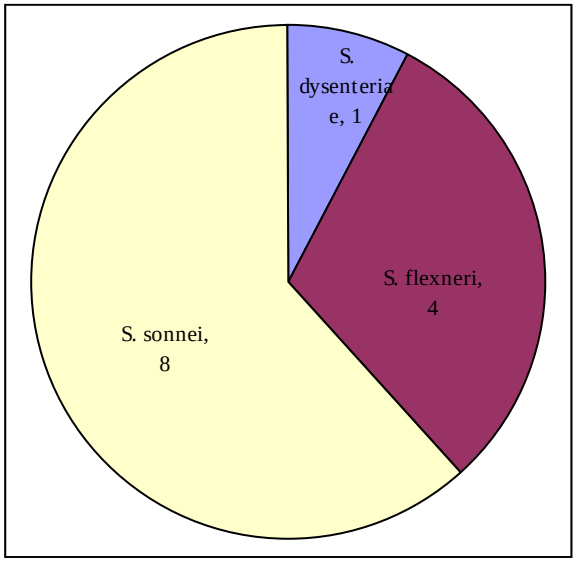
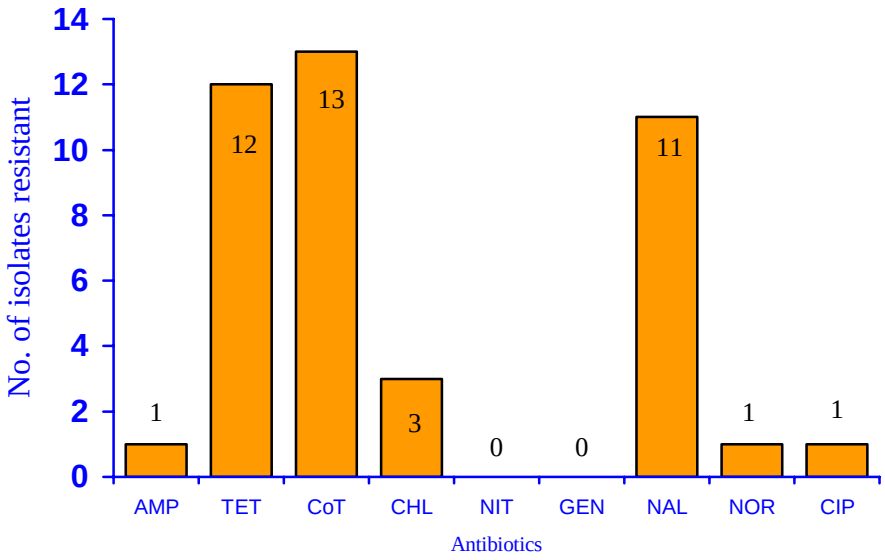



Fig. Relative proportions of the different species of *Shigella* isolated during 2003-2004.

The commonest bacterial pathogen isolated from the hospital over the years has been*Shigella spp*. Isolation rate of *Shigella* varied between 4.2% to 27.9%. During most of the years, *Shigella flexneri* has predominated as the most frequently isolated species. During the past three years, *Shigella sonnei* is accounting for a higher proportion of cases than it used to before. In 2001-2002 *Shigella sonnei* was the predominant infecting strain. In this reporting period (2003-2004), 88 stool samples were processed and 13 *Shigella* isolates were obtained. *Shigella sonnei* again emerged as the most dominating species amongst the isolates accounting for 61.5% (n=8). The dominance of *Shigella sonnei* during the past few years appears to be an unusual phenomenon particularly in developing countries where *S.flexneri* is the commonest isolate. Dominance of S.sonnei is observed in the developed countries. The shift in the most common isolate from *S.flexneri* to *S.sonnei* might be an indication of changing socioeconomic situation in the islands.

Antibiotic sensitivity tests revealed that a quite some proportion of *Shigella* isolates continue to be resistant to ampicillin and most to co-trimoxazole. Emergence of Nalidixic acid resistance in *S.sonnei* isolates were reported last year. All *S. sonnei* isolated during this year were found to be resistant to nalidixic acid. Nalidixic acid resistance has been

acquired by the endemic *S. flexneri* also, as this year, 2 out of 4 *S. flexneri* isolates were resistant to this antibiotic.



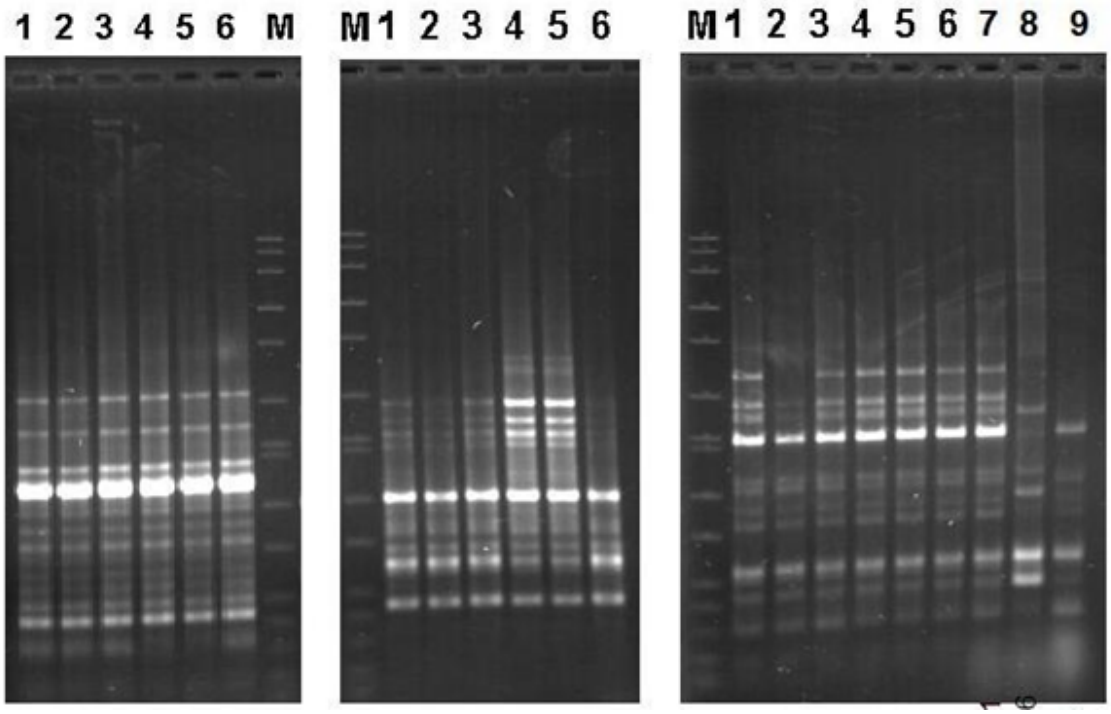

The first ciptofloxacin and norfloxacin resistant *Shigella* was isolated during this reporting period. The isolate was a strain of *S. dysenteriae* type 1, which made an appearance after a gap of four years. Over the years, resistances to new antibiotics have been observed first in *S. dysenteriae* 1, from which it had spread to other species and serotypes. Although the case was an isolated one and no other *S. dysenteriae* was isolated during the reporting period, isolation of this strain perhaps forecasts the impending resistance of shigellae to these fluoroquinolones.

**Molecular epidemiology of shigellosis in Andaman and Nicobar Islands**

To understand the epidemiology of shigellosis, a common cause of childhood dirrhoea in the islands, isolates obtained during the hospital based surveillance have been characterized using various molecular tools and their genetic relatedness was studied. *Shigella flexneri* was the dominant isolate over the years. *S. dysenteriae* became predominant in 1995 when there was an outbreak of shigellosis. *Shigella sonnei* replaced *S. flexneri* in 2001-2003. Results of characterization using plasmid profile and RAPD were reported earlier. During the current year further characterization was performed using REP, BOX and ERIC-PCR.

On the basis of genetic differences obtained using these techniques, isolates were grouped and isolates representing each group were further characterized using PFGE.

Comprehensive molecular typing using these techniques revealed that NAL sensitive *S. sonnei* isolated during 1994-1999 belonged to 4 clonal types with 2 types predominating. The *S. sonnei* strains isolated immediately after developing resistance to NAL during 1999-2001 also showed 2 clones that were identical with the predominating NAL sensitive clones. However PFGE did not reveal any difference between the sensitive and resistant clones. However those strains isolated later during 2002-2004 belonged to at least 4 other clones. The predominant clone was also found in isolates from mainland and might point towards epidemiological links.




Comparison of rep-based fingerprints of Shigella sonnei (Lane 1-Nal resistant local isolate, Lanes 2-6-different isolates from mainland, Lane 7-Local Shigella sonnei belonging to other clones isolated recently)

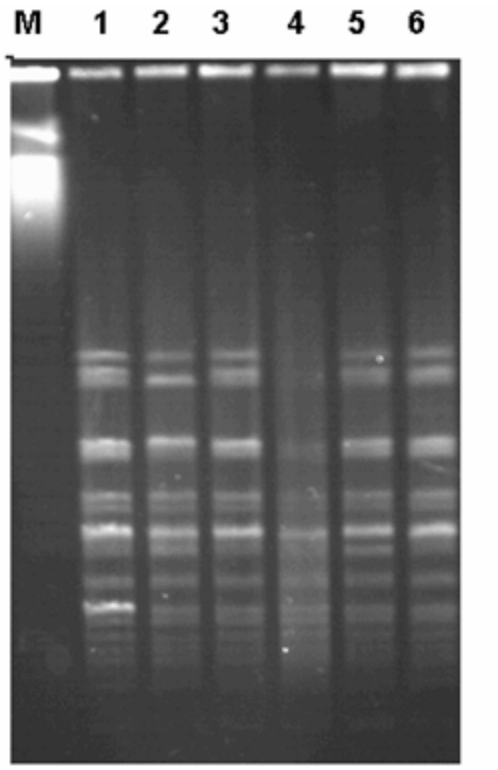



Fig. Xba 1 digested PFGE fingerprints of Nalidixic resistant and sensitive strains (M-Marker, Lanes 1-3-Nalidixic acid resistant strains, Lanes 4-6-Nalidixic acid sensitive strains)

# VIRAL HEPATITIS

**Antenatal screening for HBsAg and prevention of perinatal transmission of hepatitis B virus (HBV) infection with Hepatitis B immunoglobulin and/or hepatitis B vaccine in a tribal community**

Hepatitis B infection is an important public health problem among the tribal population of Andaman and Nicobar islands with very high HBsAg rates. As elevated carrier rates in high endemic areas are largely due to mother-to-infant transmission, it is necessary to introduce hepatitis B vaccination for the newborns among the tribal population of these islands. In view of this, a project has been initiated to compare the efficacies of two regimes, one using hepatitis B vaccine alone and the other using hepatitis B vaccine along with hepatitis B immunoglobulin. The study will be conducted as a double blind placebo controlled trial with three arms. Infants in two arms constitute those borne to HBsAg positive mothers and the third arm constitutes infants born to HBsAg negative mothers. Infants in arm one will receive hepatitis B immuneglobulin soon after birth and hepatitis B vaccine on $0^{th}$ day, 1 m, 2 m and 6 m of age. Those in second arm will receive placebo and hepatitis B vaccine in the same schedule as that for arm one. Infants in the third arm will receive hepatitis B vaccine alone. Follow-up samples would be collected from these infants at 1 m, 2 m 6 m and 1 year of age and would be screened for anti-HBs and HBsAg in order to assess the sero-protection and carrier rates. The findings of the study would be useful in formulating the strategies for interrupting the perinatal transmission among other tribal communities of these islands. Till now 117 pregnant women have been screened and 21 (17.9%) of them were positive. Administration of immunoglobulin and placebo will be started soon. The study is in progress.

# GENETIC STUDIES

**Malaria susceptibility and SNPs among the primitive tribes**

Genetic variants are powerful tools for reconstructing human evolution and for understanding the genetic basis of disease susceptibility / resistance. The most common sequence variations are single-nucleotide polymorphisms (SNPs), which provide the most comprehensive resource for ascertaining genetic diversity. The variation occurs in both coding and non-coding sequences at a frequency of approximately 1 per 1000 base pairs. A database documenting these SNPs in global populations is dbSNP. Analysis of the data in dbSNP revealed that many populations from Indian subcontinent remain undersampled. The identification of SNPs across geographically and ethnically diverse populations is important because the frequency of SNPs can vary substantially between populations, as will their usefulness as markers for gene-mapping studies. A study on SNPs on two functionally coding regions was conducted among Onges, a hunter-gatherer tribe of Andaman and Nicobar Islands.

The target DNA regions- tumor necrosis factor- α (TNF- a) and intercellular adhesion molecule 1 (ICAM-1) - play important roles in a variety of human diseases. Blood samples were processed and the genomic DNA sequences including 1000 bp upstream region of TNF- α (3085 bp) and ICAM-1 (15 kb) were assembled from UCSC Genome Browser. The genes map to the chromosomal region 6p21.3 and 19p13.2-p13.3 respectively. The TNF- α gene contains four and ICAM-1 gene contains seven exons. The ICAM-1 genomic region was repeat- masked using the Repeat-Masker2 program. Primers for PCR were designed by using the Primer Express 2.0 software package. The 5'and 3' UTRs, exons and introns were completely sequenced. A total of 3 SNPs were detected and none of which is reported in the NCBI's dbSNP database.

On the TNF-α locus three new SNPs were identified, two of which were shared by Jarawa population also as reported earlier. These variant sites lie either in the promoter or in the intronic region of the gene. One of these private sites C500T is highly polymorphic. The G-433A SNP found exclusively in the Onges population is highly monomorphic with minor allele frequency ~5%. At the ICAM-1 locus, 4 biallelic variant sites were identified, which have already been reported in the dbSNP (Table 1). One of these SNPs (rs5030382) harbors a non-synonymous change from amino acid glutamic acid (E) to lysine (K) at position 469 of the CD54 protein, while another SNP (rs3093032) lie in the exon-7 (3'UTR) of the gene. The remaining two variant sites were

located in the intron- 2 of the gene. Like the SNPs present in TNF-α , the frequencies of ICAM-1 SNPs are also moderate to high (Table 1). A total of seven haplotypes were observed among which HT1 and HT7 are the most frequent strings contributing 50 % and 20% of the gene pool respectively. HT2 & HT4 and HT5 & HT6 are more or less equally frequent in the Onges population.

**Immunological studies on Tribal population of Andaman Islands.**

There are several tribal populations (Jarwas, Onges, Shompens, Nicobaries, Great Andamanese) in Andaman and Nicober Islands. Out of these tribes Nicobaries are the only civilized tribe. Jarwas and Onges have features like Negroid pigmies and the remaining tribes are having mongoloid features. Recently Jarwas have come in contact with the general population. In the recent past, outbreaks of viral hepatitis have been reported in Jarwas. Considering this fact, it would be worthwhile to determine the background health status of the tribal population, especially of Jarwas. To understand the immunological status of the Jarwas, different health parameters need to be investigated.

The objectives of the study were to determine the basal values of Hb, TLC, DLC, the total T cell number and CD4/CD8 ratio of peripheral blood, serum antibody levels to various common infections like viral hepatitis, leptospira, candida, tetanus, diphtheria, pertusis, *Salmonella*, *M. tuberculosis* and estimation of zinc and copper in serum.

A total of 44 jarwas serum sample were collected and tested for leptopsiral antibodies. Out of 44 samples tested leptospiral antibodies were seen in 17 sera (39%). Collection of more samples are in progress and other parameters are being investigated.