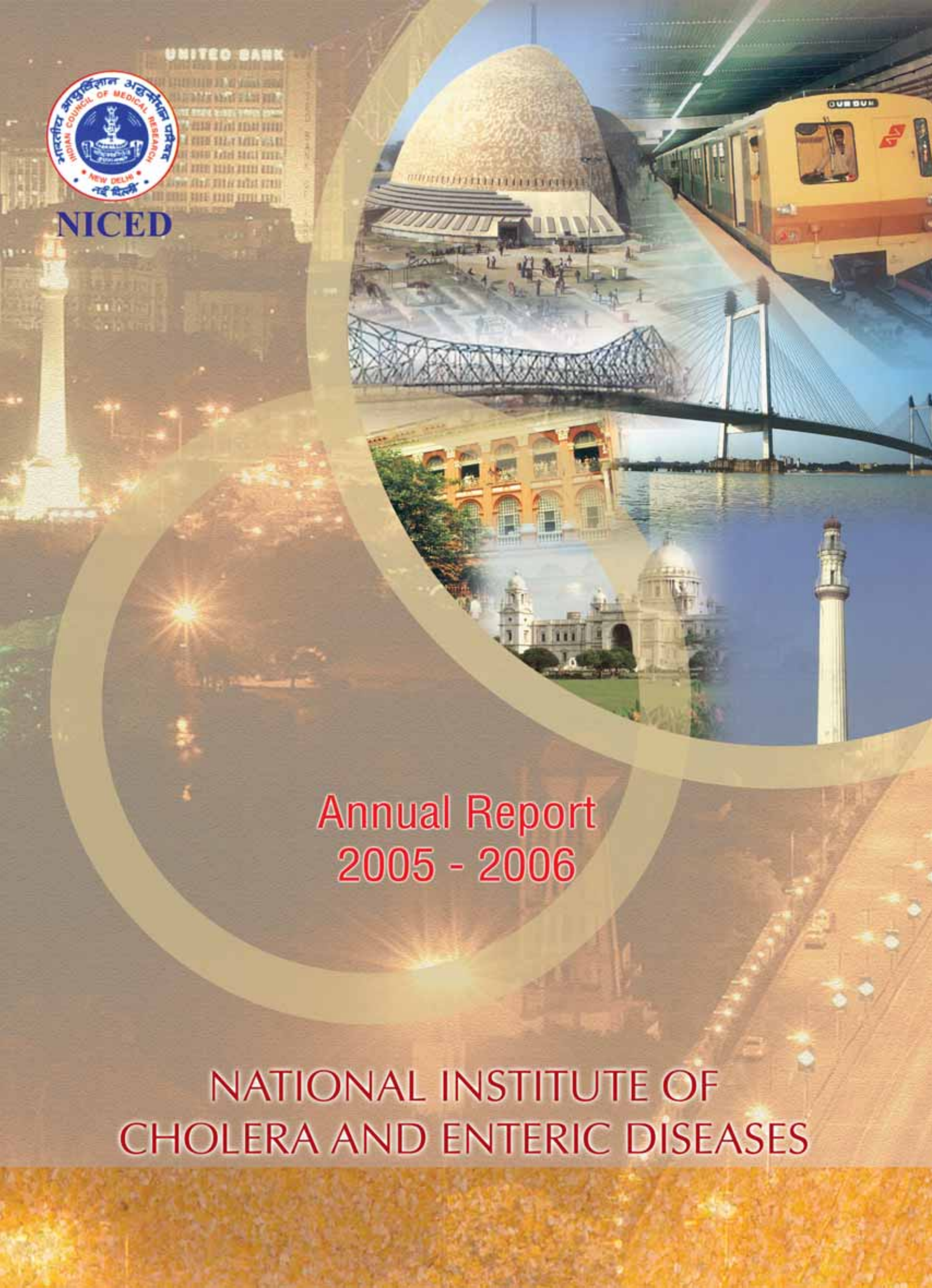

Annual Report
2005 - 2006

NATIONAL INSTITUTE OF CHOLERA AND ENTERIC DISEASES

Annual Report
2005-2006

National Institute of Cholera and Enteric Diseases
(Indian Council of Medical Research)
P-33, CIT Road, Scheme XM, Beliaghata, Kolkata – 700 010

Preface

As in the previous years, National Institute of Cholera and Enteric Diseases, Kolkata have been unrelenting in its efforts to control diarrhoeal diseases through research and training, as well as dissemination of findings of public health importance to appropriate authorities. The institute also assisted various state governments in investigating outbreaks of diarrhoea, dengue and hepatitis, in different areas of the country and provided laboratory supports at different levels for isolation and detection of various enteropathogens. In addition, scientists of this institute also investigated and suggested control measures for possible disease outbreaks in tsunami-affected areas of Andaman and Nicobar Islands.

The on-going hospital-based surveillance of various diarrhoeagenic pathogens facilitated detection and characterization of the organisms involved and helped in identification of diarrhoea-prone areas in and around Kolkata. The institute also conducted community-based studies in rural West Bengal on epidemiology of typhoid fever and to find out relations between feeding practices and occurrences of diarrhoea and acute lower respiratory infections in children. In the face of emerging antibiotic resistance in *Vibrio cholerae*, newer antibiotics were tried for cholera patients and role of lactobacillus has also been evaluated in acute childhood diarrhoea.

There have been a number of molecular biologic studies on *V. cholerae*, *Shigella spp.*, *E. coli* and other enteropathogens including enteric parasites to elucidate different aspects of their mechanisms of action, transmission, antibiotic resistance and immune responses. Studies on Vibriophages, *H. pylori* and a host of enteric and other viral infections, including HIV, opened up newer insights into these disease processes.

The ongoing JICA-NICED collaborative project, now in phase 2, provided opportunities to build necessary infra-structures and hosted a number of training programmes both in this institute and in Japan that helped in development of highly skilled manpower at different levels.

The institute also successfully continued collaborative research programmes with the International Vaccine Institute, Korea in evaluating vaccines for typhoid fever and cholera, to reduce the burden of these two dreadful diseases in Kolkata and much beyond. During the year, the institute also received financial and technical supports from CSIR, DBT, WHO and several other national and international agencies.

The guidance, support and cooperation received from the office of the Director-General, ICMR and members of the scientific and various other advisory committees are gratefully acknowledged. The dedicated and sincere efforts of our scientists, technical, administrative and support staff, along with untiring works of the research fellows will undoubtedly take the institute at a new high and thus deserve my sincere and heartfelt appreciation.

Dr. S. K. Bhattacharya, MD, FNASc, FNA, FAMS, FIPHA, WBFHC
Director

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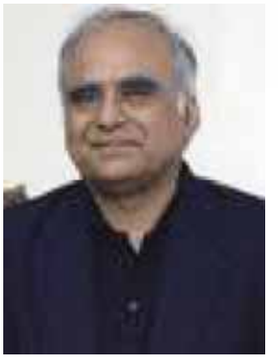

Message

7th January, 2006

I am extremely pleased to extend my deep appreciation for **National Institute of Cholera and Enteric Diseases (NICED), Kolkata** for its significant research works on different aspects of diarrhoeal diseases, other enteric pathogens including hepatitis, typhoid fever and *H. pylori*, and HIV/AIDS. The institute's achievements and continued enthusiasm are highlighted through its high quality publications and its ever-increasing collaborations with different national and international organizations, which deserve much applause.

As in the past, the Council will make all endeavour to help this institute to achieve its ultimate mission.

Prof. N. K. Ganguly

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1 Study on Hospital Surveillance

1. Study on hospital surveillance

1.1 Hospital based surveillance system for diarrhoeal diseases

Institutional Project:

This project is continuous hospital based systematic surveillance (every 5th patient on two randomly selected days per week) of diarrhoeal diseases at Infectious Diseases Hospital, Kolkata. The main objectives of this study are to monitor changes in disease patterns including drug sensitivity, to create a database on diarrhoeal diseases, to provide regular report to the Government and other relevant agencies, also to develop an early weaning system for forecasting an epidemic and to furnish information to be applied for improvement in patient care and better preventive measures.

During the period under study a total of 1265 diarrhoea patients were enrolled in the surveillance system. The isolation of different enteropathogens are depicted in Table 1.1.1.

Antimicrobial susceptibility

Vibrio cholerae 01 strains are resistant to ampicillin, co-trimoxazole, furazolidone, nalidixic acid and streptomycin. Reduced susceptibility was observed for chloramphenicol, ciprofloxacin and neomycin. Strains were sensitive to gentamycin, norfloxacin and tetracycline. *Vibrio cholerae* 0139 strains were resistant to ampicillin, furazolidone and nalidixic acid and were sensitive to chloramphenicol, gentamycin, neomycin, norfloxacin and tetracycline.

Vibrio cholerae non 01 non 0139 strains were found resistant to ampicillin and furazolidone. They were sensitive to gentamycin, tetracycline, chloramphenicol, ciprofloxacin and reduced susceptibility was found against neomycin. *Shigella dysenteriae* strains were uniformly resistant to ampicillin, co-trimoxazole, tetracycline, nalidixic acid, chloramphenicol and had reduced susceptibility to norfloxacin, ciprofloxacin and ofloxacin. *Shigella boydii* and *Shigella sonnei* were totally resistant to co-trimoxazole, tetracycline and nalidixic acid.

Table 1.1.1 Enteropathogens detected in Hospital Based surveillance system during April 2005 to March 2006 at ID Hospital, Kolkata.

Enteropathogens	Number tested	Number identified	Percentage
Bacteria			
Vibrio spp.	1265	322	25.5
Vibrio cholerae O1	1265	235	18.6
Vibrio cholerae O139	1265	4	0.3
Vibrio cholerae nonO1 nonO139	1265	68	5.4
Vibrio parahaemolyticus	1265	15	1.2
Shigella spp.	1265	2	0.2
Non typhoidal *Salmonella* spp.	1265	3	0.2
Diarrhoeagenic *Escherichia coli*			
Enterotoxigenic *Esch.coli*	402	14	3.4
Enteropathogenic *Esch.coli*	402	9	2.2
Enteroaggregative *Esch.coli*	402	35	8.7
Virus			
Rotavirus	382	58	15.1
Protozoa and Helminth			
E. histolytica	355	14	3.9
G.lamblia	355	10	2.8
C.parvum	355	13	3.7
Ascaris	355	22	6.2
H.nana	355	8	2.2
T.trichiura	355	11	3
T.hominis	355	8	2.2
Hookworm	355	6	1.7

2 Community based studies:

2. Community Based Studies

2.1 Acute lower respiratory tract infection (ALRI) and diarrhoea in rural children below two years in relation to feeding practices with particular reference to breast feeding: A community based study.

Investigator: S.K. Mondal

Acute lower respiratory tract infection (ALRI) and Diarrhoea are two leading causes of mortality and morbidity in children below five years of age in the developing countries. There is a lack of community-based information on the disease burden caused by different pathogens responsible for causing ALRI, and the epidemiological information regarding their magnitude in the community is also scanty. Breast feeding, particularly exclusive breastfeeding in infants up to 6 months and complementary feeding along with proper weaning are known to protect infants from diarrhoea as well as ALRI. However there is very little information regarding extent of exclusive breast feeding in children <6months / complementary feeding as well as weaning feeds given to children. Therefore this study with the objectives to know the prevalence of ARI and Diarrhoea in rural children and to see the impact of exclusive breast feeding / weaning in ALRI & Diarrhoea was undertaken.

The study has been initiated in 11 villages of Kalikapur Gram Panchayet 1 & 2 area of Sonarpur block of south 24-parganas District, in a population of 29000 approximate.

A baseline demography of the families have been done and data are being entered in computer. A module on ARI for health personnel was prepared in local language. West Bengal Government health staff and NICED field workers were given one day orientation training on ARI and Breastfeeding. In turn they trained Voluntary Health Workers under close supervision of investigators. So far 1297 infants have been identified and enlisted for weekly follow up of which 448 have have been excluded for study completion/leaving the area. Anthropometric measurements of infants have been conducted at the subcentres. Out of total 1297 children 867 were new born; of them 80.0 % were Hindu and 20% were Muslims. 46.6% were delivered at Hospital, 8.4% at Nursing Home and 45.0% at home. Normal delivery were 87.0%, rest 13.0% were LUCS. Average birth weight for male was 2.72 kg, for female it was 2.61kg. Median time for initiation of breast milk was 3hrs among Hindus and 2 hrs among Muslims. 81.0% of mothers were literate.

Till Dec 05 out of 1742 detected diarrhoea episodes, 9.9% were in 0-5 months age group, 41% in 6-11 months and 48.9% in 12-23 months; out of 2237 ALRI episodes, 18.9% were in 0-5 months, 40.2% in 6-11 months and 40.8% in 12-23 months. 54.4% of males and 45.6% of females suffered from diarrhea, whereas 53.1% of males and 46.9% of females suffered from ALRI.

2.2 Epidemiology of typhoid fever in a rural and urban slum community of West Bengal

Investigator: S. Ghosh

The study was initiated to know the magnitude of problem due to Typhoid fever in rural community of West Bengal. Age specific incidence may indicate appropriate age for initiation of typhoid vaccine if it is included at

National Immunization Schedule. Drug sensitivity pattern of circulating strains of Salmonella enterica *serover typhi* in the community may help the doctors for rational use of antibiotic for treatment of typhoid fever. The study area and methodology were described in last year annual report.

The study area is 35 Km away from NICED and situated in Sonarpur block of South 24-parganas. There is a total population of about 29,000 living in 5967 families in 11 villages under four sub-centers of Kalikapur PHC. Majority (81%) of them are Hindu, rest (19%) are Muslim. Only 30.4% families live in Pucca houses. Drinking water source is mainly tube wells, where as 30% use tap water. About 50% of people use open field for defecation. Majority of the families fall within the income group of <= Rs. 2000/-per month. Overall literacy rate is 72.9%. 56.8% of male and 43.2% of female are literate.

Since April 2003, a total of 556 blood samples were collected, of which 19 (3.4%) were positive for *Salmonella typhi*. Isolation rates were 4.6% in January to March, 4.5% in April to June, 2.5% in July to September and 2.7% in October to December. Sub-center wise isolation rate of *S.typhi* is significantly higher (P<0.05) in Raipur (7.80%) as compared to that of Beniabou (0.94%), Kalikapur (2.27%) & Sahebpur (2.25%).

Isolation reates in the age groups of <5, 5-9, 10-14 and >=15 years were 3.17%, 1.98%, 8.99% and 2.31%. However incidence of typhoid fever per 1000 person year was found to be the highest in the age group of < 5 yrs (11.81) compared to that of 5-9 yr (2.03), 10-15 (4.06) & >=15 yr (0.87). Headache, pain abdomen, anorexia were main associated complain along with >= 3 days duration of fever.

Salmonella typhi strains were found to be resistant to Chloramphenicol, Co-trimoxazole, and Ampicillin but sensitive to Gentamycin, Norfloxacin, Ciprofloxacin, Pefloxacin, Cefotaxime and Amikacin.

2.3 Identifying environmental risk factors for endemic diarrhoeal diseases in West Bengal, India : a remote-sensing geographic information system(GIS) approach

Investigator: A. Palit

Data of acute diarrhoeal disease incidences in Kolkata and its adjoining areas with special reference to cholera and shigellosis for at least one year (2002/2003) will be taken into account. Disease surveillance data (based on records of I.D. Hospital, Kolkata, Institutional surveillance and D.H.S records) will be analysed. Particular foci of epidemic outbreaks as well as of endemic ones in and around Kolkata, India will be identified.

Baseline ecological data on temperature, humidity, surface water temperature of water bodies, land covers etc. will be collected. The type of human habitations in relation to water resources, water bodies, drinking water supply structures etc. will also be identified for macro-stratification of geo-environmental factors. Collection of above data are meaningful to ascribe the relation of disease transmission.

Retrospective satellite data (2002/2003) of IRS ID LISS III and LISS IV will be procured in the form of CCT (Computer compatible tapes) from NRSA, Hyderabad by RRSSC (ISRO), Kharagpur, W. Bengal for the study foci. The data will be analyzed at RRSSC, Kharagpur. False colour composite (FCC) images will be generated. Ground truth validation for the land use features of interest will be done.

Classification of land use categories will be done and statistics will be generated. Analysis of satellite data will be carried out by digital image processing facilities available at Regional Remote Sensing Service Center (RRSSC), Kharagpur.

Ground truth of the study areas for analyzing the spatial variation in diarrhoeal disease dynamics will be carried out using IRS ID LISS III and LISS IV data. Mapping of temporal variation of environmental parameters for the purpose of correlating them with changing pattern of disease incidence with regard to selected foci will be done.

The land cover details within the study region at sample locations will be collected simultaneously for use in classification and interpretation of satellite data. The information available in Survey of India (S.O.I.) topo maps on 1: 50,000 scale will be used for field traverses and base map preparation. Qualitative studies of water use and the organization of water supply will also be looked into to understand drinking water epidemiology.

Monitoring, identification and recording of disease incidence will be tallied in the same months of satellite pass. The correlation between disease incidence and land use features will be worked out to identify the most relevant land use features and environmental factors that may contribute to epidemic outbreak of cholera/ shigellosis etc. Based on this information, the disease distribution map of those selected foci will be prepared.

RS-GIS Steps involved:

- Interpretation of Satellite data for geo-environmental parameters and its conversion into vector layer
- Interpolation of hydro-meteorological data & density of diarrheogenic pathogens into spatial layer
- Integration of Socio-economic and collateral data in GIS frame work
- Development of GIS model by giving weightage to each parameter for possible disease incidence
- Generation of diarrheogenic maps (levels)
- Overlaying of diarrheal disease incidence data on the above for possible correlation in relation to seasonal variation.

Data of acute diarrhoeal disease incidences in Kolkata and its adjoining areas with special reference to cholera and shigellosis for two years (2002 and 2003) have been taken into account as per objectives. Disease surveillance data has been analysed to identify particular foci of epidemic outbreaks as well as of endemic ones in and around Kolkata. Baseline ecological data on temperature, humidity, surface water temperature of water bodies, land covers etc. are being collected from meteorological office, Kolkata. The type of human habitations in relation to water resources, water bodies, drinking water supply structures etc. are in the process of identification for ground truth evaluation.

Data have been recorded in designed formats for drinking water epidemiology along with its microbiological characteristics (water quality testing, monitoring etc.) at different sample locations in preselected foci in relation to environmental and land cover variables.

Retrospective satellite data of IRS ID LISS III and LISS IV are in the process of classification for mapping out the images of Kolkata as well as some of its worst diarrhea affected foci based on the disease surveillance data. The study is in progress.

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3 Clinical studies

3. Clinical studies

3.1 Evaluation of comparative efficacy of Cefuroxime axetil and Tetracycline in the treatment of cholera in adults.

Investigator: M.K. Bhattacharya

Acute watery diarrhoea caused by *Vibrio cholerae* is an important cause of hospitalization at the I.D. Hospital, Calcutta. The number of children dying each year from diarrhoea has decreased over the past decade from 5 million mainly because of the success of oral rehydration solution (ORS) therapy. Several drugs, namely tetracycline, furazolidone and trimethoprim-sulfamethoxazole (TMP-SMX), have been found to be effective in reducing stool volume, duration of diarrhoea and faecal excretion of vibrio in patients with cholera. Recently, we observed from our hospital based surveillance system for diarrhoeal diseases that *Vibrio cholerae* strains (O1 and O139) are uniformly resistant to furazolidone, nalidixic acid and partially resistant to ciprofloxacin and norfloxacin due to indiscriminate use. Moreover, the isolated strains of *Vibrio Cholerae* nonO1 nonO139, which constitutes 30% of the total isolation of *Vibrio cholerae* strains are resistant to TMP-SMX (53%) and tetracycline (56%).

Cefuroxime is a second generation cephalosporin. It is highly active against gram-negative and gram-positive bacteria *in vitro*. Isolated strains of *Vibrio cholerae* are uniformly susceptible to cefuroxime. A study was undertaken to evaluate the safety and efficacy of cefuroxime in the treatment of cholera in adults with moderate to severe dehydration. A total of 128 cases of acute watery diarrhoea were studied. Out of 128, cases 68 cases received cefuroxime and 60 cases received tetracycline. Of these 128 cases,46 cases were positive for *V. cholerae*. Out of 46 cases, 27 cases received Cefuroxime and 19 cases received Tetracycline. Both the drugs were equally effective in the treatment of cholera in adults in respect to duration of diarrhea, volume of stool and consumption of ORS. Hence, Cefuroxime can be kept as an alternative to Tetracycline for treating Cholera in adults caused by multi-drug resistant strains of *V. cholerae.*

3.2 Impact of Lactobacillus (Lactic acid bacteria) in children with acute watery diarrhoea

Investigator: P. Dutta

This is a hospital based, randomized, double blind clinical trial to evaluate the role of Lactobacillus (Lactic acid bacteria) therapy on the outcome variables (stool output, duration of diarrhoea, consumption of fluid therapy) in acute watery diarrhoea in children.

The study is in progress at the Dr. B.C. Roy Memorial Hospital for Children, Kolkata in double blind fashion. One hundred forty male children of all nutritional groups (except severe malnutrition) aged between 6-24 months suffering from acute watery diarrhoea (Passage of more than 3 liquid stools within last 24 hours period) of < 3 days duration with some dehydration were included in this study.

Children who were exclusively breastfed, aged less than 6 months or more than 24 months, associated with other systemic infection or any

complication or chronic underlying disease or severe malnutrition for which they need extensive care were excluded from this study. Children who received antibiotic before admission and whose parents refused to give consent were also excluded from the study.

Informed written consent was obtained from the parents of the children after explaining the details of the study procedure before inclusion in the study.

After selection, complete history was taken from parents and thorough physical examination was done and findings were recorded in a pre-designed proforma. Stool samples were collected in sterile MacCartney's bottle on admission for detection of various established enteropathogens. Children were weighed unclothed on admission using weighing scale with a sensitivity of 1 gm, length and mid arm circumference of the children were also recorded.

After selection of the patient, two dispersible Lactobacillus tablets (each Lactobacillus tablet contains 60 million spores of Lactobacillus (Lactobacillus sporigen). or placebo tablets were administered two times a day according to a random number in a double blind design for a period of 5 days even after cessation of diarrhoea. Lactobacillus and placebo tablets were identical in colour, shape, size and test and were prepared in blister strips and these strips were numbered for specific patient.

After enrollment in the study, the degree of dehydration was assessed by the WHO criteria. All the patients received standard (WHO recommended) oral rehydration therapy at the rate of 75-100 ml/kg body weight for 4 hours for correction of initial dehydration. Oral rehydration solution at the rate of 50-100 ml per loose stool was also given to maintain the hydration status till cessation of diarrhoea. The patient, who developed severe dehydration during the study period, received I.V. fluid (Ringer's lactate solution) as per WHO guideline (treated as recipient of unscheduled IV fluid). The patient who developed complication was withdrawn from the study and treated according to the hospital treatment protocol.

All the children received the normal hospital diet including breast-feeding. Patients were weighed after rehydration and then every morning between 10.00 - 10.30 AM and also followed up till recovery or up to 5 days even if they did not fulfill the criteria of recovery within that period. Daily records were noted on the pre-designed proforma. Intake and output charts were maintained every 8 hourly till recovery or up to 5 days. Urine was separated from stool and volume was measured. Amount of fluid intake (ORS, plain water, I.V. fluid and other liquid food) was also measured. Stool was measured after collection in pre-weighed diaper and the measurement weight was sensitive up to 1 gm. Patients were discharged from the hospital when they fulfilled the recovery criteria (recovery was defined as passage of soft stool or normal stool or no stool for last 18 hours) even before 5 days. Prescribed tablets were supplied to the parents to continue upto 5 days if discharged before 5 days. If the patients condition deteriorated in spite of 3 days therapy, then they were considered as treatment failure and were treated according to the standard treatment of the hospital. Patients who developed complication during the study period and deviated from the study were also termed as treatment failure. Parents received nutritional advice for their children (at least one extra meal or liquid food/day) during the recovery period. Parents were also advised to attend hospital if the patient develops any complication within 15 days of discharge. Study is in progress.

4 Studies on Vibrio Cholerae

4. Studies on *Vibrio cholerae*

4.1 Molecular epidemiology of cholera in India

Investigator: T. Ramamurthy

Resurgence of cholera has been reported in many countries with several sporadic infections and outbreaks. In India, several cholera outbreaks occurred recently and it appears that the trend in the prevalence of *V. cholerae* is very dynamic with constant change in the phenotypic and genotypic traits. Conventional characterization using methods such as serology, antibiogram and phage typing though gives some idea on phenotypic features, the detailed molecular analysis reveals constant changes at the gene level, which are easy to perform and less time consuming. We are carrying out molecular epidemiological aspect for the past several years with typing methods such as ribotyping, randomly amplified polymorphic DNA PCR, pulsed-field gel electrophoresis etc. to supplement the results obtained by conventional methods. In addition, we have included the simple PCR method for the identification of classical and El Tor biotypes, differentiation of O1 and O139 serogroups, and detection of several virulence genes. *V. cholerae* O1 strains have been responsible for epidemic cholera for many years, but in 1992 an epidemic clone of serogroup O139 appeared in southern India, but its prevalence was short. From 2000, the O1 serogroup dominated in cholera endemic areas. In this study we have characterized *V. cholerae* O1 Ogawa, Inaba, serotype, which caused several cholera outbreaks and made a retrospective analysis to confirm the emergence of new clonal types.

We analyzed 402 *V. cholerae* O1 isolates from cholera patients from 17 different areas during 2004 and 2005. Among these, 43.2 and 56.7% of the strains were identified as Ogawa and Inaba serotypes, respectively. Except for Delhi, and Manipal, the serotype Inaba was exclusively identified in 5 cholera outbreaks. However, in Delhi and Manipal cholera outbreaks, Inaba was the predominant serotype with 66.0 and 67.0%, respectively. During March-June 2004, Ogawa and Inaba serotypes were detected in Ahmedabad, Goa, Chennai, Madurai, Ludhiyana, and Chandigarh. In Kolkata, there was a total replacement of Ogawa serotype by the Inaba from June 2005 onwards (unpublished data).

All the strains harbored *ctxA* and the El Tor allele of *tcpA* and *rstR* in the PCR assays. In the phage typing analysis, almost all the Ogawa as well as Inaba isolates included in this study belonged to type T4 and type 27 with new set of phages. Polymyxin B susceptibility test confirmed that the isolates are El Tor biotype. In the antimicrobial susceptibility pattern, isolates of Inaba were more resistant to chloramphenicol and streptomycin compared to Ogawa. Majority (91%) of the Inaba isolates showed reduced susceptibility for ciprofloxacin, whereas 32% of Ogawa isolates remained resistant to this drug.

We went on to detect the mutations in the *wbeT* gene of recent Inaba isolates and to find the uniformity in such changes with previous isolates. Inaba isolates from Tripura, Madurai, Ludhiyana, Ahemedabad and Kolkata had identical sequence homology in the sequenced region of *wbeT*. As reported before, silent substitution of C for A at nucleotide position 553 prevailed in all the tested recent Inaba isolates. In addition, substitution of C for T at

nucleotide position 538 leads to change of the amino acid from serine to proline, which was not reported before. The other mutations reported among previous Inaba isolates from India were not found in the recent Inaba isolates.

Following the ribotyping scheme of Sharma et al, we have tested 28 representative isolates (20 Inaba and 8 Ogawa). This analysis showed that most of the Inaba isolates of 2004 belongs to the new ribotype RIV and isolates of Ogawa during the same period and old Inaba were identified as ribotype RIII. Prevalence of ribotype RIII was also detected among some of the 2004 Inaba isolates (VC187, GO13610, and DO1358). In the PFGE, 27 *V. cholerae* isolates (20 Inaba and 7 Ogawa) were tested following the PFGE typing scheme established by Yamasaki et al., which consisted of 11 pulsotypes (A through K). Majority of the Inaba isolates belongs to 'H' type (12 isolates) or 'Ha' type (4 isolates), and 2 Ogawa isolates were identified as pulsotype 'Ha'.

The new pulsotypes viz., L (Ogawa isolates DO5465, LU626), M (UP1/13.9-Inaba), N (K5919-Ogawa), O (CHN 5/04-Ogawa) were differed with H type with 4 bands. In the other new pulsotype P (DO1272-Inaba), DNA band at about 242 Kb was absent and there was an additional band at about 290 Kb region compared to H type. In pulsotype Q (VC187-Inaba), an additional band at about 200 Kb was detected, which was absent in the pulsotype H.

Our findings are congruent with earlier studies when the new serogorup *V. cholerae* O139 emerged in India. The phenomenon of seroconversion is a great challenge to the existing surveillance work, which needs careful monitoring. The study is in progress.

4.2 Vibriocidal assay on rough *Vibrio cholerae* strains with acute and convalescent sera collected from cholera patients and implication of gene(s) for the rough phenotype

Investigator: R.K. Nandy

The diarrhoeal disease cholera, which continues to be a global threat, is caused by *Vibrio cholerae* belonging to the O1 and O139 serogroups and the two serogroups are also responsible for epidemics and pandemics. The virulence factors of O139 strains are similar to strains belonging to the O1 serogroup and detailed molecular analysis have shown that *V. cholerae* O139 resembles the O1 El Tor biotype strains with the exception of the presence of capsular polysaccharides in O139 strains. *V. cholerae* non-O1, non-O139 strains represent heterogeneous serogroups, which so far have not been recognized as having epidemic potential and are generally devoid of most of the virulence genes associated with epidemic serogroups (O1 and O139) of *V. cholerae.* However, association of non-O1, non-O139 *V. cholerae* with sporadic cases of diarrhoea has been reported. Immunogenic specificity of the long repetitive units of 'O' antigenic polysaccharides formed the basis of *V. cholerae* serotyping scheme as R-antigens of *V. cholerae* are identical to each other. *V. cholerae* rough strains are characterized by the presence of only R-antigen and they lack the long repetitive units of polysaccharides. Although much attention has been focused on studying the biochemical constituents of R-antigens of *V. cholerae*, very limited information is available on the virulence genes and pathogenic properties of rough *V. cholerae* strains isolated from cholera patients. Previous studies on rough mutants derived from wild type smooth virulent strains of *V.*

cholerae showed that these mutants are comparatively less virulent due to their severe defect in small bowel colonization properties when tested in the infant mouse model. Although, the rough strains are considered as less virulent, isolation of rough *V. cholerae* strains as sole pathogen from diarrhoea cases has also been reported. Furthermore, initial characterization study showed increased virulence by the isogenic rough strains as compared to smooth counterparts. The present study was undertaken to characterize rough strains of *V. cholerae* with respect to their serological reactivity patterns as well as sensitivity towards vibriocidal antibodies present in sera samples collected from acute and convalescent cholera patients.

Well characterized rough *V. cholerae* strains were included in the study. These strains were generated in the laboratory from smooth counterpart and used as indicator organisms in vibriocidal assay against five paired sera samples collected from cholera patients. The paired sera samples were collected from cholera patients admitted to Infectious Diseases Hospital (IDH), Kolkata. The sera samples were collected on the day of admission and considered as acute sera, while convalescent sera samples were collected within one week to 4 weeks post period of admission. During this study period 5 paired sera samples were collected after obtaining informed written consent from the patients. These paired sera samples were tested for their serological reactivity to whole cells of *V. cholerae* rough strains and corresponding smooth strains. Induction of IgM subclass specific immune response in convalescent sera was analyzed by whole cell ELISA. More than 2-folds rise in serological reactivity was observed in all five paired sera samples against O1 El Tor Ogawa strain VC20 while only two-fold rise was noted against O1 classical Ogawa strain O395. Interestingly, rough strains of VC20R and O395R reacted in a similar way to each other and no seroconversion (more than 2-fold rise) was observed. These results were extended further by determining vibriocidal activity of these sera samples to smooth and isogenic rough strains. Analysis of the vibriocidal data showed decrease of vibriocidal antibodies in the convalescent sera samples as compared to acute sera. It is difficult to explain such decrease of vibriocidal antibodies in the convalescent sera samples where induction of immune response in the IgM level was observed. The assays were extended further using *V. cholerae* strains that were isolated from respective patients. All three *V. cholerae* strains showed reciprocal vibriocidal titer <5 when tested against acute sera. However, more than 4 folds rise was noted in one of the three convalescent sera. Induction of IgG subclass specific anti-CT antibody response in was noted in all these paired sera. This study also pointed out the fact that recent *V. cholerae* strains are not sensitive in the assay for the detection of vibriocidal antibodies present in the sera samples. It may be possible that recent *V. cholerae* O1 strains are somewhat resistant to vibriocidal lysis or may have altered antigenic expression to escape vibriocidal antibodies.

4.3 Studies on the structure-function relationship of *Vibrio cholerae* hemolysin (HlyA)

Investigator: K.K. Banerjee

Vibrio cholerae hemolysin (HlyA), an extracellular water-soluble membrane-damaging protein with a native molecular weight of 65,000, belongs to a unique class of dimorphic proteins that can exist in two stable states, a water-soluble monomer and an oligomeric amphipathic protein capable of spontaneous insertion into the membrane lipid bilayer. These

proteins, commonly referred to as pore-forming toxins (PFTs) for their ability to lyse target eukaryotic cells by punching holes in the plasma membrane, interact with specific target membrane components and self-assemble by noncovalent interactions involving oligomerization domains into exceptionally stable and rigid β–barrel oligomers that eventually insert into the membrane by using amphiphilic β-hairpins as anchors to the nonpolar core of the lipid bilayer. Previously, we showed that perturbation of the native tertiary structure of HlyA under certain conditions was sufficient to induce complete and irreversible conversion to the oligomer. This led us to postulate that the hemolytically active HlyA monomer represents a quasi-stable conformation corresponding to a local free energy minimum and the transmembrane heptameric pore represents a stable conformation corresponding to an absolute free energy minimum.

The present work is a continuation of the study on the structure-function relationship of *Vibrio cholerae* hemolysin (HlyA) and the molecular interpretation of the membrane permeabilization by the toxin. Experiments in the past year indicated that interaction of the pre-pore oligomer of the toxin with cholesterol-sphingolipid rich lipid microdomains played a decisive role in inducing its transition to an insertion-competent configuration. The fully active 65 kDa toxin and its 50 kDa variant, generated by a 15 kDa carboxy-terminal deletion, were incubated with phosphatidylcholine-cholesterol vesicles, dispersed in 1% Triton X-100 and fractionated on Sepharose CL-4B in presence of the detergent. It may be recalled that the truncated toxin was hemolytically100-fold less active but otherwise very similar to the 65 kDa toxin. It was observed that the 65 kDa oligomer preferentially associated with the phospholipid-cholesterol vesicles that were not solubilized by the detergent. In contrast, the 50 kDa toxin was completely solubilized. This observation suggested a correlation between raft-association and efficiency of membrane permeabilization activity. We are making efforts to repeat the experiments with biomembranes.

Earlier, we proposed the hypothesis that the hemolytically active toxin represented a quasi-stable state corresponding to a local energy minimum and the amphipathic heptamer a thermodynamically stable terminal configuration. Recent evidence suggests that the survival of the hemolytically active quasi-stable monomer might depend on its association with a chaperone-like accessory molecule. The study is in progress.

4.4 Exploring the mechanism of the immunomodulatory functions of cholera toxin

Investigator: S.S. Das

Cholera is the most severe form of watery diarrhoea caused by the non-invasive gram-negative bacilli *Vibrio cholerae*. The major cause for diarrhoea is an enterotoxin called cholera toxin (CT). CT is an oligomeric protein comprising of a single A subunit (CT-A) and five identical B subunits (CT-B). Following its release into the extracellular environment of the gut after *V. cholerae* infection, CT binds to the ubiquitously expressed host cell-surface molecule GM1-ganglioside receptor via its B subunit and gets internalized by the host cell. Following trafficking to ER, the A subunit enters the cytosol and displays ADP ribosyltransferase activity that results in irreversible activation of adenylate cyclase and

an increase in intracellular cAMP concentration. Increased levels of cAMP activate PKA, leading finally to chloride secretion and osmotic diarrhoea.

Although CT generates a strong antibody response, the antitoxic antibody remains non-protective. Interestingly, CT and the structurally and functionally analogous heat-labile enterotoxin (LT) of enterotoxigenic *E. coli* (ETEC) show profound immunomodulatory activity that led to numerous attempts to test these molecules as adjuvants to mucosal vaccines against a variety of bacterial, viral and fungal pathogens. Several mechanisms have been proposed to explain the immunomodulatory functions of CT; many are contradictory and none is completely satisfactory.

Cholera toxin has been shown to upregulate the co-stimulatory molecule B7.2, but not B7.1, expression on murine B-cells and macrophages (6), induce isotype switching of B-cells, result in apoptosis of $CD4^+$ Th1 cells and $CD8^+$ intraepithelial lymphocytes and induce maturation of blood monocyte-derived dendritic cells (DCs), promoting upregulated expression of HLA-DR and B7.1, B7.2 and CD134. CT treatment of LPS-stimulated macrophages increases the production of IL-6, IL-10 and IL-1 and decreases the production of IL-12, TNF- and nitric oxide. While CT by itself releases low levels of IL-1 and IL-8 from human monocytic cell line THP-1, it potently regulated cytokine induction in cells activated by bacterial LPS or fimbriae, induction of TNF- and IL-8 were downregulated while IL-10 and IL-1 were upregulated. Pre-incubation of J774.A2 macrophage cell line with CT significantly downregulated LPS- induced nitric oxide synthesis and PMA-induced respiratory burst, whereas simultaneous addition of CT and LPS enhanced the production of TNF-. CT synergized with low doses of LPS to induce IL-10 production by immature DCs, but inhibits LPS driven induction of CD40 and ICAM-1 expression and secretion of inflammatory cytokines/chemokines IL-12, TNF-, MIP-1, MIP-1 and MCP-1; thus, CT suppresses the generation of Th1 cells and promotes the induction of T-regulatory (T_{reg}) cells.

Cholera toxin also acts through the intestinal epithelial cells that form a critical component of the mucosal innate immune system. CT increases the permeability of the murine intestinal epithelium to low molecular weight peptides. It also enhances IL-6 secretion by IEC-6 intestinal epithelial cell line. CT-B caused selective dose-dependent increase in IL-10 production by several intestinal epithelial cell lines. Several recent reports showed that infection of intestinal epithelial cell lines with *V.cholerae* result in secretion of a number of cytokines and chemokines, but the bacterial factors responsible for such effects have not been conclusively identified.

Molecular mechanism behind the immunomodulatory effect of CT is unclear. An early report from Lycke and co-workers suggested that mutant recombinant CT and LT that lacked ADP-ribosylating activity were deficient in the immunomodulatory effect and as a result, failed to act as adjuvants upon oral administration. More recently, several groups have reported that ADP-ribosylating activity contributes to, but is not essential for, the adjuvant properties of CT and LT. Thus, CTB/LTB or enzymatically inactive or partly active mutants of the holotoxin (LTA72R, LT T63K, CTT63K, LT R192G, CTS106) have been shown to retain the immunoenhancing functions.

NF-κB and ERK MAPK are two major signal transduction pathways that play critical roles in the immune response to microbial pathogens.

These signal transduction pathways regulate the release of a number of cytokines and chemokines including IL-6, IL-8, TNF-α, Gro-α, MCP-1, RANTES, MIG and IP-10 from various immune cells as well as the epithelial cells. These pathways are activated by LT and may contribute directly to its immunomodulatory effect. LT-B induces $CD8^+$ T-cell apoptosis by transcriptionally activating caspase-3 in an NF-κB-dependent manner. It was also shown that GM1 binding by recombinant LT-B activates ERK MAPK that is dependent on phosphoinositide 3-kinase (PI-3K) and protein kinase C (PKC) activity. PI-3K activity is also critical for the upregulation of CD25 and MHC class II expression. However, little is known about the CT-induced regulation of NF-κB and MAPK pathways. One recent study documented the involvement of the NF--κB pathway in the interaction between CT and the targeted APC, suggesting effects on gene transcription associated with inflammatory responses, but whether this activation was a prerequisite for an adjuvant effect was not shown.

We propose to investigate whether NF--κB and ERK signaling pathways are regulated by cholera toxin and whether they contribute to cholera toxin induced immunomodulation.

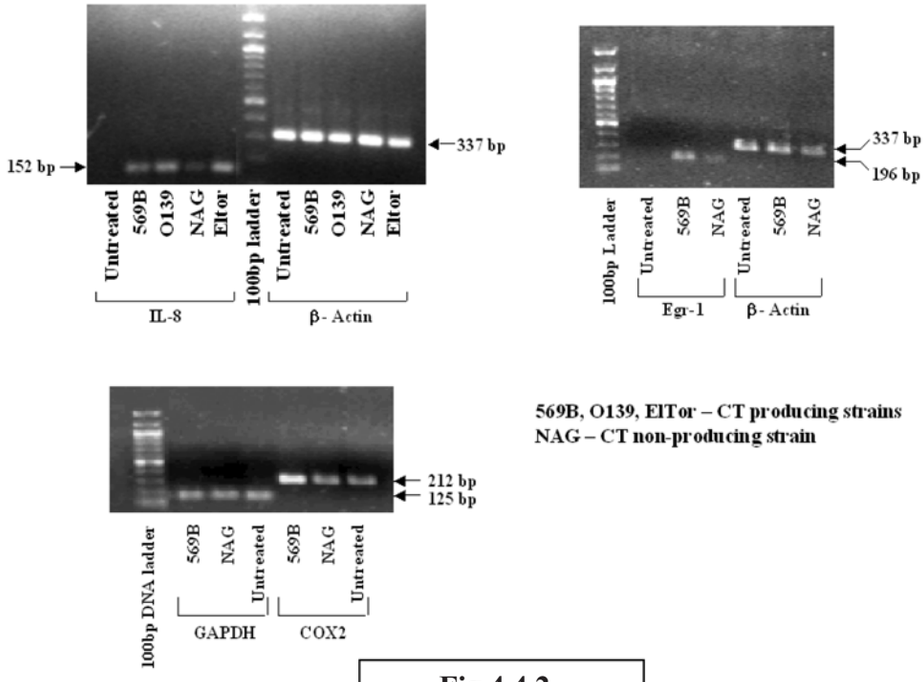
Exploring the mechanisms behind the immunomodulatory role of CT will help in better understanding of the pathogenesis of cholera. At the same time, it will promote improved designing of vaccines against *V. cholerae* and other mucosal pathogens.

Role of cholera toxin (CT) in the regulation of the immunological functions of intestinal epithelial cells and its contribution to the overall immunoregulatory / adjuvant functions of CT is not well studied. We planned to use human intestinal epithelial cell lines HT-29 and Caco2 and pursue two different methods to generate purified CT. Firstly, we designed experiments to express recombinant CT in *E. coli* and purify it using Ni-NTA agarose columns. For this purpose, we cloned CT into prokaryotic inducible expression vector pET28a (Fig. 4.4.1) and transformed *E. coli* BL21 (DE3) cells with this vector. IPTG induced low levels of CT expression that exerted significant toxicity against *E. coli*. We are currently working on maximizing the expression of CT and minimizing its toxicity on *E. coli*. Secondly, we are working on purifying CT from the culture supernatants of *Vibrio cholerae* 569B and a hypertoxigenic strain of *V. cholerae* O395. This is currently at its early stage. We planned to follow the protocol as described by Mekalonos et al (29). Briefly, CT will be purified by ion exchange chromatography using phosphocellulose P11 columns and will be eluted out of the columns by altering the pH of the buffer.

In absence of the availability of purified CT at the present moment, we planned to investigate its role in the regulation of the immunological functions of intestinal epithelial cells by stimulating post-confluent HT-29 cells with the culture supernatants of CT- positive and CT-negative strains of *V. cholerae*. Expression of immune response genes was studied by semiquantitative RT-PCR of total RNAs prepared from the cells stimulated in the presence and absence of CT, using amplification of -actin as a loading control. We found that pro-inflammatory genes like IL-8, Cox-2 and Egr-1 were upregulated in presence of CT (Fig.4.4.2). However, our results may have been influenced by the presence of other secreted molecules in the culture supernatants. This issue will be addressed in future when we will use

Fig.4.4.1

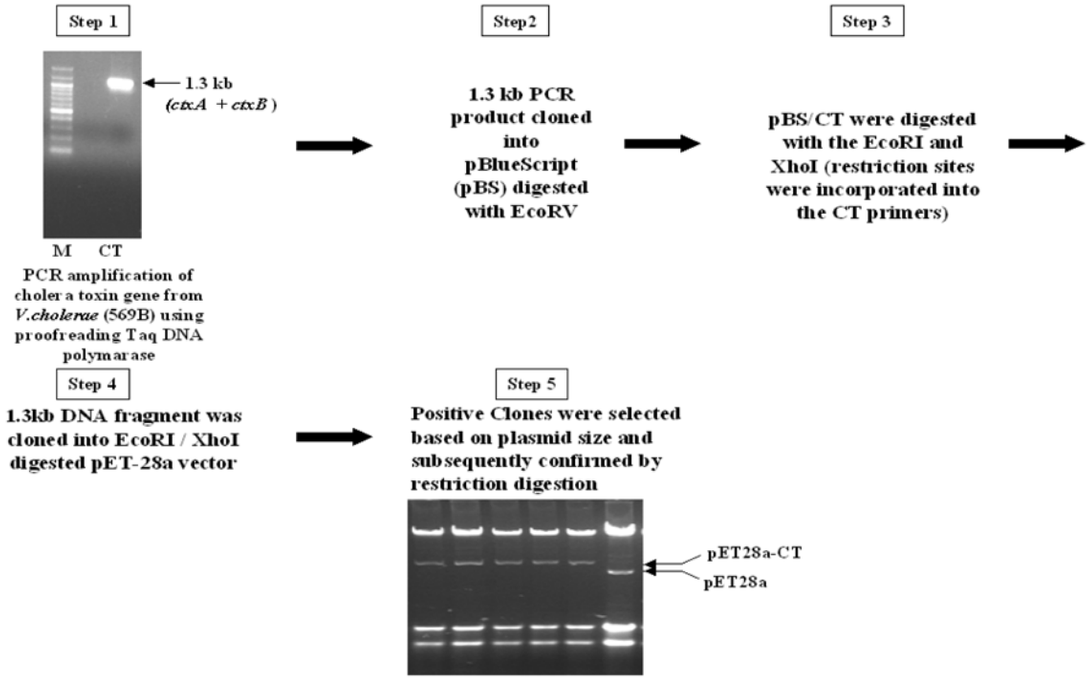
RT-PCR amplification of genes after treating HT-29 cells with cholera toxin



569B, O139, ElTor – CT producing strains
NAG – CT non-producing strain

Fig.4.4.2

Cloning of cholera toxin gene from *V.cholerae* (569B) into pET28a expression vector



4.5 Enterotoxigenicity of 45-kDa matured and 35-kDa processed forms of hemagglutinin protease purified from a cholera toxin gene negative *Vibrio cholerae* non-O1, non-O139 strain

Investigator: A. Pal

Cholera toxin gene negative *Vibrio cholerae* non-O1, non-O139 strain PL-21 is the etiologic agent of cholera like syndrome. Hemagglutinin protease (HAP) is one of the major secretory proteins of PL-21. The 45-kDa matured and the 35-kDa processed forms of HAP were purified in the presence and absence of EDTA from culture supernatants of PL-21. *V. cholerae* O1 classical and ElTor biotypes as well as non-O1 serotypes produce HAP. The secreted HAP has been purified and sequenced. The *hap* gene consists of 1827 nucleotides with a predicted molecular weight of 69.3 kDa. This is larger than the molecular weight of the purified HAP (32 kDa). When the protease is purified in the presence of protease inhibitors, a larger form of HAP (approximately 45-kDa) is isolated. The protease undergoes several steps of processing including cleavage of the signal peptide of the proprotein (69.3-kDa) to generate the mature N terminal form (45-kDa) and a further proteolytic processing of its C-terminal region generating the 32-kDa form. Ours is the first study where both the 45-kDa mature and the 35-kDa C-terminally processed forms of HAP have been purified from a *ctx* negative *V. cholerae* non-O1, non-O139 strain, PL-21. We have shown that the 35-kDa processed form of HAP induced in a dose dependent hemorrhagic response in the RIL assay, a decrease in the intestinal short circuit current in Ussing's chamber and a cell rounding effect on HeLa cells. The 45-kDa mature form showed an increase in intestinal short circuit current in Ussing's chamber and a cell distending effect on HeLa cells. PL-21 gave rise to severe watery diarrhea in the RITARD model. Histopathological examination of the ileal section derived from the RITARD and RIL assays showed infiltration of inflammatory cells into the gut mucosa. Our results strongly suggest that HAP plays a major role in the pathogenesis of the disease caused by PL-21.

5 Studies on Vibrio Cholerae Phages

5. Studies on *Vibrio cholerae* phages

5.1 Nationwide screening of phage types of *V. cholerae* O1 biotype ElTor

Investigator: B.L. Sarkar.

During the period under study, a total of 981 strains of *V.cholerae* were received from different parts of the country including West Bengal for serotyping, biotyping and phage typing. Of these, 678 (69.11%) representative strains confirmed as *V. cholerae* O1 biotype ElTor were included in phage typing study. This year, highest number of strains was received from Maharashtra. Majority of the strains belonged to Ogawa. For the last couple of years, Ogawa was the dominant serogroup. A total of 30 strains were found to be untypeable with the conventional scheme of Basu and Mukerjee. Using the new scheme, all of these strains were found to be typeable and could be clustered into a number of distinct types of which majority were grouped under type 27 (88.2%) followed by type 26 (3.69%), type 24 (3.24%), type 20 (0.74%) respectively. It has been observed that type 27 was the predominant phage type circulating in this country.

5.2 Molecular analysis of *Vibrio cholerae* bacteriophages: cloning and sequencing of phage DNA

Investigators: B.L. Sarkar

The N-4Φ is one of unique O1 lytic phages of *V. cholerae* O1 biotype ElTor phage typing scheme. Most of currently prevailing *V. cholerae* O1 strains are sensitive to this phage. Therefore, this phage was selected for complete nucleotide sequencing. Genomic DNA was purified from a stock of high titre phage (10^{11}pfu/ml). Subsequently, phage DNA was subjected to enzymatic digestion with twenty eight restriction enzymes. Additionally, the analysis was further supported by the digestion profile generated with *EcoT*14*I*, *EcoT*22*I*, *TthHB* 8*I* and *Msp*I. Based on the estimated sizes of the DNA fragments obtained with *Hin*dIII and E*co*RV, genome of the phage was estimated to be about 40 kb in size. The purified phage DNA (free from host genomic DNA) was randomly sheared using an ultrasonic disintegrator. Shearing of DNA was controlled in such a way to generate fragments ranging from 0.2-1kb with a majotity between 500-600bp region. Sheared DNA fragments were treated with Mungbean nuclease to generate blunt ends and electrophoresed onto agarose gels. DNA fragments ranging between 500 and 600bp sizes were cut out from the gel and recovered from the agarose blocks. Gel-eluted fragments ranging between 500 and 600 bp was ligated to *Eco*RV digested cloning vector pZero-2.1. Transformants were selected on kanamycin (50µg/ml) plates containing 1 mM IPTG. It may be mentioned here that the vector pZero-2.1 contains lethal gene *ccd*B under IPTG inducible promoter. The strategy involves insertion of the DNA fragments within the *ccdB* locus. Therefore, selection of transformants on plates containing both kanamycin and IPTG will reduce the chance of getting false positive clones. A total of 470 transformants were arbitrarily picked up for further study. Purified plasmid DNA was isolated form randomly

selected 170 clones and tested for the presence of insert. Results showed that among these 170 clones, contained inserts with sizes ranging between 500 and 600bp. Nucleotide sequencing were carried out using these plasmids as a source of template DNA while universal M13 primers were used as sequencing primer. So far, 130 clones were sequenced and nucleotide sequence data were assembled using the software DNASTAR. Assembly of the reads resulted into 26 contigs with maximum and minimum sizes are 4000 and 500 bp respectively. Numerical addition of the sizes of all the contigs could cover 30 kb region of the phage. Despite the fact that existence of many gaps to join these contigs, BLAST searches were carried out with individual contigs. Interestingly, nucleotide sequence data generated from most these contigs did not show any significant match to the data available in the public domain databases. The studies are underway to complete the whole genome sequence of said phage.

6 Studies on *Shigella* species

6. Studies on *Shigella* species

6.1 Molecular characterization of multi-drug resistant *Shigella flexneri* In Kolkata

Investigators: S.K. Niyogi

Shigellosis is a major public health problem in developing countries. Increased incidence of antibiotic resistance in *Shigella spp*. constitute a major concern. High frequency of resistance of *Shigella flexneri* to many of the first line antimicrobial agents (multi drug resistant) have been reported in recent years from Kolkata. Most of the conventional typing methods are based on the phenotypic properties of the micro-organisms and offer little strain discriminatory information. The objective of this study is to analyze clonal relationships among isolates of multi-drug resistant *Shigella flexneri* using different molecular typing methods to determine changes at the genetic level and to understand their implications in the epidemiology of the diseases. During the period under study a total of 589 stool samples from Dr. B.C. Roy Memorial Hospital for Children were screened for detection of *Shigella spp*. To isolate *Shigella spp.*, stool samples were inoculated onto MacConkey, XLD, HEA and SS agar plates (Difco, USA) and the resulting colonies which exhibited characteristics of *Shigella spp* were identified by conventional biochemical methods. Subsequently serogroups and serotyping were identified by slide agglutination using commercially available poly and monovalent antisera (Denka Sliken Co., Japan).

Antimicrobial susceptibility tests were performed by an agar diffusion disk method as advocated by the National Committee for Clinical Laboratory Standards. Mueller Hinton agar was obtained from Difco, Detroit, USA and antimicrobial disks were obtained from Difco (Detroit, USA). MIC of the strains against different antimicrobial agents were determined by E-test (AB BIODISK, Solna, Sweden) following manufacturers instructions.

Out of 355 stool specimens 33 (9.2%) were positive for *Shigella spp.* Among Shigella strains 17 (51%) were *Shigella flexneri*, 3 (9%) were *Shigella dysenteriae*, 10 (30%) were *Shigella sonnei* and 3 (9%) was *Shigella boydii*. All these isolates were tested for their antimicrobial susceptibility patterns to evaluate the possible mechanism of quinolone resistance. During the study period *S. flexneri* was the most prevalent serogroup and *S. flexneri* serotype 2a was the predominant serotype among the strains isolated. All *S. flexneri* were found to be multiple antibiotic resistant, few strains of *S. flexneri* type 2a were resistant to fluoroquinolone and the MIC of these strains were >256, 4-8, 10-16, and 12-16µg/ml for nalidixic acid, ciprofloxacin, norfloxacin, and ofloxacin respectively. Few strains were also found resistant against gatifloxacin. However, all were found susceptible against azithromycin and ceftriaxone. All the tested strains uniformly harbored *ipa*H, *ial*, *Shigella* enterotoxin 1 genes. Digestion of chromosomal DNA with the restriction endonuclease Xba1 produced clearly resolvable restriction endonuclease analysis (REA) pattern after PFGE (Fig. 6.1.1).

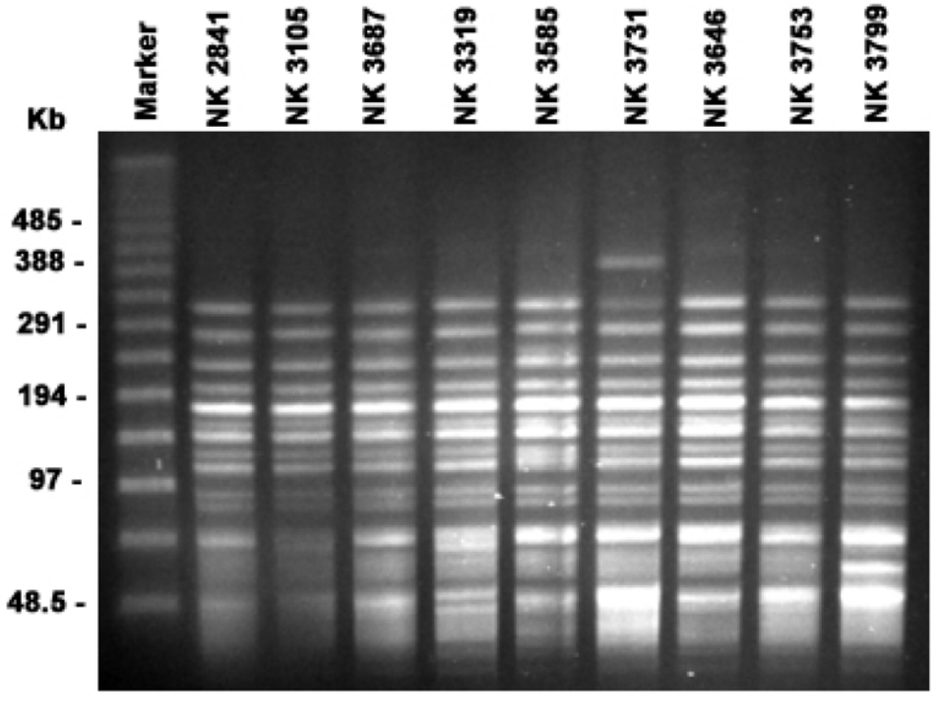


Fig. 6.1.1

6.2 Antigenic recognitation of *Shigella dysenteriae* outer membrane proteins using human convalescent sera and to evaluate their role in cell-mediated immune response in Shigellosis

Investigator: A.K. Sinha.

The study was undertaken with the objectives of identification of major antigenic outer membrane proteins (OMPs) from *S. dysenteriae* 1 using acute and convalescent sera from human and evaluation of the T-cell function in cell-mediated immune (CMI) response and finally demonstration of the protective role of such immunodominant components if any, will be carried out in animals.

Previously, it was reported that sharing of IL2 to form a high-affinity of receptor complex with $CD4^+$ T-cells through motive signals suggested a generalized T-cell activation with increased humoral responses. Macrophage migration inhibition factor (MIF) and IL4 response during anti-CD3 stimulation of immunized mice indicated the role of anti-CD3 in generation of O^-2 is due to synergistic effect by Th1 subsets of Th0 cells. The above findings should have implications for understanding the immuno-regulatory role of anti-CD3 associated with 57kDa antigen in immunoprophylactic measures. It was also established that 57kDa major antigenic OMP is immunogenic for MHC II-restricted T-cell response to acquire host defense against *Shigella* infection. The above findings were also published.

In continuation to earlier study, the antigen-specific T-cell signalling via T-cell antigen receptor stimulation was carried out in BALB/c mice immunized with the 57kDa major

antigenic component of Shigella dysenteriae type 1 outer membrane proteins. In presence of anti-CD3, the 57kDa major antigen increases the level of IL-2 significantly instead of IL-4. IL-2 production in T-cells was consistent with an increase in intracellular free Ca 2+ [$(Ca^{2+})i$] concentration. The antigen-specific modulation was observed during T-cell signalling, with enhanced release of [$(Ca^{2+})i$]. Furthermore, the phosphatidylinositol-specific kinases are regulated by phospohorylation of tyrosine kinase through the activation of the T-cell antigen receptor. The above findings indicate that phosphotidylinositol-3 kinase -mediated signals are up-regulated through [$(Ca^{2+})i$] which is essential for Th1-type responses. The above findings were published. Further, an effort was also made to analyze the effect of in-vitro stimulation on macrophages using killed *Shigella dysenteriae* type-1 (KSD1) coupled with anti-Interferon Gamma (anti-IFN-γ) antibody. The stimulated macrophages were co-cultured with primed or non-primed T-cells from Shigella infected patients. T-cell cultures were also established by co-culturing KSD1 coupled with or without PHA stimulated macrophages. Emulsified KSD1 coupled with anti- IFN-γ antibody was found to act as a potent immunogen, inducing the release of Th1 cytokine from primed T-cells cultured in acute stage of the disease and was also associated with substantial production of superoxide ions (O-2), which probably inhibits the colonization of intracellular Shigella due to the presence of anti- IFN-γ antibodies. The above findings reflect that in the presence of anti- shigellosis.

All the above findings were published recently.

6.3 Porin Induced Polarization of Peritoneal Macrophage and Activation of T cells for Th1/Th2 Response

Investigator: T. Biswas

Porin of *Shigella dysenteriae* type 1 coexpressed Toll-like receptor (TLR) 2 and TLR6 on peritoneal cavity (PerC) macrophages (Mϕ of C57BL/6 mouse implicating that both the TLRs are essential as a combinatorial repertoire to recognize the protein. Besides TLRs, mRNA for MyD88, TRAF6 and NK- κB was enhanced that indicate their involvement in tandem in the activity of porin. The protein selectively up-regulated CD80 on the activated Mϕ together with MHC class II molecule and CD40, and had no effect on CD86 expression. The porin-induced profile of MIP-1α, MIP-1β and RANTES showed strong bias for chemokines correlated with M1 polarization. Intracellular expression of TNF-α and IL-12 in presence of porin suggests type I polarization of the Mϕ that would influence Th1-type response (Fig.6.3.1).

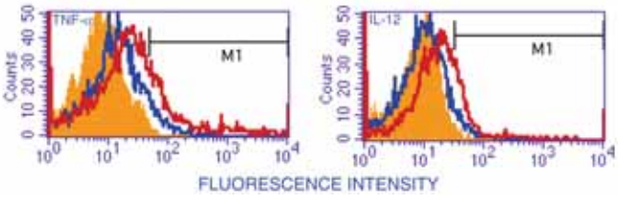


Fig. 6.3.1 Induction of intracellular cytokine expression by porin

7 Studies on *Escherichia coli*

7. Studies on *Escherichia coli*

7.1 Studies on the binding of *Escherichia coli* heat-stable enterotoxin to the intestinal epithelial cells and brush border membranes of different animals.

Investigator: M.K. Chakrabarti

The principal objective of this study was to determine the presence and density of the STa receptor in intestinal epithelial cell and brush border membranes of different animals and to purify and characterize the receptor for STa from a high-density receptor system. Moreover the exact mechanism of action of STa was also to be explored. We reported earlier that binding of ^{125}STa to the brush border membranes of rat, rabbit, hamster and guineapig was specific, time and temperature dependent. A single class of receptors was present in all the tested animals and the number of receptors remained lower in hamster in comparison to rat, rabbit and guineapig. Autoradiographic demonstration of SDS-PAGE of intestinal brush border membranes showed STa binding proteins of apparent MW of 160 KDa in rat, 118 KDa in guineapig, 140 and 38 KDa in rabbit and 65 KDa in hamster. We also reported that STa binds to a single class of receptors in COLO-205 human colonic carcinoma cell. Binding was specific, time and temperature dependent. STa binding protein with MW of 95 KDa was detected in this cell line. STa was found to stimulate G-cyclase in COLO-205. It has been found that besides stimulating cGMP STa also involves two potential intracellular signal. It increases rapidly inositol triphosphate and cytosolic free calcium in COLO-205 cells prelabelled with myo[2-^{3}H] inositol resulted in a rapid rise of [^{3}H] inositol triphosphate . Using fluorescent indicator, Fura 2AM, intracellular free Ca^{2+} has been found to increase 5.12 fold compared to control. Suspension of cells in calcium was chelated with EGTA. This effect was not observed with cells that were pretreated with dantrolene which suggest that the intracellular calcium rise might be due to mobilisation of intracellular stores. This study demonstrated for the first time a change in cytosolic calcium in cultured human colonic cell by STa, which was accompanied by inositol triphosphate activation. The involvement of protein kinase C (PKC) in the mechanism of action of STa in COLO 205 had been shown. STa treatment causes translocation of PKC from cytosol to membrane fraction of COLO 205 in a Ca^{2+} dependent manner and PKC might have some role in the regulation of guanylate cyclase. These findings have been further supported by the fluorescence ratio imaging studies by using fura-2AM. Moreover, involvement of nitric oxide in the mechanism of action of Ecoli-STa in COLO-205 cells was determined by using the nitric oxide probe 4,5 diaminofluorescein-2 diacetate (DAF 2DA) as a probe. It was found that Ecoli STa increases the intracellular nitric oxide level in COLO-205 cell line. It was found that Ecoli STa increases the intracellular nitric oxide level in COLO-205 cell line by activation of intracellular nitric oxide synthase. For the better yield and characterization of Ecoli STa receptor of COLO-205 cell line an attempt had been made to amplify and clone the Ecoli STa receptor gene of COLO-205 cell line to a suitable eukaryotic system. At first the receptor gene was amplified and the product was found to be of molecular size of 3.5kb.

During the reported period an attempt has been made to clone the PCR amplified STa receptor gene into a suitable cloning vector. 3.5kb

amplified fragment (2-4l) was mixed with TOPO-TA cloning vector (Invitrogen Corporation) and was incubated at room temperature for 20 mins.Then 3l of TOPO reaction mixture was added to *E.coli* (DH5) competent cell for transformation by following standard protocol. After that, transformed colonies of *E.coli* was selected by blue-white selection procedure. For confirmation of cloning, plasmid DNA was isolated from the transformed *E.coli* cells and was subjected to agarose gel electrophoresis and shifting of band compared to the control vector was observed. For further confirmation restriction digestion by EcoR1 enzyme was performed. Then the digested product (STa receptor gene, size-3.5kb) was partially sequenced by using M13 forward and reverse primers. Further studies are in progress.

7.2 Electron microscopic study of ultra structures associated with Intestinal cell adherence of enteroaggregative *Escherichia coli* isolated from acute hospitalized diarrhea cases and healthy control children from Kolkata

Investigators: S. Dutta

Objectives of the study was to demonstrate the ultra microscopic structure of attachment and penetration of EAEC strains into intestinal cells at various culture conditions and to compare the attachment to intestinal cells by EM study among two groups of EAEC strains : one group from diarrhoeal children and another from healthy controls

Strains of EAEC were selected from two groups of the study for comparison. These strains have already been tested for virulence markers by PCR and for a number of phenotypic properties by conventional techniques. One set of the strains was isolated from hospitalized children with acute diarrhoea and the other was from comparable healthy controls. Both groups of strains showed typical stacked brick appearance by light microscopy on in vitro cell culture.

Intestinal cells would be gently scrapped off and prefixed with 3% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.20, post fixed with 1% buffered osmium tetroxide dehydrated through graded series of ethanol and embedded in agar 100 resin. Ultra thin sections were cut, stained with uranyl acetate and lead citrate and examined under EM.

On examination, the bacterial cells were found adherent to the intestinal cells. The brush borders of epithelium have been destroyed by the bacteria and internalization of the bacteria by epithelial cells was observed. But there was no obvious difference was found between two groups of strains. Hence it may be concluded that in vitro interaction of the bacteria with cell was more or less similar in case and control groups and it does not have any effect on disease progression and persistence.

As the objectives have been achieved the study has been proposed to be concluded with SAC members approval.

7.3 Detection and characterization of different colonization factors for molecular typing of Enterotoxigenic *Escherichia coli*

Investigator: N.S. Chatterjee

Enterotoxigenic *Escherichia coli* (ETEC) are an important cause of diarrheal disease in humans, affecting children and adults. In particular, ETEC is a cause of morbidity and mortality in children up to 5 years of age in developing countries. ETEC strains have two major virulence determinants: the enterotoxins (the heat-labile

toxin [LT] and the heat-stable toxin [ST]) and the colonization factor antigens (CFAs).

Over 20 distinct, human-specific ETEC adhesins or colonization factor antigens (CFAs) have been described. In many geographic areas, the most common CFs individually expressed by ETEC strains are CF antigen I (CFA/I), CFA/II, which is composed of coli surface antigen CS3 alone or in combination with CS1 or CS2 and CFA/IV, which is composed of CS6 alone or in combination with CS4 or CS5. To cause disease, ETEC must first adhere to the epithelium of the small intestine by means of the CFAs and then produce secretory diarrhea due to the effects of the enterotoxin(s). The overall goal is to develop a simple and specific method for detection of different colonization factor antigens (CFAs) for typing enterotoxigenic *Escherichia coli* (ETEC), identify the most prevalent CFAs and characterize them. This will also help us in tracking the movement of ETECs round the globe.

We wanted to develop a PCR-based method to determine the colonization factors present in the enterotoxigenic *E. coli* (ETEC) namely, CFAI, CFAII (CS1, CS2, CS3), CFA III (CS8), CFA IV (CS4, CS5, CS6), CS14, CS17, CS19. The data obtained will be analyzed for a possible association between toxin types and CFAs on ETEC isolates and the severity of diarrhoea in patients. Till today, serology-based techniques, using a variety of mouse monoclonal antibodies against 12 different CFAs, are used to determine prospectively the toxin types and CFAs on fresh isolates of *E. coli*. This study is aimed to establish a PCR-based detection method. Once the method is standardized, this could be used for surveillance studies using clinical isolates.

The CFAs have been implicated as protective antigens and have accordingly become the focus of ETEC vaccine development efforts. As specific vaccine candidates advance to field evaluation, it becomes imperative to characterize the extent of phenotypic diversity of ETEC with respect to these virulence factors to permit predictions of the theoretical extent of vaccine coverage. Since the relative distribution of ETEC toxin and CF phenotypes can vary from one geographic location to another, specific data are needed for each site where vaccine testing is contemplated.

Based on the ETEC cDNA sequences available in the GenBank, primers were designed to detect 10 of the CFAs and CS antigens. Calculated amplicon size is listed below:

COLONIZATION FACTOR ANTIGEN (CFA)	COLI SURFACE (CS) ANTIGEN	PRODUCT SIZE (bp)
CFA/ I		364
CFA/ II	CS1	324
	CS2	368
	CS3	264
CFA/ III	CS8	437
CFA/ VI	CS4	250
	CS5	453
	CS6	321
	CS14 (PCFO166)	394
	CS17	290
	CS19	-

Initially, we used seven strains that were previously classified by serology, were used to standardize the PCR conditions. All seven strains were correctly identified.

Next, we did PCR using DNA as the template isolated from the same strains. Interestingly, multiple and additional CFAs were identified; the result suggested that the strains contained multiple CFA genes but did not express all the genes that were present, suggesting that environmental factors play a role in their expression.

Then we analyzed several strains that were previously analyzed by serology and were classified as non-typeable by this method. PCR based method resulted in specific amplicons. The amplicons were randomly confirmed by automated DNA sequencing. The PCR-based assay was able to identify the ETECs based on the colonization factors expressed by them. Thus, our results suggested that ETEC strains could be classified on the basis of the newly developed PCR-based method.

CS6 was found to be more prevalent in the strains classified by PCR-based method. 15 strains were sequenced for cssA (a subunit of CS6). Variations of amino acids were found in different strains. Analysis of the data revealed that these variations might lead to change in structure and thus antigenicity. Currently used antibody might fail to interact with these regions, suggesting that not all strains may not be typeable by serology method. We are currently analyzing cssB, the other subunit of CS6. We are also in the process of raising antibody against the CFAs using synthetic peptides to confirm our result.

8 Studies on *Helicobacter pylori*

8. Stduies on *Helicobacter pylori*

8.1 Characterization of the *cag* pathogenicity island of *Helicobacter pylori* isolated in Kolkata with reference to interleukin-8 secretion.

Investigator: A.K. Mukhopadhyay

CagA is a 120-145 kDa protein with a carboxy terminal variable region. Length polymorphism observed at the 3' end of *cagA* gene of *H. pylori*, which results in variation in the number of phosphorylation sites of the encoded protein (CagA), is of great interest in recent times since higher number of phosphorylation sites in CagA was described to be associated with stronger biological function and disease manifestation. To understand the length polymorphism at the 3' end of the cagA gene, one PCR assay was performed to amplify the 3' end region using two primers namely CAG1 and CAG2. A total of 75 strains, 40 isolated from duodenal ulcer patients, 20 isolated from healthy volunteers and 15 from non-ulcer dyspeptics were included in this study. PCR results showed that all these strains carry *cagA* gene but yielded amplicons with different sizes.

Further analysis of the amino acid sequences predicted from nucleotide sequences of *cagA* 3' end revealed some distinct features present in this repeat region. *H. pylori* strains isolated from Calcutta carried *cagA* genes capable of coding proteins, which may contain EPIYA motifs as low as 1 to as high as 5 in number. All these EPIYA motifs are preceded and followed by distinct, short stretch of amino acid sequences.

The first EPIYA motif (termed in this study as Y1 to denote tyrosine phosphorylation site) in this CagA primary structure was preceded by a stretch of 10 amino acids (NNNNNNGLKN), which contain 6 successive asparagines in tandem and was termed as S1 to denote the starting sequence for the repeat region, responsible sites for CagA phosphorylation. The first and the second EPIYA (termed as Y2) motifs are separated by a stretch of 13 amino acids (denoted as V1 in this study), as was described and named as R2 by Yamaoka *et al.* (1998), in all CagA sequences included in this study that carry A-B-C or A-B-C-C or A-B-C-C-C type EPIYA motifs.

A very exciting observation has come out from this study while analyzing the intervening sequences of third, fourth and fifth EPIYA (termed as Y3, Y4 and Y5) motifs. These intervening regions, originally described as R3 by Yamaoka *et al.* (1998) and described as repeat region containing WSS or ESS by Higashi *et al.* (2002), contained even shorter, discrete repeats as it was observed in the current study. The sequence, termed in this study as I1 (QVAKKVNNAKIDRLNQIASGLGGVG QAAG) was present immediately after the second EPIYA motif (Y2) and unlike CagA sequences found in Japan was never repeated. This stretch was followed by another stretch of amino acids, termed as I2 (FPLKKHDKVDDLSKVGLS) and a very short domain of only 3 amino acid residues, termed as I3 (ASP), which was present just before the third EPIYA motif (Y3).

After the third EPIYA motif (Y3), another distinct domain was noticed and termed as W1 (TIDDLGGP), which was again followed by I2 and I3 in A-B-C-C and A-B-C-C-C type CagA. In A-B-C type CagA, though W1 and I2 were always present, I3 was replaced by another domain, termed as T1 having sequence REQQL (or REQNL in some strains). However, in A-B-C-C-C type CagA, I3 is repeated for 3 times along with EPIYA motifs (at C positions) and after the last EPIYA at C position (Y5), I3 was replaced by T1. The comparative analysis of all the CagA primary

structures revealed that this T1, which must be present after I2 (and in the place of I3) indicates the termination of this repeat sequences and after T1 no EPIYA site was observed in any CagA primary structure.

To check whether the above variation in the 3' end of the cagA gene has any effect in the expression of the CagA protein, representative *H. pylori* strains suspended in PBS, carrying different CagA primary structures were boiled, run on polyacrylamide gel and the total proteins were transferred to nitrocellulose membrane. After blocking with 3% bovine serum albumin, the membranes were incubated with blocking solution containing rabbit polyclonal anti-CagA and then anti-rabbit IgG. All the strains were found to express CagA including strain San77 (AB-C-C-C type), which was isolated from HV. We also have performed the RT-PCR assay to check the expression of *cagA* mRNA taking representative *H. pylori* strains with varying *cagA* 3' end structure using *ureB* as internal control. All the H. pylori strains expressed cagA, which means variable repeat regions at the carboxy terminal have little effect in the expression of the cagA gene. Same set of strains when used to infect AGS cells, CagA was detected from the infected cell lysates by the western blot analysis except for PCR24 where the intensity was significantly diminished.

We investigated the capacity of CagA to bind SHP-2 in different *H. pylori* strains containing variable 3' end structures by co-immunoprecipitation and western blotting experiments. We have demonstrated here that *H. pylori* virulence factor CagA, which is translocated from the bacteria into gastric epithelial cells, can perturb mammalian signal transduction machineries and modify cellular functions by physically interacting with a host cell protein, SHP-2. SHP-2 is known to play an important positive role in the mitogenic signal transduction that connects receptor tyrosine kinases and ras. Also, SHP-2 is actively involved in the regulation of spreading, migration, and adhesion of cells. Deregulation of SHP-2 by CagA may induce abnormal proliferation and movement of gastric epithelial cells, promoting the acquisition of a cellular transformed phenotype. Thus, the potential of CagA to disturb host cell functions as a virulence factor could be determined by the degree of SHP-2 binding activity. Our results provide a molecular basis for the pathological actions of CagA on gastric epithelial cells.

Finally, amino acid sequence diversity in CagA among different *H. pylori* strains from Kolkata has been well documented. The phosphorylation of the EPIYA motif is located in the repeat region of CagA and is expanded by duplication. Accordingly, the number and sequence polymorphism of the CagA phosphorylation sites, which collectively determine binding affinity of CagA to SHP-2, may be important variables in determining the clinical outcome of infection by different *cagA H. pylori* strains.

We intend to continue this study for another year to understand more closely the translocation of different types of CagA through type IV secretion system and its binding efficiency with SHP-2 by immunoprecipitation assay along with their roles in IL-8 production.

8.2 Correlation of histology with genotypes of *Helicobacter pylori* isolated from cases of Peptic ulcer, Non ulcer dyspepsia, Gastric carcinoma and Lymphoma

Investigator: D.R. Saha

Helicobacter pylori, a gram negative spiral bacterium, is of major concern today because of its causal relationship with gastroduodenal diseases. The bacteria are prevalent worldwide and more than half of the people throughout the world are infected with *H. pylori.* Nearly 80-90% of the

population in developing world acquire *H.pylori* infection in early childhood and if untreated the infection lasts for decades. Most of them remain asymptomatic though a certain percentage develop peptic ulcer diseases including gastric lymphoma and even carcinoma. The bacteria have direct influence in the disease process though the actual pathology behind the disease is not clear.

This project was undertaken to determine the association and tissue response to *Helicobacter pylori* with different diseased conditions and in healthy volunteers (HVs) and to correlate the histologic findings with *CagA, VacA* and other virulent genetic pattern of the organism. Endoscopic biopsy samples were collected from fundus and antrum of the stomach from S.S.K.M hospital, Kolkata. Five bits of tissue were taken – one for Rapid urease test, two in Brucella broth with 15% glycerol for culture and two in buffered formalin for histopathological examination. About 80 healthy volunteers (40 from urban area and 40 tribal) were selected in the study. Volunteers from urban area belonged to different socioeconomic status of the society ranging from day labourers to medical students. The tribals were mostly cultivators and Santhals and Orans in origin. Each of these two groups of healthy volunteers had no specific gastric complain. Formalin treated tissues were processed for paraffin embedding and serial thin sections were cut, stained by Haematoxylene and Eosine (H&E) to see the histological changes by light microscopy. For better visualization of *H pylori* in gastric biopsies, few special stains like modified Giemsa and Immunohistochemical stain were done in addition to routine H&E stain. With the help of H&E stain gross morphological changes were detected. To know the genotypes of the strain, culture positive *H.pylori* specimens were further analysed by PCR using specific primers. Activity was frequently observed in *H.pylori* associated gastritis, which was evaluated by the presence of inflammatory cells in lamina propria and in glands. Histologically *H.pylori* positive active gastritis was detected in almost 80-90% of the HV subjects. A mixed genotyping pattern like *vacAs1 s2*, *vacA m1m2, iceA1* and *iceA2* allelic pattern were observed in few cases though most of the subjects studied so far were infected with a single strain. The major histopathological changes in the two groups and their relation with the genotypes of the strains are in progress.

9 Studies on *other bacterial pathogens*

9. Studies on other bacterial pathogens

9.1 Mode of action of *Yersinia enterocolitica* heat stable enterotoxin (YSTa) in rat intestinal epithelial cell.

Investigator: M.K. Chakrabarti

The principal objective of this study was to evaluate the mechanism of action of heat stable enterotoxin secreted by *Yersinia enterocolitica.* Yersinia enterocolitica heat stable enterotoxin (YSTa) was purified from the culture filtrate using ammonium sulfate precipitation, DEAE Sephacel and Sephacryl S-100 HR column chromatography. Fractions were tested for enterotoxicity by suckling mice assay. It was found that purified YSTa raised $[Ca^{2+}]_i$ in a dose dependent manner and the optimal level of $[Ca^{2+}]_i$ was achieved by incubating cells with 10ng YSTa. We reported earlier that Y-STa stimulated phopsholipase C activity. It was also found that Y-STa induced rise in intracellular calcium level by calcium influx from extracellular environment as well as IP_3 mediated calcium moblization from intracellular calcium store. In further support of the involvement of IP_3 mediated calcium mobilization in the mechanism of action of Y-STa evidence we have directly measured the intracellular IP_3 level and found that Y-STa increased the intracellular IP_3 level. Moreover, it was found that PLC- isoform might have a direct role in calcium influx across the plasma membrane.

During the reported period an attempt has been made to evaluate the involvement of nuclear calcium signaling in the mechanism of action of Y-STa. Calcium imaging with Time Series Confocal Microscopy shows that Y-STa stimulates both the nuclear and cytosolic calcium level in rat intestinal epithelial cells where rise in nuclear calcium precedes the cytosolic events. Western blot analysis reveals higher density of IP3 receptor (IP3R) type II in nuclear membrane compared to cytosol, which may be the cause of early rise of nuclear calcium level. Moreover, immunofluorescence study in Laser Scanning Confocal Microscope with anti-Protein kinase C- (PKC-) antibody shows that nuclear PKC- translocates earlier from nuclear interior to nuclear envelope in comparison to the translocation of cytosolic PKC- to plasma membrane. Inhibition of PKC- translocation by chelation of nuclear and cytosolic calcium with BAPTA (intracellular calcium chelator) has suggested that nuclear and cytosolic PKC-translocation are calcium dependent. So, we propose for the first time that Y-STa regulates the nuclear and cytosolic calcium signals in a distinct temporal manner in rat intestinal epithelial cells.

10 Studies on *diarrhoeagenic parasites*

10. Studies on diarrhoeagenic parasites

10.1 Cloning and Characterization of Collagenase genes of *Entamoeba histolytica*

Investigator: A. Mazumder

Isolated electron dense granules (EDG) were characterized by biochemical and immunological parameters. The purified EDG showed 8 times more collagenolytic activity than the whole *E.histolytica* trophozoites. Purified EDG showed six polypeptide bands with apparent molecular weights of 108, 106, 104, 97, 68 and 59 kDa that were not detected in whole *E.histolytica* extracts. Similarly, two protease activities with apparent molecular weights of 40 and 85 kDa were detected only in EDGs. Scanning transmission electron microscopy clearly demonstrated that EDGs were highly complex molecule, mainly made of cations. Collagenolytic activity from EDG has been purified by FPLC MONO-Q HR 5/5-matrix and maximum activity was observed in fraction 16. However, no further functional studies could be conducted because, the purified enzyme was highly unstable. To overcome the problem several approaches were made. One that is working better is solubilization of EDG, using 1%Triton X-100 and demonstration of collagenolytic activity in the soup. The confirmation of collagenolytic activity of the soluble fraction was done by zymography analysis. A broad single clear zone compared to parent EDG in soluble fraction was seen at 85 kDa by zymography technique.

To purify the collagenolytic activity, the solubilized fraction from EDG has been applied on to the size exclusion matrix (Sephacryl 300). Fractions were collected and activity was tested by zymography in polyacrylamide co-polymerized collagen gel. Five fractions showed collagenolytic activity. These fractions were further purified by ion exchange chromatography. Purified fractions were collected and activity was once again tested by zymography. This purified fraction was transferred to PVDF membrane and send for sequencing.

Antibody raised against EDG was also used in screening a pathogenic *E. histolytica* cDNA library constructed in ZAP plus II vector. Ten plaques showing intense colour were digested with EcoRI and XhoI to release insert. Sequencing and BLAST search analysis of plasmid DNA of ten clones revealed homology with four genes in GenBank viz. Actinin like protein (ALP) (Acc.No. AF208390), Grainin 1 (Acc.No. AF085196), Serine rich protein (Acc.No. M34438), gEh29 gene for alkyl-hydroxyperoxidase reductase (Acc.No. X70996). Primers were designed to get the full-length gene coding for grainin I to study its function and localization in EDG. Clone PMLrfr5 representing the full-length protein (Grainin I) was digested with BamHI and HindIII and the released fragment was ligated to pMALC2X expression vector and transformed into competent *E. coli* XLI Blue cells. Plasmid from the positive clone having in frame orientation with the vector sequence was confirmed by sequencing. The right orientated plasmid was then transformed into *E.coli* BL21 (DE3) competent cells. Transformentant were screened by blue white screening on LA plate containing ampicillin and one of them harboring pMALC2X-grainin DNA was selected for recombinant protein expression.

In optimization experiment, LB 0.6 OD, 0.6 mM IPTG concentration, 6 hours, 26°C was optimal in expression of recombinant protein.

The recombinant protein was purified from soluble fraction using maltose affinity chromatography column as a maltose-tag fusion protein. The recombinant protein was eluted with 5 volumes of elution buffer containing 200mM maltose. Over expressed band appeared in 64 kDa in 10% SDS-PAGE analysis. After expression, affinity purification and proteolytic removal by factor-Xa protease of maltose binding protein, bands appeared at 42kDa and 22kDa in commassie stained SDS-PAGE. These 42kDa and 22kDa proteins represent the maltose binding and Grainin 1 protein respectively.

Characterization of recombinant purified protein

I. Antibody production in rabbit:

Rabbit immunized with recombinant Grainin 1 showed high antibody titre (1:6,400) by ELISA, with the expected specificity. Immunoglobulin IgG was the most represented serum antibody isotype.

II. Western blotting of Grainin 1 antigen:

The antibody raised against the recombinant purified protein showed a single immunoreactive band at 22kDa position against both crude soluble and plasma membrane antigen, thereby confirming that recombinant protein has similar epitopes with that present in the parasite.

III. Localization of Grainin 1 protein:

Intracellular localization of Grainin 1 protein was observed by confocal microscopy. Surface localization of recombinant 22-kDa antigen was observed by confocal microscopy. The specific fluorescence was found to be localized on the surface and mainly concentrated in vesicles of the *E.histolytica* trophozoites.

IV. Localization of Electron Dense Granules (EDGs) within *E. histolytica* using laser scanning confocal microscope:

The cells were examined under confocal laser microscope (Carl Zeiss, LSM 510 laser scanning microscope; Germany). FITC labeled EDG was visualized green after excitation at 514nm. The results of fluorescence staining with anti-EDG antibody to collagen treated trophozoites showed minimum fluorescence during first 3 hrs in cytosol, 6hr at membrane and almost no fluorescence after 10 hr in cells. Thereby suggesting the secretion of EDG to the extracellular milieu by 10 hrs of incubation.

V. Localization of Grainin 1 protein within EDG using laser scanning confocal microscope:

The cells were examined under confocal laser microscope (Carl Zeiss, LSM 510 laser scanning microscope; Germany). Cy5 labeled EDG was visualized red after excitation at 543nm and FITC labeled Grainin 1 was visualized green after excitation at 514nm. The localization and co-localization experiments clearly demonstrate that Grainin 1 protein is present within the electron dense granules.

VI. Immunoblotting:

The antibody raised against the recombinant purified protein showed a single immunoreactive band at 22kDa position against both in EDG and recombinant protein, thereby confirming that recombinant protein has similar epitopes with that present in the EDG.

11 Studies on *Viral Pathogens*

11. Studies on Viral Pathogens

11.1 Molecular characterization of a porcine group A rotavirus strain with G12 genotype specificity

Investigator: T.N. Naik

A porcine Group A rotavirus strain (RU172) was detected and molecularly characterized during a surveillance study conducted for rotavirus infection in a pig farm located in suburban area of Kolkata City, India. The G12 genotype specificity of RU172 was revealed by PCR based genotyping assays following addition of a G12 type specific primer (designed in our laboratory to pick up G12 isolates from field samples), and was confirmed by sequence analysis of the VP7 encoding gene. RU172 strain exhibited maximum VP7 identities of 93.6% to 94.5% with human G12 strains at deduced amino acid level. Inspite of its G12 genotype nature, RU172 appeared to be distinct from human G12 rotaviruses, and on phylogenetic analysis, formed a separate lineage with human G12 strains. Among the other gene segments analyzed, RU172 belonged to NSP4 genotype B, had a NSP5 and VP6 of porcine origin, and shared maximum VP4 identities with porcine P[7] rotaviruses (94.3%-95.4% at deduced amino acid level). Therefore, to the best of our knowledge, RU172 is the first report of detection of an animal rotavirus strain with G12 genotype specificity. Detection of strains like RU172 provides vital insights into the genomic diversity of Group A rotaviruses of man and animals.

11.2 Genomic diversity of Group A rotavirus strains in Eastern India.

Investigator: T.N. Naik

Rotavirus genotypes, G1-4 and G9 are important serotypes associate with childhood diarrhoea throughout the world. To determine G- and P-types of rotaviruses associated with dehydrating diarrhoea and to study the appearance and disappearance of G- and P- genotypes during the study period, an active surveillance was conducted for elucidation of rotavirus infection in two leading hospitals in Kolkata, West Bengal and Berhampur (GM), Orissa, India separated by 603 Kms from May 2005 to February 2006. The rotaviruses were detected by RNA electrophoresis in polyacrylamide gel. G- and P- typing of the positive samples were accomplished by amplifying VP7 and VP4 genes by RT-PCR and genotyped by multiplex PCR methods. The genotypic distribution varied remarkably well from our earlier study period (January 2002 to April 2005) with G1 (64%) was the most predominant strain followed by G2 (19%), G9 (11.1%) and G12 (5.5%) and not a single G3 or G4 was detected. On the other hand, 68% samples exhibited P[8] followed by mixed P-types (21.6%), P[4](5.4%), and P[6](5.4%). The three G9P[8] strains and one G9P[4] were identified by type-specific primes and then were confirmed by nucleotide sequencing. In contrast to the earlier study, G12 strains were replaced by G9 rotaviruses as the third most predominant strain in this part of the country.

11.3 Genogrouping rotaviruses of human and animal origin in India

Investigator : Triveni Krishnan

In course of the study faecal samples were collected from (a) acute watery diarrhoea cases admitted to the ongoing active surveillance programme at the Infectious Diseases & Beliaghata General Hospital and (b) diarrhoeic children brought for treatment to the NICED Unit at Dr B.C. Roy Memorial Hospital for Children in Kolkata. The rotavirus positives detected by PAGE and silver staining were documented to record their electropherotype and ascertain the Group A, B, or C nature of the rotaviruses respectively.

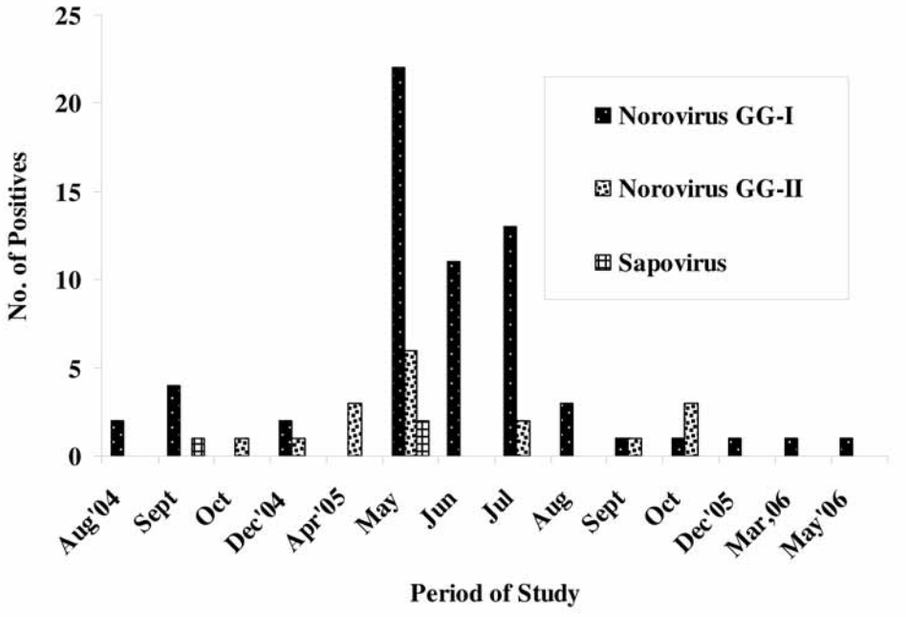
Rotaviruses are one of the major pathogens causing life threatening dehydrating gastroenteritis in children and animals where the trans-membrane glycoprotein NSP4 functions as a viral enterotoxin, capable of inducing diarrhoea in young mice. NSP4 gene of rotaviruses showing different electropherotype patterns of dsRNA was taken up for molecular characterization. RT PCR and direct sequencing of specific PCR product provided sequence data for full length of NSP4 genes from the different rotaviruses. Sequence analysis was carried out with different softwares to study the phylogenetic nature of the NSP4 genes sequenced and compare their sequence homology with others reported earlier in DNA databases. The sequence analysis showed distinct molecular diversity among NSP4 genes when the sequence homology was compared at nucleotide and predicted amino acid level among the different NSP4 genes. Hence the study indicates that genetic diversity of NSP4 genes serves as a very useful tool to understand molecular epidemiology of rotaviruses in circulation.

11.4 Molecular characterization of Human Caliciviruses, Astroviruses and Picobirnaviruses

Investigator : Triveni Krishnan

Human Caliciviruses and Astroviruses are single stranded, positive sense RNA viruses known to cause epidemic and sporadic gastroenteritis in humans. Epidemiological studies conducted in Kolkata also indicate that Caliciviruses are one of the most frequent causes of viral gastroenteritis besides rotaviruses (Fig. 11.4.1, 11.4.2). Among the Caliciviruses which belong to the family *Caliciviridae*, only two genera Norwalk-like Virus [NLVs] and Sapporo-like Virus [SLVs] are human pathogens with two genogroups [I & II] for the former and three genetic groups [(1) Sapporo, (2) London and (3) Parkville types] for the latter. The Stockholm strain might form the fourth group but its complete antigenic characterization is still awaited. Astroviruses belong to the family *Astroviridae* comprising of two genera Mamastrovirus with eight different serotypes and Avastrovirus. The molecular epidemiology of astroviruses in Kolkata shows that the strain is causing acute watery diarrhoea among children and a few adults (Fig. 11.4.3, 11.4.4). Human picobirnaviruses are bisegmented double stranded RNA viruses and are also found to be associated with acute watery diarrhoea in Kolkata (Fig. 11.4.5). The phylogenetic relationship of the Kolkata strains of the different diarrhoeagenic viruses is being studied to compare their genetic diversity with other strains of human caliciviruses, astroviruses and picobirnaviruses prevalent elsewhere in different parts of the world.

Fig. 11.4.1


RT-PCR positives for Human Calicivirus from ID & BG Hospital
No. of Positives
25
20
15
10
5
0
Norovirus GG-I
Norovirus GG-II
Sapovirus
Aug'04
Sept
Oct
Dec'04
Apr'05
May
Jun
Jul
Aug
Sept
Oct
Dec'05
Mar'06
May'06
Period of Study

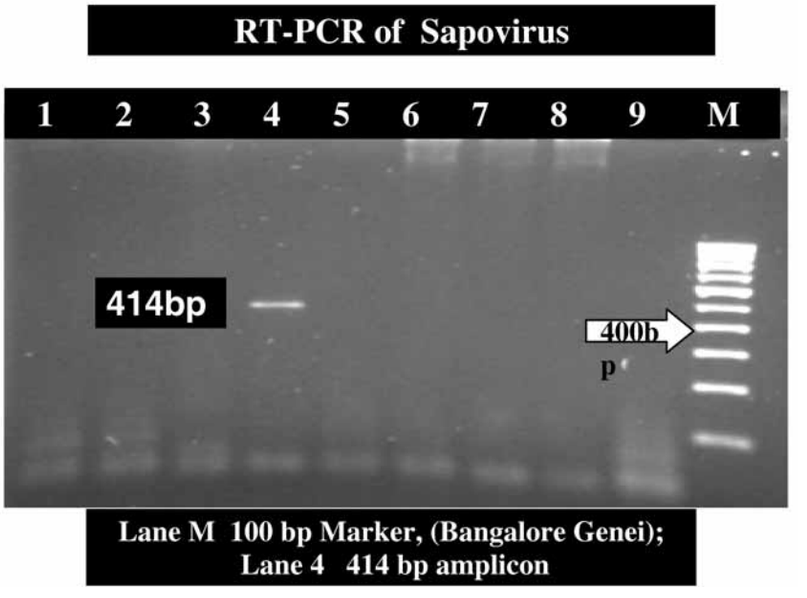
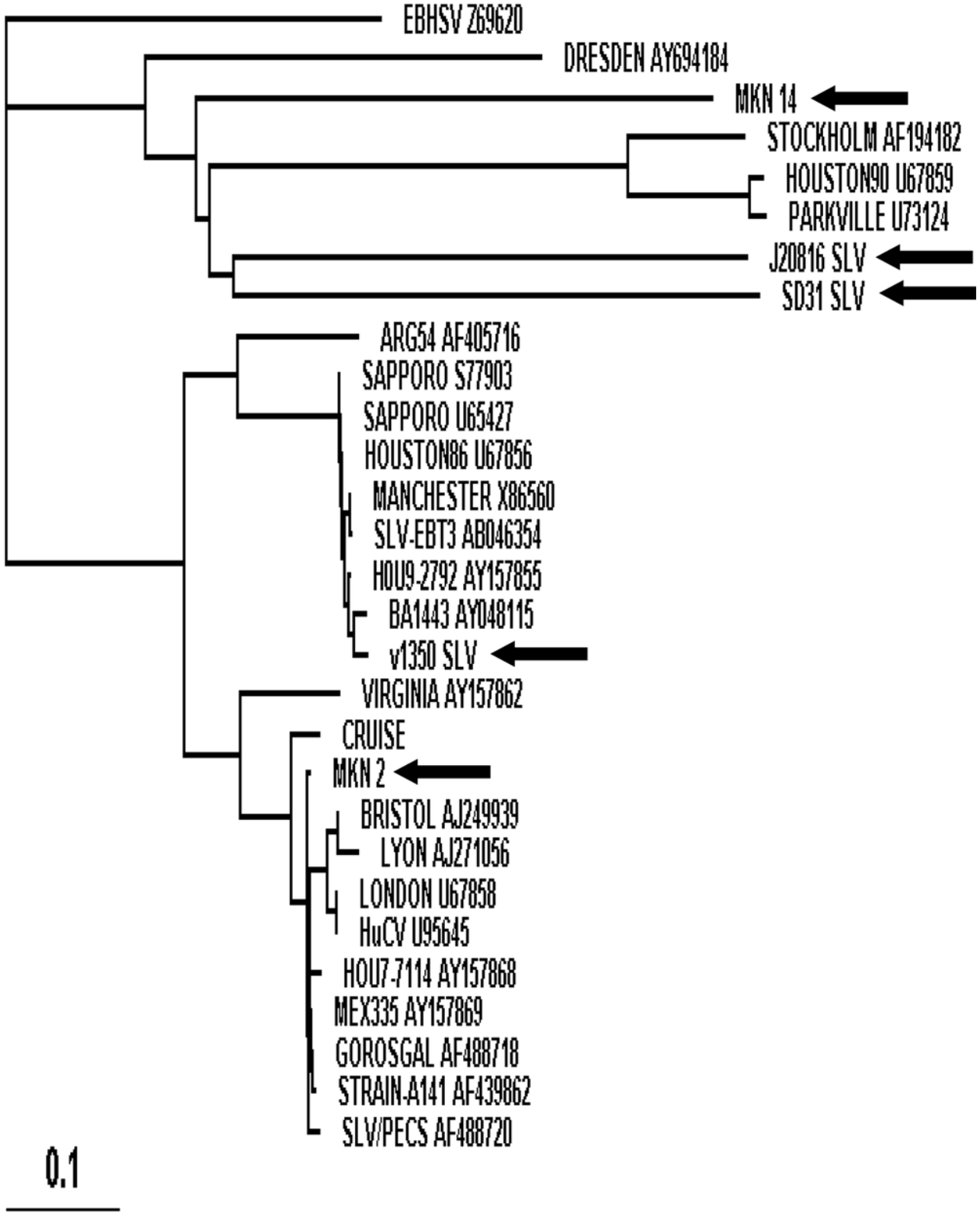

RT-PCR of Sapovirus
1 2 3 4 5 6 7 8 9 M
414bp
400bp
Lane M 100 bp Marker, (Bangalore Genei);
Lane 4 414 bp amplicon

Fig. 11.4.2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SD31 SLV
ARG54 AF405716
SAPPORO S77903
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HOUSTON86 U67856
MANCHESTER X86560
SLV-EBT3 AB046354
HOU9-2792 AY157855
BA1443 AY048115
v1350 SLV
VIRGINIA AY157862
CRUISE
MKN 2
BRISTOL AJ249939
LYON AJ271056
LONDON U67858
HuCV U95645
HOU7-7114 AY157868
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STRAIN-A141 AF439862
SLV/PECS AF488720
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Fig. 11.4.3

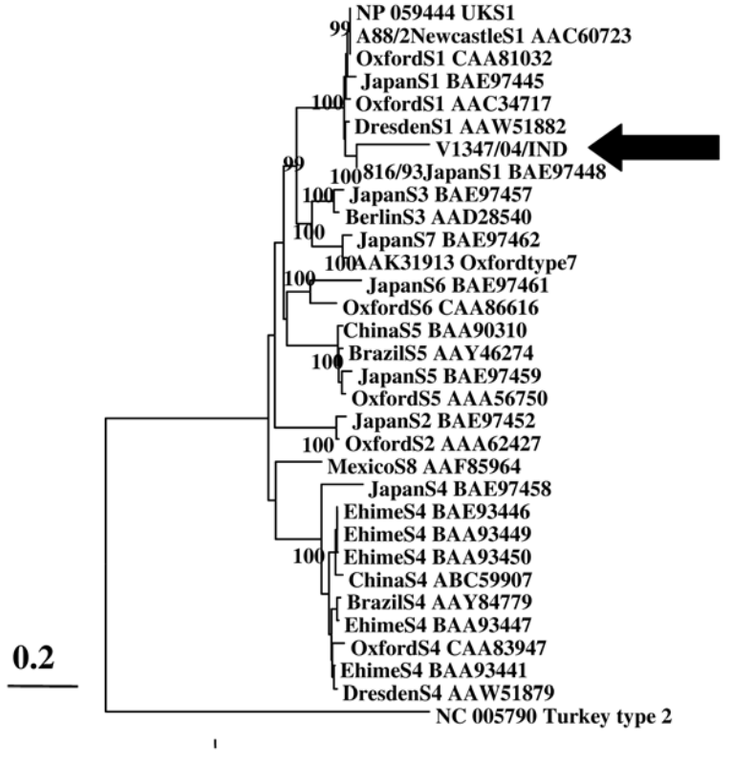


Fig. 11.4.4

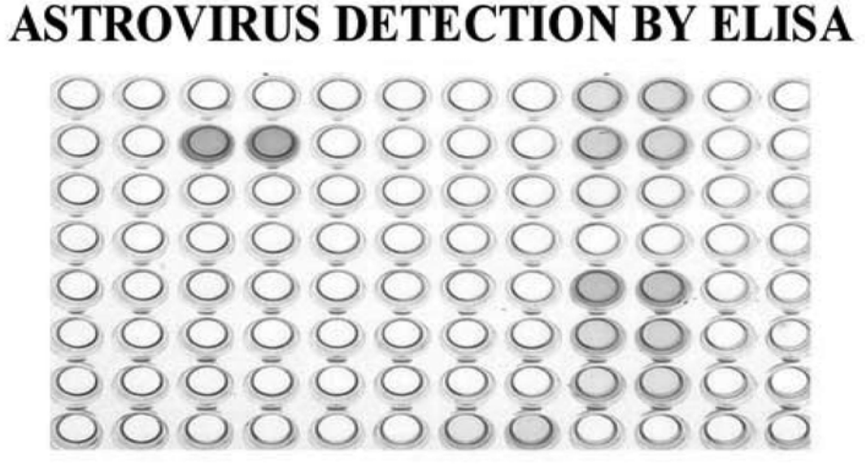
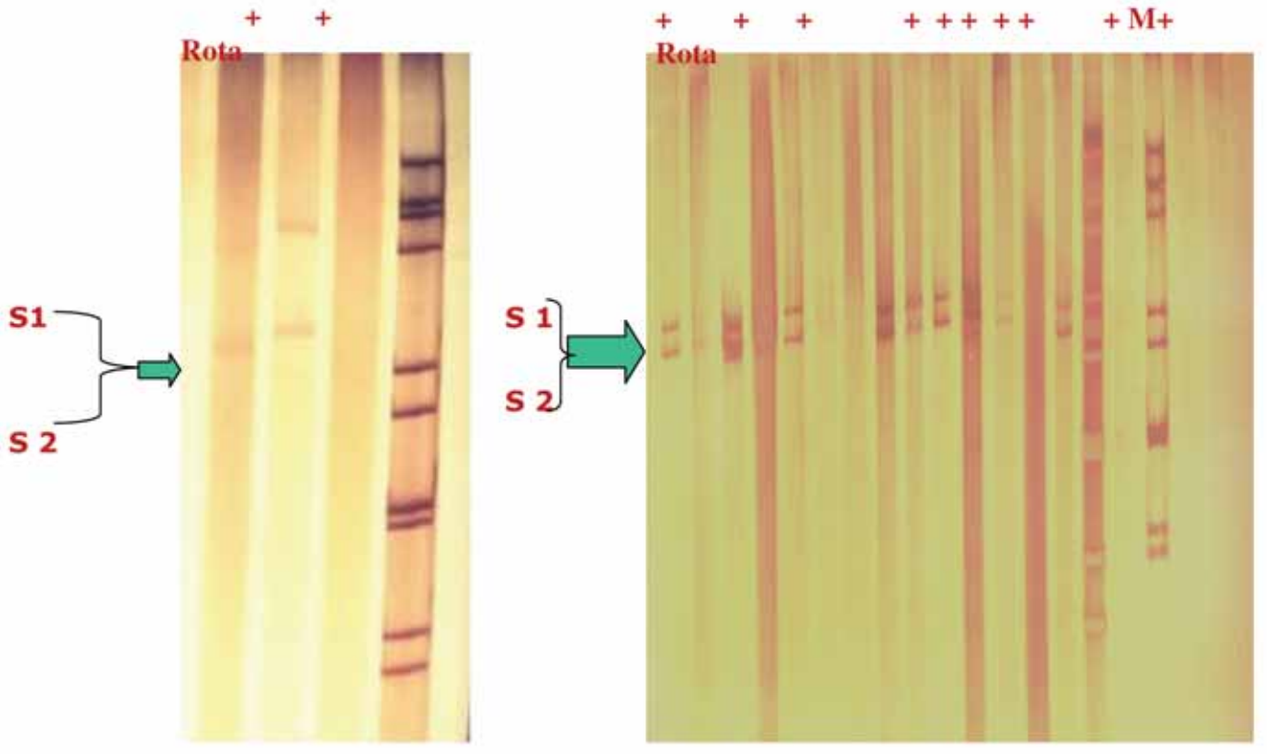


Fig. 11.4.5



Picobirnavirus detection in Kolkata

PAGE gel of picobirnaviruses with 'large profile' detected from diarrhoeic children in Kolkata

PAGE gel of the picobirnaviruses with 'small profile' detected from diarrhoeic children in Kolkata

12 Studies on HIV/AIDS

12. Studies on HIV/AIDS

12.1 Distribution of HIV-1 subtypes on the basis of *gag* gene

Investigator: S. Chakrabarti

The magnitude and complexity of the HIV-1 genetic diversity are one of the major challenges for vaccine development. Earlier reports from India identified the preponderance of subtype C and small proportion of subtype A and Thai B viruses (only among the IDUs in Manipur- see below for details). Besides the envelope C2-V3 region, gag p24-p7 region has become important to study the genetic diversity of HIV-1 since the development of *gag* based heteroduplex mobility assay (*gag*-HMA) followed by sequencing and phylogenetic analysis. Based on envelope C2-V3/V5 region, we previously identified subtype C viruses among the female sexworker in Calcutta, the most populous city in the eastern part of India . In this study, we report the distribution of HIV-1 subtypes on the basis of gag p24-p7 region.

320 female sex workers (between an age group of 20-35 years) in Calcutta were included in an unlinked anonymous study during the study period following the ethical guidelines. Blood samples were collected in Na-citrate solution after obtaining the informed consent and pre- and post-test counseling. HIV-1 seropositivity was determined by rapid spot test (Immunocomb HIV-1/2 Bi-spot, Orgenics, Israel), followed by ELISA (Immunogenetics, Belgium) and line immunoassay (Inno-LIA, HIV-1/HIV-2). 51 out of 320 samples (16%) were HIV-1 positive. Peripheral blood mononuclear cells (PBMCs) were separated from whole blood by Ficoll-Hypaque gradient centrifugation and the DNA was extracted by using the QIAamp DNA Blood Mini Kit 250 (QIAGEN, Germany) according to the manufacturer's protocol. The HIV-1 DNA fragment comprising of a 460 bp gag gene fragment corresponding to the region from amino acid 132 of p24 till amino acid 40 of p7 was amplified by nested polymerase chain reaction (PCR) in a thermal cycler (GeneAmp PCR system, 2400, Perkin Elmer). Primers used for the amplification were- H1G777 – 5'TCACCTAGAACTTT GAATGCATGGG 3' (outer forward), H1P202 – 5'CTAATA CTGTATCATCTGCTCCTGT 3' (outer reverse), H1Gag1584- 5'AAAGATGG ATAATCCTGGG 3' (inner forward) and G17 – 5'TCCACATTTCCAACAGCCCTTTTT3' (inner reverse).

PBMC DNA (1 g) was used as a template for PCR in the presence of 1.5 mM $MgCl_2$, 0.2 mM dNTPs (Perkin Elmer), 10 pmol of each primer and 2.5 unit of Taq DNA polymerase (Ampli Taq gold, Perkin Elmer) in a total volume of 50 l. PCR conditions followed were: 94^{0}C- 2 min; 35 cycles consisting of 94^{0}C- 30 sec, 50^{0}C- 30 sec, 72^{0}C- 1 min 30 sec with a final extension at 72^{0}C- 7 min in the first round and 94^{0}C- 2 min; 35 cycles consisting of 94^{0}C- 30 sec, 50^{0}C- 30 sec, 72^{0}C- 1 min with a final extension at 72^{0}C- 7 min in the second round. A heteroduplex mobility assay (HMA) was performed following the standard protocol. A 460 bp *gag* p24-p7 region was amplified from the reference samples (NIH AIDS Research and Reference Reagent Program, NIH, USA) using the same sets of primers used to amplify p24-p7 from PBMC. Amplified *gag* DNA fragments from reference strains were mixed separately

with the amplicon obtained from the sample DNA (4.5 µl each) in the presence of annealing buffer (100 mM NaCl, 2 mM EDTA, 10 mM Tris-Cl, Ph 7.8). Denaturation and renaturation of DNAs were done by heating the mixes at 94C for 2 minutes, followed by rapid cooling in ice. Heteroduplexes formed were then analyzed in a 5% polyacrylamide / 20% urea gel in 1X TBE buffer at 250 V for about 2 hr 30 minutes. A 14" x 16" vertical gel apparatus (ATTO, JAPAN) was used for the HMA. Heteroduplex molecules formed between the unknown sample and the most closely related subtype exhibited the fastest mobility. Analysis of heteroduplex mobility of fifty-one samples with respect to the reference strains showed the prevailing subtype of HIV-1 in Calcutta is C.

PCR amplicons were purified by a QIAGEN PCR purification kit (QIAGEN, Germany) and the purified products were subjected to cycle sequencing reactions in both directions using fluorescent dye-labeled dideoxy nucleotides in an ABI PRISM 310 automated sequencer following the manufacturer's protocol. Sequences were submitted to GenBank and the accession numbers were assigned. The p24-p7 sequences were edited by using the BioEdit sequence alignment editor program (version 5.0.6.; Department of Microbiology, North Carolina State University) and were subsequently analyzed on the BASIC BLAST program, which revealed a close relatedness to subtype C. At least 5% divergence was shown by each sequence from those in the database suggesting an absence of sample mix-ups with previously published sequences. All the fifty-one sequences were then aligned with a reference panel of reported sequences and/or related sequences of strains isolated from different geographic regions available in the HIV sequence database provided by the Los Alamos National Laboratory, operated by the University of California to generate the nucleotide substitution pattern among them. The reference panel included thirty sequences of different global strains with respect to the same p24-p7 region of HIV-1 consisting of all subtypes (A-K). At least two to three sequences of each reference subtype were taken for comparison. The multiple alignments were done by the Clustal W program. Phylogenetic and molecular evolutionary analyses were conducted using MEGA version 2.1. Evolutionary distances were measured by a Kimura two-parameter distance matrix method. Mean genetic diversity among the Calcutta samples was found to be 7%, ranging from 2% to 13%. The mean genetic diversity between the strains from Calcutta and other subtype C global strains was also found to be 7%.

A phylogenetic tree was constructed by Neighbor-joining (NJ) method using the Interior Branch Test of Phylogeny – a t-test that is computed using the bootstrap procedure. Bootstrapping was done for 1000 replicates and finally the tree was viewed and edited by the Tree Explorer in MEGA 2.1. Sequences from Calcutta belonged to the subtype C. However, no rigid monophyletic cluster was observed and all were placed in discrete groups. Some sequences from Calcutta were found to be dispersed among the Indian C and some non-Indian C strains like from South Africa, Botswana, Brazil, Kenya, Israel, Zambia, and Myanmar etc. However, in most of the cases, they formed small groups among themselves characteristic exclusively of the C type from Calcutta.

This result propelled us to study the distribution and phylogenetic pattern of the HIV-1 type C gag p24-p7 sequences from Calcutta compared to other Indian C strains from different regions. All the fifty-one sequences from Calcutta were analyzed with another forty-four sequences from

India (mainly western and northern parts) as available in the database (Fig. 12.1.1). Samples from Calcutta represented eastern India. Clustal W multiple sequence alignment program was used and a phylogenetic tree was computed using the NJ method by MEGA version 2.1. The radial pattern of the tree was viewed for better observation. Two main clusters were formed. Majority (thirty-five out of forty-four) of the Indian strains other than Calcutta formed the cluster – I while another group of sequences (cluster – II) were comprised mostly of the Calcutta strains (forty-six out of fifty-one). Only five strains from Calcutta were laying within cluster - I while nine Indian strains resided within cluster – II with other Calcutta strains. This result showed the distribution pattern of the HIV-1 strains from the eastern part to be a little different from rest of the country.

12.2 HIV and sexually transmitted infections in sex workers of West Bengal

Investigators: K. Sarkar

West Bengal has long been considered as a low HIV prevalent state based on sentinel surveillance data, which was supported by National AIDS Control Organisation (NACO). It is obvious that clinic based surveillance data does not reflect community picture. Hence, a cross-sectional community based study was conducted among brothel based sex workers of West Bengal to understand the existing HIV/STI status and associated risk factors for HIV in them.

Initially all districts of West Bengal were categorized in to high, medium and low sex worker prevalent districts based on rate of sex workers per 100,000 population in the district. Two districts from each of high, medium and low sex worker prevalence were selected for surveying sex workers for HIV. Most sex workers were contacted at brothels of selected districts through local community based organizations (CBOs) working with them. In two places, street based sex workers were contacted through local CBOs as there was no brothel. All subjects were explained the purpose of this study and requested to participate in it. Informed verbal consent was taken from all willing participants. A subset of above subjects was interviewed with the help of pre-tested questionnaire to study their socio-demographic variables and risk behaviors. This was followed by collection of 4-5 ml blood samples using unlinked anonymous method. All blood samples were tested for HIV, VDRL, TPHA and for hepatitis-B infection (HBV).

Table-12.2.1 District-wise HIV sero-prevalence among sex workers of West Bengal

District Category	Districts	No. tested	No. +ve	HIV sero-prevalence with CI at 95% level
High	Darjeeling	109	17	15.6% [9.3 – 23.8]
	Kolkata	622	60	9.6% [7.4 - 12.2]
Medium	Midnapore E	590	26	4.4% [2.8 - 6.3]
	24 parganas N	194	9	4.6% [2.1 - 8.6]
Low	Purulia	50	1	2% [0.1 - 12]
	Murshidabad	511	9	1.7% [0.8 - 3.3]
All Districts	Total	2076	122	5.9% [4.9 - 6.9]

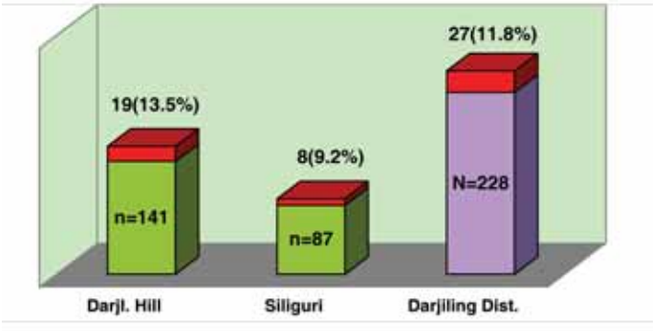
Results revealed that overall HIV sero-prevalence was 5.9%, VDRL was 11.6% and HBV was 4.2% in sex workers of West Bengal. All were infected with HIV-1 except 4 sex workers from Kolkata, who were infected with HIV-2. Sero-prevalence of HIV was highest in high sex worker prevalent districts [Kolkata & Darjeeling], moderate in medium prevalent districts [24 Pargana (N) & Midnapore-East] and low in low prevalent districts [Purulia & Murshidabad]. These differences were statistically significant. About one third of the studied sex workers (31.3%) of the state were detected to be positive with TPHA, indicating past or present syphilis infection. HIV infection was found much higher (12.5%) in younger sex workers of 20 year or less compared to older age group (5.9%). Odds Ratio was found 2.04 with 95% CI: 1.29 – 4.38.

12.3 A study on HIV and HCV infection among injecting drug users of Darjeeling district, West Bengal

Investigators: K. Sarkar

This was a community based cross-sectional study. A total of 228 study subjects were included from all four sub-divisions of Darjeeling district through secondary informant technique followed by snowballing. Personal interviews followed by collection of 3-ml. blood samples were done after obtaining informed consent, using unlinked-anonymous method. The study revealed that overall HIV sero-prevalence among IDUs was 11.8% (N=228) [95% CI: 7.9 – 16.7], while sero-prevalence of hepatitis C was found to be 47.7% (n=203). Prevalence of HIV was higher in hills (13.5%) compared to that of the plains (9.2%).

Fig. 12.3.1 Sero-prevalence of HIV-1 in IDUs of Darjeeling District, West Bengal



It also revealed that most IDUs (75.3%) used `brown sugar' as their major addictive substance followed by injection Norphine. Sharing of injecting equipment was found to be as high as 67% among IDUs (N=93) and sharing of drugs from common ampoules was found to be 35.5% (N=93). Most of them (96%) were found to clean their injecting paraphernalia by plain water. Most IDUs (98%) [N=93] were found to inject intravenously. About 52% IDUs (N=93) visited sex workers within last 1 year and 15% of the total subjects (N=93) reported to suffer from sexually transmitted diseases (STDs) during the same period. All the IDUs heard about HIV/AIDS. About 69% of the subjects (N=93) knew that apparently healthy looking person might have HIV infection. HIV was found to be associated significantly with age of the injectors and duration of injecting practices. The study revealed the alarming situation of HIV and HCV among IDU population at this bordering district of West Bengal and requires urgent intervention at local, national and international level.

13 Epidemic investigations

13. Epidemic investigations

13.1 Investigation of outbreak of Dengue, Leptospirosis and gastroenteritis

Investigators: Dr. D. Sur, Dr. K. Sarkar, Dr. S. Kanungo

Background:

After unprecedented and torrential rains during the last week of July 2005 in Mumbai, the whole of Mumbai was in a very grave situation and on the verge of an epidemic of certain diseases. To combat this emergency it was imperative to build a system of early case detection and management in the community and to take adequate measures for prevention and control of communicable diseases, namely Dengue, Leptospirosis and Gastro-enteritis. With this objective, NICED team was requested to develop a system of containment of possible post-flood outbreak in Mumbai municipal areas for UNICEF, Mumbai. NICED epidemiologists developed a micro planning with following objectives

- Early detection of Dengue, Leptospirosis and Gastro-enteritis in the community
- Management of cases at community level
- Control and prevention of the outbreak, if any, caused by the above mentioned diseases
- To establish an ongoing community based surveillance system even after containment of possible outbreak.

The community health volunteers of BMC were trained for

Early Case detection of Leptospirosis, Dengue and gastroenteritis (including hospitalized cases)

Prevention of the communicable diseases and their basic management

Follow up of cases

Referral and back Referral of cases to local G.P.s and municipal hospitals

Providing Basic Health education to the community

NICED team comprising of Dr. D. Sur, Deputy Director, Dr. Kamalesh Sarkar, Asst. Director and Dr. S. Kanungo, consultant epidemiologist, IVI monitored the surveillance for a week in Mumbai.

13.2 Report of diarrhoeal outbreak in different wards of Garulia, North 24-Parganas, West Bengal

In response to the request of the Ministry of Health & Family Welfare, Govt. of West Bengal, an investigation of diarrhoeal outbreak in different wards of Garulia, N 24-Parganas was conducted by NICED (ICMR), Kolkata on 25th April 2005. The team consisted of Dr. Dipika Sur (Deputy Director, NICED), Dr. Mihir Kr. Bhattacharya (Assistant Director, NICED), Dr. Ananya Saha (SRF, Med), Dr Reshmi Dutta (SRF, Med) and Mr. S. Turi (staff of NICED).

The total population of the area is 79926
No. of wards - 21

Method of investigation:

The team met the members of the health authority, who briefed them about the latest outbreak situation (index case, onset of outbreak, worst affected blocks, outcome of cases, presenting features). They also accompanied the investigating team to the wards and the hospitals. On 25th April 2005, the picture

of outbreak was as follows:

Population at risk	-	32999
Total number of affected wards	-	7(1,2,3,4,5,7,8)
Most affected ward	-	2 & 3
Date of first attack	-	22.04.2005
Total number of Hospital admission	-	286
Total number of death	-	2

A total of 28 patients were enlisted in the survey, out of which 14 cases were taken from Garulia emergency dispensary. Another 14 cases were taken to I.D. &B.G. Hospital, Kolkata. Biological samples of stool and rectal swabs were collected from hospitalized cases and dispensary clinic respectively. A total number of 6 water samples were collected from different pumping stations and source of drinking water of different wards of Garulia, North 24 Prg.

Total patients enlisted

Ward No. 1	-	3
Ward No. 2	-	13
Ward No. 3	-	2
Ward No. 4	-	7
Ward No. 5	-	1
Ward No. 7	-	2
Total	**-**	**28**

Before survey, a definition was made of the case and it was "**history of diarrhoea irrespective of age group".**

A rapid epidemiological survey was conducted in the affected wards to understand the clinical presentation and transmission dynamics of this outbreak.

Geographic Information of affected area:

Garulia is situated in North 24 Parganas and is presently under Garulia Municipality.

Population consisted of people speaking different languages and sharing different occupation and lifestyle. The area is congested and comprising of both poor and rich.

Now Garulia Municipality is supplying treated drinking and potable water from its Treatment Plant, twice in a day, in the morning and afternoon, in a major portion of the region. Most of the pipelines are ill maintained & chances of contamination are high. Most houses have sanitary privies, though few katcha privies remain till date. Drainage system is not adequate and is not properly functioning in most of the areas.

Age Distribution of Examined Cases:

Age	Cases at Garulia	Cases in I.D.&B.G.
(0-5)	2	4
(>5-10)	2	
(>10-20)	3	
(>20-30)	3	4
(>30-40)	1	2
>40	3	4

Sex Distribution of Examined Cases:

Sex	Cases at Garulia	Cases in I.D.& B.G.
Male	7	9
Female	7	5

Clinical presentation of 14 cases in Garulia dispensary:

Watery stool	14(100%)
Vomiting	2(14.2%)
Fever	Nil
Tenesmus	Nil
Similar cases in family	7(49.9%)
Degree of dehydration: No	2(14.2%)
Some	12(85.7%)
Severe	Nil

Clinical presentation of 14 cases in I.D. Hospital:

Watery Stool	14(100%)
Vomiting	9(64.28%)
Fever	Nil
Tenesmus	Nil
Similar cases in family	1(7.14%)
Degree of dehydration: No	Nil
Some	Nil
Severe	14(100%)

Particulars of death:

No.	Age	Sex	Ward	D/O/A	D/O/D	Hospital
1	40	M	1	24.04.05	24.04.05	Balaram
2	45	F	1	23.04.05	24.04.05	B.N.Bose
3	65	M	2	28.04.05	29.04.05	Naihati S.G.
4	76	F	2	29.04.05	30.04.05	Naihati S.G.

Acute patients were mostly sent to Barasat General Hospital, Kalyani General Hospital, Panihati General Hospital, Balaram Hospital, Golghar Hospital, Bhatpara, Naihati Hospital & I.D. Hospital, Kolkata.

Results:

All together 8 water samples and 22 stool samples were taken,out of which 8 samples were taken from the patients visiting emergency dispensary clinic at Garulia and 14 samples were taken from patients who were sent to I.D.&B.G. Hospital for treatment**.**

Out of 8 water samples, 5 samples were positive for *V.Cholerae01 Inaba* and fecal E.coli. It was observed that all the samples, which were collected from taps supplying potable water for domestic use and from the leakage point in ward 2, were positive. 3 water samples collected from pumping stations were negative.

All the stool samples taken from Garulia emergency clinic were positive for *V.Cholerae 01 Inaba*. Out of 14 stool samples collected from I.D.& B.G. Hospital, 12 samples were positive for *V.cholerae 01 Inaba***.** Stool samples of 2 cases did not confirm presence of any *Vibrio cholerae* may be due to receive of appropriate antimicrobial agent before hospitalization.

Drug sensitivity tests were carried out in NICED laboratory and it was observed that the organism was sensitive to gentamycin and tetracycline (which is the drug of choice for treating cholera). The organism was intermediately sensitive to fluroquinolones and completely resistant to ampicillin, cotrimazole, nalidixic acid and furazolidone.

Conclusion:

It is observed that the character of stool was watery in 100% cases. 64.8% cases (9 out of 14) in I.D. & B.G. Hospital were associated with vomiting. It was not associated with fever or tenesmus in both groups. Degree of dehydration were found to be severe in 100%

cases who were admitted and treated with i.v.fluids and inj.Tetracycline &/or Metrogyl or fluoroquinolones &/or Metrogyl and inj.Ampicillin and inj.Amikacin in I.D.Hospital. Similar cases in family were found in 50% cases (7 out of 14), which were taken from Garulia dispensary.

It is also observed that there was no variation in disease frequency between sexes and all age groups were equally affected.

Recommendation:

Primary:

- Chlorination of piped water supply.
- The district health authority must be alert so that all the steps would be taken to control this outbreak and to arrange for the proper management of cases.
- Adequate supply of drugs, posting of personnel should be done to take care of cases and prevent development of carriers.
- An effective surveillance system is to be established to monitor the disease with such symptom complex and for the early prediction of such outbreak.
- Construction of deep tube wells for safe potable water.
- Local Health authority (Dy. CMOH II/ CMOH) should be equipped with additional funds to tackle the epidemic situation.

Secondary:

- Regular check-up and maintenance of pipelines. Establishment of effective Sanitation Barrier.
- Daily collection of house wastes.
- Properly treated drinking water, containing free residual chlorine should be made available to all households.
- Ensuring 100% establishment and utilization of sanitary latrines.
- Establishment of appropriate and adequate drainage system and their proper maintenance.
- Proper record keeping with surveillance.
- Setting up of back up Microbiological Laboratory for early diagnosis and treatment.
- Improvement of personal hygiene particularly use of sanitary latrine, hand washing after ablution and before feeding of child.
- Use of good quality bleaching powder for disinfection. Careful handling of cases.
- Collection of drinking water in narrow –mouthed container and covering of storage water.

Proper disposal of garbage.

13.3 Investigation on Jaundice outbreak at Durgapur, West Bengal

Investigator: Dr. M.K. Saha

Jaundice outbreaks at two wards (No. 20 and 21) of Durgapur Municipality, and National Institute of Technology (formerly Regional Engineering Collage), Durgapur, of Burdwan District, West Bengal were investigated on 8th April 2005 and 9th May 2005. On investigation it was learnt that the cases started on as early as first week of March 2005. Cause was detected as contamination of sewage water through leakage of drinking water distribution pipes. Repair of the leakage points stopped the occurrence of new cases. Altogether the affected population was 10,200; more than 500 jaundice cases were reported and only one death was recorded. Cases were mostly adults or young adults. Laboratory investigation revealed that the causative organism was Hepatitis E Virus (HEV).

Continuous monitoring of bacterial contamination of supply water recommended. Additionally proper chlorination of drinking water was advised beside strengthening continued health education.

14 Activities of Training and Extension Division

14. Activities of Training and Extension Division

14.1. Phase IIA of VA1.3 candidate Cholera vaccine DBT Meeting on 2nd May 2005

Cholera vaccine developed by IMTECH, Chadigarh, IICB and NICED, Kolkata was in position to undergo a clinical trial. In this meeting, Dr. D. Mahalanabis, Director, Society of Applied Sciences made a presentation on Phase IIA clinical trial. Dr. T. Ramamurthy, Deputy Director presented the *Vibriocidal* assay validation report made by ICDDR, B, Dhaka. Discussion was also made on toxicology report of VA1.4. The session was chaired by Prof. M.K. Bhan, Secretary, Department of Biotechnology, Govt. of India, New Delhi.

14.2. Two training Programmes for German Doctors on Tropical and Travel Medicine jointly organized by Jadavpur University during 4th to 16th April 2005 and 14th to 25th November, 2005

Two training programmes were organized in this Institute in collaboration with Institute of Tropical Medicine and Centre for Travel Medicine, Berlin, Germany and Jadavpur University, Kolkata. The aim of the course was to provide knowledge and skill in applied clinical tropical medicine in hospitals in Kolkata, India. Ten German Doctors attended in each of the training courses. Inauguration of both the courses were graced by Prof. S. Sanyal, Pro-Vice Chancellor and Prof. Subrata Pal, Dean, Life Sciences, Jadavpur University, Kolkata. Dr. S.K. Bhattacharya, Additional Director General (ICMR), Dr. S. Chakrabarti and Dr. P. Dutta, Deputy Directors (Senior Grade), Dr. D. Ganguly , Dr. D. Sur, Deputy Directors, Dr. K. Sarkar, Assistant Director delivered the talk. Dr S.S. Dutta, Director of Pasture Institute, Dr. A. Chowdhury from S.S.K.M. Hospital, Dr. S.K. Roy from Medical College, Dr. M. Lahiri Ex Prof., Department of Pediatrics, Calcutta Medical College & Hospital, Dr. P.P. Bhattacharya, Dr. P. Bhattacharya consultants were invited to deliver talk as external lecturers. The participants also visited the following hospitals to see the patients : Dr. B.C. Roy Memorial Hospital for Children, Dr. B.C. Roy Hospital for Crippled Children, Infectious Diseases Hospital, Skin Department of National Medicial College, Pasteur Institute, Leprosy Home. Dr. U. Mitra and Dr. MK Bhattacharya assisted in the visit of the hospitals. They also visited Jadavpur University to get an idea of research activities on indigenous medicine.

14.3. National Technology Day Celebration

National Technology Day was celebrated on 11th May 2005 at the auditorium of the Institute. Prof. Arunava Mazumdar, Head, Department of Public Health Engineering, All India Institute of Hygiene & Public Health, Kolkata delivered a talk on "Water Management". He described his experience about the water purification adopted in West Bengal. He showed many photographs. All the staff of this Institute attended and actively participated in this programme.

14.4. Workshops on Water Quality Monitoring held on 27.10.2005 at Siliguri, 11.11.2005 at Burdwan, 18.11.2005 at Kolkata, 08.12.2005 at Malda and 22.12.2005 at Midnapore

Water Quality Monitoring is one of the most effective public health interventions to reduce childhood morbidity due to water borne diseases. Mid level managers are responsible

for effective implementation of such activities. They must be updated their knowledge in this field. Their updated knowledge will equip them further to strengthen the control of water borne diseases of this state. With this view in mind, Ministry of Health and Family Welfare, Govt. of West Bengal proposed to train the district level officers.

14.5. 3rd Country JICA-NICED Training Programme on Molecular Epidemiology of Diarrhoeal Diseases with special reference to cholera organized by NICED and Japanese International Cooperation Agency (JICA) during November 21 to December 4, 2005

JICA-NICED 3rd Country Training Programme on "Molecular Epidemiology of Diarrhoeal Diseases with Special Reference to Cholera" was conducted between November 21 to December 4, 2005. The following ten participants were selected as forwarded by the JICA India office, New Delhi through proper diplomatic channel:

Table 14.5.1. List of participants of NICED-JICA 3rd country training programme

Sl. No.	Name of the participants	Country
1.	Mr. William Dei-Alorse	Ghana
2.	Mr. John Mwaba	Zambia
3.	Ms. Mary Eugne Kauma	Tanzania
4.	Mr. Bo Pang	China
5.	Dr. (Ms.) Thida San	Myanmar
6.	Mr. Ugyen Dorji	Bhutan
7.	Dr. (Mrs.) Devika Jayawardhana	Sri Lanka
8.	Mr. Mohan Prasad Dahal	Nepal
9.	Dr. Maria Margarita M. Lota	Philippines
10.	Mr. Nawa Raj Banjade	Nepal

This course was inaugurated on 22nd December, 2005 by Toshifumi Sakai, Resident Representative, JICA India Office, New Delhi, amidst the presence of Mr. Kenji Shimuzu, Consul General, Consulate General of Japan, Kolkata; Prof. Shinji Yamasaki, Osaka Prefecture University, Osaka, Japan and Dr. S.K. Bhattacharya, Director, NICED.

Dr. S. Yamasaki, Professor, Osaka Prefecture University, Osaka, Japan acted as an external expert. Dr. S.K. Bhattacharya, Director, Dr. P. Dutta, Deputy Director (Senior Grade), Dr. T. Ramamurthy, Assistant Director, Dr. R.K. Nandy, Senior Research Officer, and Dr. A. Mukhopadhyay, Research Officer of this Institute acted as course facilitators and they delivered talks on different subjects. Dr. S. Chakrabarti, Prof. A.C. Ghose, Dr. D. Sur and Dr. S.K. Niyogi delivered lectures as Special Lecturer. Ph.D students of the Division of Microbiology acted as instructors by assisting the faculties to conduct the practical sessions. In the practical, different aspects composed of isolation and identification of *Vibrio cholerae* and *Vibrio parahaemolyticus, Escherichia coli* and *Shigella* spp; pulsed-field gel

electrophoresis (PFGE) to determine the clonality of *V. cholerae;* simplex and multiplex polymerase chain reaction (PCR) to determine the specific genes among *V.cholerae* O1 and O139 serogroups, *V. parahaemolyticus*, diarrhoeagenic *Escherichia coli*; group-specific PCR for the detection of pandemic strains of *V.parahaemolyticus;* extraction and analysis of chromosomal DNA form *V. cholerae;* extraction and analysis of plasmid DNA containing the r-RNA probe fragment; construction of dendrogram using PFGE profiles; ribotyping of *V.cholerae* O1 and O139 serogroups were covered under this programme. The participants visited the Infectious Diseases Hospital, Kolkata to have an idea on how the diarrhoeal patients are receiving the treatment and the symptoms based diagnosis of the disease.

All the participants presented their area of research interest during the special session in the programme. During the last day of the course, certificates were distributed.

Diarrhoeal disease is still a major problem in several parts of India. Use of molecular biological tools will provide comprehensive information at the gene level, which are highly reliable, reproducible and also help in tracing the origin/reservoirs of the pathogens. Hence, it is very important to study the genetic relatedness between the strains using various molecular epidemiological tools to differentiate the isolates from epidemiologically unrelated strains. This domestic training programme organized by JICA-NICED and ICMR from 17th to 29th October, 2005 helped several researchers to initiate molecular epidemiological studies related with diarrhoeal disease in India. The major objective of this programme is to train the Indian scientists in molecular typing methods using standard techniques. The data generated by the scientists in their respective centers after undertaking the training is expected to participate in the national database program on cholera epidemiology.

14.6. JICA-NICED Domestic Training Programme on Molecular Epidemiology of Diarrhoeal Diseases with special reference to cholera organized by NICED and Japanese International Cooperation Agency (JICA) during 17th to 29th October, 2005

Table 14.6.1. Name of the candidates and nominated Institutes of NICED-JICA Domestic Training Programme

Sl. No.	Name of the Candidates	Nominated Institute
1.	Dr. Arunabha Sarkar	N.B. Medical College, Darjeeling, West Bengal
2.	Dr. S. Ramesh	Sri Paramakalyani College, Tamil Nadu
3.	Dr. Swapna Kanade	Seth G.S. Medical College & K.E.M. Hospital, Mumbai
4.	Dr. Rakesh Kumar Maheshwari	S.P. Medical College, Bikaner, Rajasthan
5.	Dr. Deepinder Kaur	Dayanand Medical College & Hospital, Ludhiana
6.	Dr. K. Sarangapani	General Hospital, Pondicherry
7.	Dr. Shakil Ahmed Wani	University of Agricultural Science & Technology of Kashmir, Jammu & Kashmir

8.	Dr. Probodh Borah	Assam Agricultural University, Guwahati, Assam
9.	Dr. Rathod Sanjay Dhanraj	Smt. H.H.L. Municipal Medical College & Sheth V.S. General Hospital, Ahmedabad, Gujrat
10.	Dr. Jyoti Bajaj (Iravane)	Govt. Medical College, Aurangabad, Maharashtra
11.	Dr. Beena Uppal	Maulana Azad Medical College, New Delh
12.	Dr. Anuradha Agarwal	Kothari Medical Centre, Kolkata, West Bengal
13.	Dr. Shashi Gandhi	M.G.M. Medical College, Indore, Madhya Pradesh
14.	Dr. K. Nagameni	Gandhi Medical College, Secunderabad, Andhra Pradesh
15.	Dr. P. Bangar Raju	Kasturba Medical College, Manipal, Karnataka

The course was inaugurated on October 18, 2005 by Dr. Meena Basak, Principal, Dr. B.C. Roy Memorial Hospital for Children, Kolkata; Mr. Nobuaki Koguchi, Assistant Resident Representative, JICA India Office, New Delhi; Dr. S. Chakrabarti, Deputy Director (Senior Grade), NICED, Kolkata; Dr. S. Yamasaki, Professor, Osaka Prefecture University, Osaka, Japan, Dr. P. Dutta, Deputy Director (Senior Grade), Kolkata.

Dr. S.K. Bhattacharya, Dr. P. Dutta, Dr. T. Ramamurthy, Dr. A.K. Mukhopadhyay of this Institute and Prof. S. Yamasaki, Professor, Osaka Prefecture University, Osaka, Japan acted as Resource Persons. Prof. Asis Dutta, Dr. S. Chakrabarti, Prof. A.C. Ghose, Dr. D. Sur and Dr. S.K. Niyogi delivered lectures as Special Lecturer. For practical, different aspects of isolation and identification of *Vibrio cholerae, Vibrio parahaemolyticus* and other diarrhoeagenic pathogens were demonstrated and the candidates had their opportunity to do these tests of their own.

14.7. INDO-US Workshop on diarrhoea protogoans parasites-New chalanges on the era of HIV/ AIDS on 3-5th October 2005

This training programme was approved by INDO-US Science and Technology Forum. Ten International Delegates and another twenty National Delegates were attended this workshop. The programme was held at Hyatt Regency, Kolkata and inaugurated by Dr. Surja Kanta Mishra, Hon'ble Minister of Health & Family Welfare, Government of West Bengal in presence of Prof. N.K. Ganguly, DG, ICMR, New Delhi; Dr. S.K. Bhattacharya, Director, NICED, Kolkata; Dr. Mark Eberhard, Director, Parasitology Division, CDC, USA; Dr. Arbinda Mitra, Executive Director; Dr. Y. Ortega, Georgia University, USA

14.8. Training programme of informedia.co.in on biomedical collection on 7th July 2005

This programme was organized on 7th July, 2005. All the scientists and research scholars of this institute attended this training programme. J-Gate, our global e-journal gateway platform, provides access to the most comprehensive database network of peer-reviewed scholarly journals as well as professional and industry journals and full text articles retrieval from OVID Database. They provide access to online tutorials and training tools to help scientists and researchers refine their search skills on OVID.

14.9. Indo –US Workshop on Water Quality Monitoring on 21 –22 February 2006

This workshop was organized jointly by All India Institute of Hygiene and Public Health and NICED, held at Hyatt Regency, Kolkata on 21–22 February 2006. Forty national and international scientists attended this workshop.

15 JICA-NICED Collaborative Project

15. JICA-NICED Collaborative Project

The, Phase 2 of the Project was launched from 1st July, 2003, with the purpose of Technology being established and improved at the National Institute of Cholera and Enteric Diseases (NICED), and expanded throughout the country for the control of diarrheal diseases.

Activities of the Project

Project activities are going on at NICED with the three-fold program of dispatching Japanese experts to NICED, accepting Indian counterparts for training in Japan and providing state-of-the-art equipment, to carry on the activities of manpower training and development directed at improvement of analytical techniques and modern tools for identification of diarrheal pathogens.

Moreover, with the initiative of the Indian side under the cooperation of JICA, in order to disseminate the technology which is transferred from Japanese experts to Indian counterparts, and skills already acquired and accumulated, training courses are arranged by the NICED, for concerned doctors and scientists from within the country and also from other developing countries.

Goal of the Project

Through project activities, strengthen capacities and augment capabilities at the National Institute of Cholera and Enteric Diseases (NICED), and to expand the same throughout the country for prevention and control of diarrheal diseases. The ultimate objective of the project is to contribute towards reducing mortality and morbidity caused by diarrheal diseases and improve the ability of medical institutions in India to prevent diarrheal diseases, thus improving the overall health and welfare of the Indian people.

Activities of the Project:

Table 15.1 Short term Experts to NICED

No	Universities	Name of Expected	Speciality /Field Short-term Experts	Date of visit to NICED
1.	Jissen Women's University	Dr. Y. Takeda	Microbiology	June, 2005
2.	Okayama University	Dr. S. Shinoda	Environmental Microbiology	June, 2005
3.	International Medical Center of Japan	Dr. T.Hamabata	Microbiology	June, 2005
3.	Okayama University	Dr. S. Shinoda	Environmental Microbiology	November, 2005
4.	Okayama University	Dr. K. Okamoto	Clinical Microbiology	November, 2005
5.	Osaka Prefectural University	Dr. S. Yamasaki	Molecular Biology (Third Country Training Program)	October, 2005
6.	Osaka Prefectural University	Dr. S. Yamasaki	Molecular Biology (In Country Training Program)	November, 2005
8.	Jissen University	Dr. Y. Takeda	Microbiology	January, 2006
9.	Sapporo Medical University	Dr. A. Sumi	Virology	January, 2006

2. Counterparts who visited Japan

1. Dr. M. K. Chakrabarti, Deputy Director, March, 2005 to July, 2005,
2. Dr. Ranjan Kumar Nandy, Senior Research Officer, August 24th 2005 to February 4th 2006
3. Mr. Salil Kumar Sadhukhan, Technical Assistant, 17th January, 2006 to 20th May, 2006.
4. Dr. Amit Pal, Senior Research Officer, March 27, 2006 to July 29, 2006

In the reported year research equipment worth more than Rs. 15.5 lakhs including a fully Air Conditioned bus was provided to NICED to facilitate scientific work. The other components of the Project Design Matrix including dispatch of short term experts from Japan and sending counterparts from NICED to Japan were also realized.

Two training programs were also successfully carried out to disseminate the technology established in NICED to scientists and doctors in other parts of India and to those from other developing countries as well.

Grant Aid Project: The present facilities of NICED were constructed more than 20 years ago. To create an environment for efficient research work and space to install the equipment required to carry on the molecular biological research activities of the 2nd Phase, the Indian government asked the Japanese government for Grant Aid for construction of new research facilities. Exchange of Note was signed between both the governments on 25th June, 2004. Construction work for the new building on the land procured by NICED, within the campus of the ID Hospital, started from 18th November, 2004. The construction is being carried on in full swing. Expected completion date is March, 2006.

Total cost of facilities and equipment: 2.134 billion Yen (approx. Rs. 92 crores) Outline of the facility: Four storied research building including state-of-the-art laboratories and sophisticated animal house with total floor space of 6300 m^2

Japanese Visitors

Name & Designation	Address	Date
Mr.Moa Kashiwazaki *Senior*	Faculty of Medicine,Yokohoma City University	March 22, 2005
Ms.Marie Abe *Sophomore*	"Do"	"Do"
Mr.Jun Sugihara *Sophomore*	"Do"	"Do"
Mr. Takeshi Endo *Project Manager/Architectural Planning*	Nihon Sekkei Inc.	April 03-07, 2005
Mr. Takahisa Isobe *Facility Planning*	"Do"	April 03-08, 2005
Mr. Yu Rikukawa *Architectural Planning*	"Do"	"Do"
Mr. Takao Nakamoto *Equipment Planning (Assistant)*	"Do"	"Do"

Mr. Shigetada Kayumi *Techinical Advisor on Japanese Grant Aid*	JICA, Cambodia	April 15-22, 2005
Mr. Shuzen Tanigawa *Senior Vice Minister for Foreign Affairs*	Ministry of Foreign Affairs, Japan	April 20, 2005
Mr. Kikuta *Director*	Southwest Asia Division, Ministry of Foreign Affairs,Japan	"Do"
Mr. Yokota *Secretary to Vice Minister*	Ministry of Foreign Affairs, Japan	April 20, 2005
Ms. Yoshihiro *Secretary*	Embassy of Japan, New Delhi	"Do"
Mr. Kenji Shimizu *Consul General*	Consulate General of Japan, Kolkata	"Do"
Mr. Tsuyoshi Fukaya *Vice Consul*	"Do"	"Do"
Dr. Sumio Shinoda *Professor*	Faculty of Science, Okayama University of Science	"Do"
Dr. Takashi Hamabata *Division Chief*	Division of Bacterial Infection, International Medical Center of Japan	June 03-11, 2005
Prof. Yoshifumi Takeda *Chief Advisor, Microbiology*	Director, Cine-Science Lab, Japan	June 29-July 06, 2005
Mr. Toshiyuki Kondoh *Chief Researcher*	Secretariat of the Budget Committee, House of Councilors (Upper House)	July 01, 2005
Mr. Masami Kawate, *Researcher*	Secretariat of the Budget Committee, House of Councilors (Upper House)	"Do"
Mr. Mitsuo Takamatsu, *Vice Consul*	Consulate General of Japan, Kolkata	"Do"
Mr. Azumo Kosegaki, *Administrative Officer*	Consulate General of Japan, Kolkata	"Do"
Mrs. Kazuki Nigam *Interpreter*		"Do"
Ms. Haruka Yoshida *President*	International Federation of Medical Students' Associations, Japan	July 03-06, 2005
Mr. Junichi Nitta *Treasurer*	International Federation of Medical Students' Associations, Japan	"Do"
Mr. Keiji Kamiyama *Counsellor*	Embassy of Japan	July 11, 2005
Dr. Shinichi Yoshida *Professor & Head*	Department of Bacteriology, Kyushu University	July 19 - August 15, 2005

Mr. Takehiko Uraki *4th-year*	Faculty of Medicine, Toyama Medical & Pharmaceutical University	August 10, 2005
Mr. Natsuyuki Fujisawa *4th-year*	Faculty of Medicine, Toyama Medical & Pharmaceutical University	"Do"
Ms. Ayuko Kurahashi *5th-year*	Faculty of Medicine, Toyama Medical & Pharmaceutical University	"Do"
Ms. Kozue Nogami *4th-year*	Nursing, Toyama Medical & Pharmaceutical University	"Do"
Ms.Yukiko Nakamura	Hosei University, Japan	August 26, 2005
Ms.Eriko KARIYA	"Do"	"Do"
Mr.Hajime Koga	"Do"	"Do"
Mr.Tooru Masukawa	"Do"	"Do"
Ms.Takao Sakai	"Do"	"Do"
Ms.Sayaka Sato	"Do"	"Do"
Mr.Masahiko Yamaguchi	"Do"	"Do"
Ms.Yukari Kanamori	"Do"	"Do"
Ms.Emi Harasima	"Do"	"Do"
Mr.Kentaro Tagawa	"Do"	"Do"
Ms.Ami Yamasita	"Do"	"Do"
Mr.Manabu Takahashi	"Do"	"Do"
Mr.Kosiro Fuyama	"Do"	"Do"
Ms.Mariko Hagiwara	"Do"	"Do"
Mr.Shyungo Yamamoto	"Do"	"Do"
Ms.Akane Hayakawa	"Do"	"Do"
Ms.Keiko Eda	"Do"	"Do"
Mr. Masaki Norimine *1st year,Masters Degree*	Faculty of Law, Hokkaido University	September 08-18, 2005
Mr. S.Suzuki *Staff*	Overseas Dispatch Coordination Division, JICE, Tokyo	September 10-16, 2005
Mr. Yoshihisa Ueda *Vice- President*	Japan International Cooperation Agency (JICA)	September 26, 2005
Mr. Toshifumi Sakai *Resident Representative*	JICA-India Office, New Delhi	"Do"
Mr. Kenzo Iwakami *Team Leader*	Administration Team, Regional Department II, JICA	"Do"

Mr. Mitsuo Takamatsu *Vice-Consul*	Consulate General of Japan, Kolkata	"Do"
Dr. Shinji Yamasaki *Professor*	Osaka Prefecture University	October 16-23, 2005
Mr. Nobuaki Koguchi *Asst.Resident Representative*	JICA-India Office, New Delhi	October 18, 2005
Mr. Tomoyuki Nakano *First Secretary*	Embassy of Japan, New Delhi	November 08, 2005
Dr. Sumio Shinoda *Professor*	Faculty of Science, Okayama University of Science	November 18-27, 2005
Prof. Keinosuke Okamoto *Professor*	Okayama University	"Do"
Mr. Kentaro Kobayashi *Researcher*	Okayama University	"Do"
Ms. Yuka Omi *Researcher*	Okayama University	"Do"
Prof. Shinji Yamasaki *Professor*	Osaka Prefecture University	November 19-26, 2005
Mr. Yoshikazu Takeuchi *Consul General*	Consulate General of Japan, Kolkata	November 22, 2005
Mr. Toshifumi Sakai *Resident Representative*	JICA-India Office, New Delhi	November 22, 2005
Mr. Mitsuo Takamatsu *Vice-Consul*	Consulate General of Japan, Kolkata	"Do"
Dr. Yoshifumi Takeda *Chief Advisor*	Cine-Science Lab, Japan	November 28th – December 08th, 2005
Mr. Hidetoshi Yamashita, *Leader of Delegation, Parliamentary Member*	House of Councilors, Former Vice-Minister for Finance	December, 07th, 2005
Mr. Kotaro Tamura *Parliamentary Member*	House of Councilors (LDP)	"Do"
Mr. Keishiro Fukushima *Parliamentary Member*	House of Councilors (LDP)	"Do"
Mr. Tsutomi Okubo *Parliamentary Member*	House of Councilors (DPJ) and the Shin-Ryokufukai	"Do"
Mr. Yukio Tomioka *Parliamentary Member*	House of Councilors (DPJ) and the Shin-Ryokufukai	"Do"

Mr. Mikishi Daimon *Parliamentary Member*	House of Councilors, Japanese Communist Party	"Do"
Mr. Kikuo Nakagawa *Chief Representative*	Japan Bank For International Cooperation	"Do"
Mr. Yoshikazu Takeuchi *Consul-General*	Consulate-General of Japan,Kolkata	"Do"
Mr. Toshifumi Sakai *Resident Representative*	JICA-India Office, New Delhi	"Do"
Mr. Ryoji Ono *Chief Researcher*	Research Office of the Standing Committee on Budget, House of Councilors	"Do"
Mr. Ryoji Fuji *Researcher*	"Do"	"Do"
Mr. Tsutomi Hachiya *Section Chief*	First Division of the Committees Department, House of Councilors	"Do"
Mr. Mitsuo Takamatsu *Vice-Consul*	Consulate-General of Japan, Kolkata	"Do"
Dr. Ayako Sumi *Assistant Professor*	Sapporo Medical University School of Medicine	January 21st -31st, 2006
Mr. Mitoji Yabunaka *Deputy Minister for Foreign Affairs*	Ministry of Foreign Affiars, Japan	January 30th, 2006
Mr. Yoshikazu Takeuchi *Consul General*	Consulate General of Japan, Kolkata	"Do"
Mr. Isamu Nitta *Chairman*	The Standing Committee of Japan-India Business Co-operation Committee	"Do"
Mr. Mitsuo Takamatsu *Vice-Consul*	Consulate General of Japan, Kolkata	"Do"
Mr. Azumo Kosegaki *Administrative Officer*	Consulate General of Japan, Kolkata	"Do"
Mrs. Minako Nakatani *Evaluation Analyst*	Special Development Dept. Researcher, Global Link Management	February 1st -17th, 2006
Dr. Hideo Hayashi *Professor*	Chugokugakuen University	February 8th -17th, 2006
Ms. Tomoko Shimada *Staff*	Infectious Disease Control Team,Group IV, (Health II), Human Development Department, JICA,Tokyo	"Do"
Mr. Toshifumi Sakai *Resident Representative*	JICA-India Office, New Delhi	February 10th, 2006
Mr. Tomoyuki Fujii *Resident Representative*	JICA-India Office, New Delhi	February 22nd, 2006

Mr. Nobuaki Koguchi *Asst.Resident Representative*	JICA-India Office, New Delhi	February 22nd, 2006
Mr. Shinsuke Shimizu *Director*	Southwest Asia Division,Asia & Oceania Affairs Bureau,Ministry of Foreign Affairs, Japan	February 23rd, 2006
Mr. Mitsuo Takamatsu *Vice-Consul*	Consulate General of Japan, Kolkata	"Do"
Ms. Sadako Meguro *Health Administrator*	JICA Office – Bhutan	March 16th, 2006
Mr. Tomoyuki Fujii *Resident Representative*	JICA-India Office, New Delhi	March 16th -17th , 2006
Ms. Eri Mizuno *3rd year student*	School of Medicine, University of Tsukuba, Japan	March 18th, 2006
Mr. Kentaro Iwasawa *3rd year student*	"Do"	"Do"
Mr. Gotaro Tamura *3rd year student*	"Do"	"Do"
Ms. Natsuki Kawashima *2nd year student*	"Do"	"Do"
Mr. Tomohiro Sakaguchi *2nd year student*	"Do"	"Do"
Mr. Kohei Morinaga *2nd year student*	"Do"	"Do"
Mr. Kisaburo Tokai *Director-General*	International Bureau, Liberal Democratic Party	May 05th, 2006
Mr.Kazunori Tanaka *Deputy Secretary General*	Liberal Democratic Party	"Do"
Mr.Rokuzaemon Yoshida *Member*	House of Representatives, Liberal Democratic Party	"Do"
Mr.Motoo Hayashi *Member*	House of Representatives, Liberal Democratic Party	"Do"
Mr. Ryu Ohsaki *Officer*	International Bureau, Liberal Democratic Party	"Do"
Mr. Mitsuo Takamatsu *Vice-Consul*	"Do"	"Do"
Mr. Azumo Kosegaki *Administrative Officer*	"Do"	"Do"
Mr. Ashok Bhattacharya *Economic Officer*	"Do"	"Do"
Mrs. Kazuki Nigam *Interpreter*		"Do"

16 IVI-NICED Collaborative Project

16. IVI-NICED Collaborative Project

16.1 Randomised controlled evaluation of protection by Vi polysaccharide vaccine against typhoid fever in Eastern Kolkata

Objectives of the study:

1. to assess safety and feasibility of the vaccine
2. to assess the acceptability of the vaccine by the community
3. to assess efficacy of the vaccine against typhoid fever

Major progress:

We have selected the ward no.29 and 30 of Kolkata Municipal Corporation as study sites. In census survey, we have covered 10,995 families of total 57,099 slum populations. The average family members were 6 and 5 in the ward 29 and ward 30 respectively. After census, the data have been entered into the computer and data have been checked and edited for any duplication / sequence break in family number. The Identification card including all family members in laminated form has been issued to each family. The geographical information system (GIS) of those areas for mapping the disease distribution has been completed. We have set up 5 health outposts, each covering about 12,000 populations for passive surveillance of Cholera and typhoid fever. Another 2 health outposts have been setup in the reference hospitals (Infectious Disease Hospital and B.C. Roy Children Hospital) for surveillance so that the case will not be missed from the study sites. Surveillance for Cholera and typhoid fever has been started from 21st April 2003. From April 2005 to 31st March 2006, 41 cholera were confirmed out of 5603 samples processed of which 35 (0.6%) were Inaba and 6 (0.1%) were Ogawa serotypes. For typhoid fever number positive were 99 (1.6%), for Paratyphi-A 53 (0.8%) out of 6075 samples and Widal test was positive for 1346 (22.2%) samples.

The mass typhoid Vi vaccination campaign was held in the study site from November 27 to December 31, 2004. Out of 60,615 individuals, 54,674 were considered eligible. During the vaccination, those who were considered not eligible were pregnant or lactating women, children < 2 years of age, those with a febrile illness or those travelling out of the study site. 37,686 individuals or 68.9% of 54,674 were immunized.

Surveillance for typhoid fever is continuing. The study is in progress.

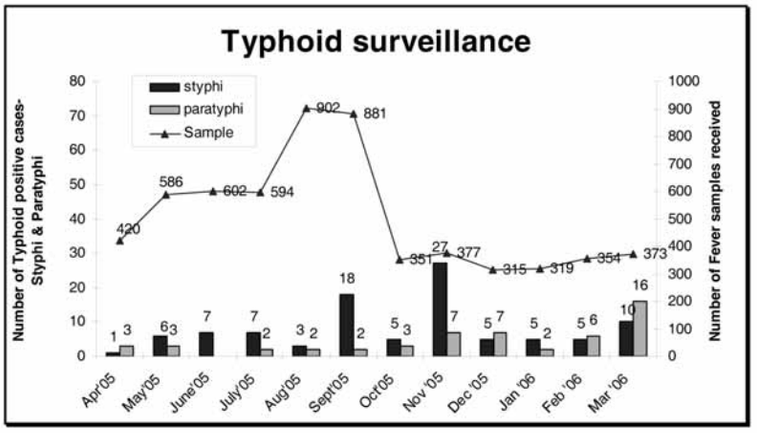


Fig. 16.1.1

16.2 Socio-behavioral and economic studies on typhoid fever and cholera

Objectives of the study:

1. To estimate the economic costs of cholera and typhoid fever in the community
2. To access the cost effectiveness of vaccination (e.g., the cost per illness episode avoided, cost per life saved)
3. To evaluate the willingness to pay (private demand) for cholera and typhoid vaccine in the community

Major progress:

We have started a willingness to pay (WTP) approach to measuring the economic benefits of the vaccines for three reasons. First, WTP is the most comprehensive measure of the private benefits of disease prevention and encompasses at least two additional components of the economic benefits of disease prevention: (1) the avoided intangible costs of disease, like pain and suffering; and (2) the household's value of avoided risk. Second, the comprehensiveness of WTP is likely to be important for the diseases because of their reputation or susceptible populations. For instance, the dread associated with cholera and typhoid fever due to mortality would be reflected in larger WTP estimates. Third, since these vaccines have not been introduced in many countries, there is no evidence of the uptake and benefits that policymakers may expect by their adoption.

Private costs of illness (COI) measures the ex-post costs associated with an episode of illness, including both out-of-pocket expenditures and indirect costs (e.g., lost wages, costs of waiting time). Private cost of illness data is being collected using structured instruments from subjects with laboratory confirmed cholera and typhoid fever. Respondents with cholera are being interviewed two times over a period of two weeks, while those with typhoid fever are being interviewed three times over a period of three months. The study covers duration of illness since the first symptom realized by patient including all sequence symptoms (sequelae) until cured. The costs include out-of-pocket expenditure such as cost of diagnosis, laboratory tests, medicines and indirect costs in terms of real income loss of patient or family members due to work absence (payment cut and/or cost of substitute labor). Institutional cost data is also being collected and includes recurrent and capital expenditures.

During cholera and typhoid fever (Vi) vaccination trials, two kinds of costs are to be collected. This will include private cost of vaccination defined as expenditure incurred to receive a vaccine and vaccine delivery cost defined as the cost for providing and administering the vaccines. The private costs will be collected from a sample of individuals who receive the vaccines using a structured survey instrument. Vaccine delivery cost including personnel, equipment and supplies will be calculated based on actual expenditure. Pre-cholera vaccine data collection is going on. The study is in progress.

16.3 A randomized controlled trial of the bivalent killed whole cell oral cholera vaccine in Eastern Kolkata, West Bengal, India

Objectives:

Primary objective: To estimate the efficacy of a two-dose primary regimen of the oral killed bivalent cholera vaccine when administered to residents at least 1 year of age of eastern

Kolkata, West Bengal, India, in preventing culture-proven *V. cholerae* O1 diarrhoea episodes severe enough to require treatment in a health care facility.
Secondary objectives:

a. To estimate the efficacy of the vaccine in preventing:
- Culture-proven *V. cholerae* O1 diarrhoea episodes associated with severe dehydration;
- Episodes of acute watery diarrhoea associated with severe dehydration;
- Episodes of acute watery diarrhoea severe enough to require treatment in a health care facility; and
- All the above endpoints stratified by age (less than 5 and over 5 years)

Major progress:

The extended area of ward 29 and ward no. 33 have been also included for the study area for vaccine trial to reach the sample size of 110,000 population. The census-1 of new area and the census-3 of the existing area have been completed. The GIS work of the new area has been completed. Premises based randomization is complete and vaccination programme will be started from 6th July, 2006

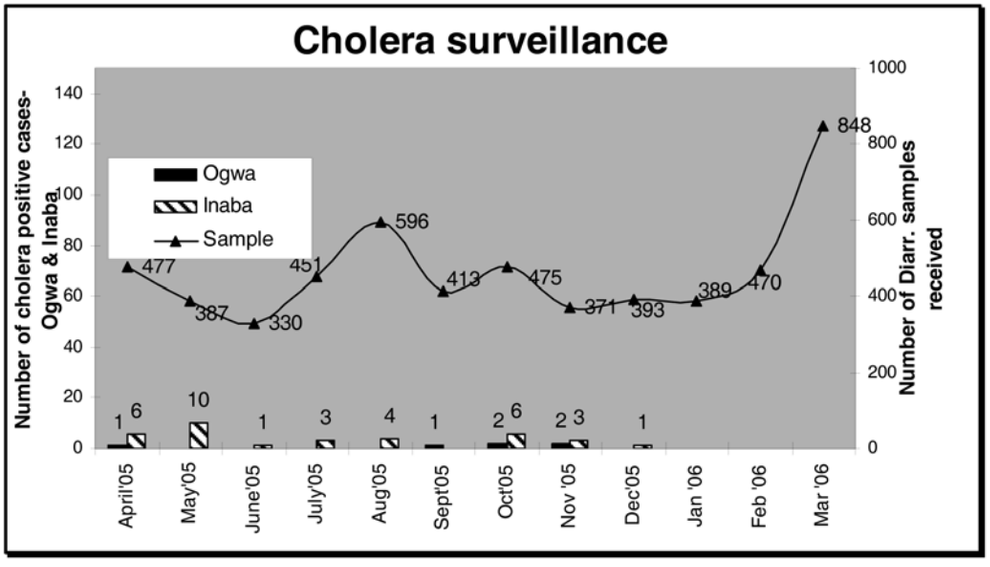


Fig. 16.3.1

17 Scientific conference/seminar/meetings attended by the Scientists / Research Fellows / Staff

17. Scientific conference/seminar/meetings attended by the Scientists / Research Fellows / Staff

Participants	Conference/seminar/meeting	Title of the papers/talks
Dr. S.K. Bhattacharya	Board of Trustees Meeting of IVI, Seoul, Korea, held at Maputo, Mozambique during April 8-10, 2005.	
	3rd International Congress on Leishmania and Leishmaniasis of Zentaris in Sicily, Italy during April 10-15, 2005.	
	Global Vaccine Research Forum and Parallel Satellite Symposia on HIV and Enteric Vaccines in Salvador da Bahia, Brazil during June 12-15, 2005.	
	Third meeting of PulseNet Asia Pacific Members at National Institute of Infectious Diseases, Tokyo, Japan during November 16-18, 2005.	
	Meeting on 'OCV use in Complex Emergencies: What next?' in Cairo, Egypt during December 14-16, 2005.	
	CAPGAN meeting held at Dhaka, Bangladesh organized by the OneWorld Health (iOWH) on February 9, 2006.	
Dr. S. Chakrabarti	Seventh Sir Dorabji Tata Symposium on "HIV Vaccines: Research Issues" at Bangalore, India during March 10-11, 2006.	MVA based Vaccines
	Tenth International Conference on Emerging Infectious Diseases in the Pacific Rim at Hanoi, Vietnam during November 16-17, 2005	
	Trilateral workshop of India-Brazil-South Africa (IBSA) on "HIV Vaccines" at Cape Town, South Africa during October 2-4, 2005.	HIV Vaccines for India
	International Conference on AIDS Vaccine, 2005 at Montreal, Canada during September 6-9, 2005.	Title of the presentation: Molecular Characterization of HIV-1 in India
	National Conference of AIDS society of India: Share & Care at New Delhi, India during April 2-4, 2005.	Title of the lecture: Development of HIV vaccine appropriate for India
Dr. T.N. Naik	Invited speaker at the Indian Science Congress held at ANGRAU at Hyderabad, India during January 2-7, 2006.	Emergence of Group B Rotavirus Infection Among Children in Kolkata

Dr. A.N. Ghosh	UGC-Refresher Course on 'Analytical Instruments and their Applications' held in Jadavpur University, Kolkata, during January 30 to February 18, 2006.	Transmission Electron Microscopy in Biological Sciences: Specimen Preparation Techniques and Applications
Dr. M.K. Chakrabarti	93rd Session of Indian Science Congress on involvement of *Yersinia enterocolitica* heat-stable enterotoxin (Y-STa) in regulation of nuclear calcium. Section of Medical Science including Physiology at Hyderabad during January 3-7, 2006.	
	Indo-US Workshop on "Diarrhea and Enteric Protozoan Parasites: New Challenges in the Era of HIV/AIDS" held at National Institute of Cholera and Enteric Diseases, Kolkata during October 3-5, 2005.	
	Mechanism of action of heat stable enterotoxin secreted by non-O1 non-O139 *Vibrio cholerae*:Role of nitric oxide, in the UGC-sponsored state-level seminar on "Recent Advances in Molecular and Integrative Physiology" held at Raja Peary Mohan College, Uttarpara, Hooghly during December 16-17, 2005.	
Dr. S.K. Niyogi	Water Quality Monitoring Workshop at Durgapur, West Bengal on June 18, 2005.	Profile of Water Borne Diseases.
	An orientation programme for primary teachers on School Health Education at Sri Balkrishna Vidyalaya, Kolkata on July 6, 2005.	Diarrhoeal Diseases
	Training programme for Vibriocidal Assay at The International Vaccine Institute, Seoul, Korea during September 21-23, 2005.	
	Steering committee meeting on vaccine probe study to estimate the preventable burden of Hib meningitis and pneumoniae in India held at ICMR Hqs on October 1, 2005.	
	Indo-US workshop on diarrhoea and enteric protozoan parasites held at Hyat regency Kolkata, organized by NICED, Kolkata during October 3-5, 2005.	
	JICA-NICED domestic training programme held at NICED, Kolkata on September 18, 2005.	
	Methods for isolation and identification of common enteric pathogens.	
	Meting on "external quality assurance system (eqas) for public health laboratories in India organized by National Institute of Communicable Diseases (NICD), Director General of Health Services at NICD, New Delhi on December 22, 2005.	

	Visited the laboratory of International Prevention of Epidemics,Graduate School of Life and Environmental Sciences,, Osaka Prefecture University, Osaka, Japan in connection with an ongoing research work on characterization of antimicrobial resistance among enteric bacteria, supported by the Japan society for the promotion of science from 20 to 25th February, 2005.	Shigellosis – the mechanism of drug resistance.
Dr. D. Sur	Attended Hib investigator's meeting at New Delhi on 19th April 2005.	
	Conference on 'Nutrition and Self care for Healthy Aging' at NIN, Hyderabad during September 2-3, 2005.	
	93rd Indian Science Congress at Hyderabad during on January 3-7, 2005.	Presented paper on 'Impact of zinc supplementation on diarrhoeal morbidity and growth pattern of Low Birth Weight infants in Kolkata – a randomized double blind placebo controlled community based study'
	Seminar of Indian National Science Academy during February 12-13, 2006.	Impact of water quality in spread of cholera and other enteric diseases
	6th International Conference on Typhoid and Other Salmonellosis held at Guilin, China November 12-14, 2005.	Presented poster on 'A comparison of risk factors for typhoid fever and paratyphoid fever in Kolkata, India'
	Conference of Commonwealth Association of Paediatric Gastoenterology and Nutrition (CAPGAN) held at ICDDRB, Dhaka, Bangladesh during February 6-8, 2006.	Presented paper on 'Impact of deworming on nutritional status and slum children of Kolkata'.
	11th Asian Conference on Diarrhoeal Diseases and Nutrition (ASCODD) held in Bangkok, Thailand March 8-10, 2006.	Presented poster on 'Incidence of cholera in children under 2 years of age in an endemic area- is cholera vaccination justified?
Dr. S. Dutta	WHO meeting of WHO Collaborating Centers and National Referral Labs held at Microbial Containment Complex of NIV, Pashan, Pune during April 13-15, 2005.	Activities of NICED, its strength and capabilities and role of NICED in disease surveillance.
	Indo-US workshop on "Diarrhoea and Enteric Protozoan Parasites: New Challenges in the Era of HIV/AIDS" held at Hyatt Rregency, Kolkata during October 3-5, 2005.	
	ICMR funded workshop on "Drinking water quality monitoring " held at NICED on January 20, 2006	Protocols for sample collection, preservation, transportation and bacteriological tests of water samples.

	Meeting of Indo-US joint working group on environmental and occupational Health water, Sanitation and Environmental Health in India held at Hyatt Regency, Kolkata during February 21-22, 2006.	Prevention of outbreaks of waterborne diseases.
	6th International Conference on Typhoid Fever and Other Salmonelloses held at Guilin, China during November 12-14, 2005.	
Dr. T. Ramamurthy	Attended the 11th Asian Conference on Diarrhoeal Diseases on March 8, 2006 at Bangkok, Thailand	Pandemic strains of Vibrio parahaemolyticus – a changing scenario
Mr. A. Palit	Indo-US Workshop on diarrhoea and enteric protozoan parasites: new challenges in the era of HIV/AIDS at NICED, Kolkata during October 3-5, 2005.	
	National Seminar on "Affordable quality based health care – opportunities and challenges" organised by IISWBM, Kolkata during August 18-19, 2005.	
	Workshop on "Application of remote sensing towards natural resource management and environmental protection" at West Bengal University of Technology, Salt Lake, Kolkata on 27th September, 2005.	
	Meeting at CMOH office, Burdwan with CMO and Dy. CMO for the proposed project on "Identifying environmental risk factors for endemic diarrhoeal diseases in West Bengal, India : a remote sensing – geographic information system(GIS) approach" on 30th June, 2005.	
	Meeting at RRSSC (ISRO), Kharagpur for initiating collaborative project work on "Identifying environmental risk factors for endemic diarrhoeal diseases in West Bengal, India: a remote sensing – geographic information system (GIS) approach" (ICMR-ISRO collaboration) during September 23-24, 2005.	
	Meeting with City Census Officer (KMC) and Dy. Mpl. Commissioner (KMC) for baseline data compilation for the NICED-RRSSCC (ISRO) collaborative project on 10th and 14th June, 2005.	
	Participated in a workshop on "Drinking water quality monitoring" at Barasat, North 24-Parganas on 24th March, 2006	Identifying environmental risk factors for diarrhoeal endemicity in West Bengal India
Dr. M.K. Bhattacharya	As a high power committee member of Govt. of India investigated Tsunami affected area at Andaman & Nichobar during January 2–6, 2005.	
	Workshop on CMC for Post Graduate Medical Education organized by Ministry of Health, Govt. of West Bengal during March 17-19, 2005.	

	Indo-US workshop on 'Diarrhoea and Enteric Protozoan Parasites: New challange in the Era of HIV/AIDS at Kolkata during October 3-5, 2005.	
Dr. B.L. Sarkar	Seminar on affordable quality based health care challenges and opportunities organized by Dept. of Health Care and Hospital Management, Indian Institute of Social Welfare and Business Management, Kolkata during August 18-19, 2005.	
	Delivered lecture for the students of Biotechnology in Cyber research and training Institute, Burdwan on January 8, 2005	
	Attended workshop on water quality monitoring at Durgapur, West Bengal on June 18, 2005	Monitoring water bodies
	Participated in a hand-on training course on Vibriocidal assay in connection with cholera vaccine study at the International Vaccine Institute (IVI), Seoul, Korea during September 2005 for seven days.	
	Water quality monitoting workshop at Durgapur, West Bengal on June 18, 2005	Monitoring water bodies
Dr. T. Krishnan	XIII International Congress of Virology.[ICV] San Francisco, California, USA. Moscone Convention Center. July 23rd-28th, 2005	i) Molecular characterization of astrovirus causing acute watery diarrhoea among adults and children in Kolkata, India ii) Molecular Diversity among Sapoviruses from eastern India
Dr. D.R. Saha	Workshop on scanning Electron Microscopy and its application in Biological Sciences'' at Jadavpur University, Kolkata organized by the Electron Microscope Society of India (Zonal Chapter, Kolkata) on 22nd February 2005.	
	Participated as a resource person in the international symposium conducted jointly by NICED-JICA on 'Cholera and other diarrhoeal diseases' at National Institute of Cholera & Enteric Diseases, Kolkata from 9th –11th June,2006	
Dr. M.K. Saha	Workshop organized by Women in Public Sector of Eastern Coalfield Limited at Kajora Area, Coal India, Govt. of India on 27th January 2005.	How to stop the spread of HIV.
	Symposium on HIV / AIDS organized by West Bengal Society for prevention & Control of HIV/AIDS at Dr. B.C. Roy College of Pharmacy and Allied Health Sciences on 15th February 2006.	HIV the Menace.
	Kalyani University on 3rd March 2006.	Bird flu- the challenge.
Dr. N.S. Chatterjee	International Symposium on Teaching, Research & Exploration in Biochemistry" held at the department of Biochemistry, Calcutta University during January 6-8, 2006.	
Dr. A. Deb	Indo-US Joint Programme Workshop on "Children's Environment and Health" held in Goa during April 03-06, 2005.	Presentation on "Water and Sanitation as Risk Factors for Diarrhoea in Children.

	First meeting of the Advisory Committee for the proposed Hib Probe Study held at ICMR Hqrs., New Delhi during April 18-19, 2005.	
	An investigators' meeting on Hib vaccine probe study (Part 'A') at NICED on 10th May, 2005.	
	School of Public Health, University of California, Los Angeles, USA during July-December 2005 to prepare, submit and defend doctoral dissertation in Epidemiology.	
Dr. S. Ganguly	Indo US workshop and International symposium on Diarrhoea and enteric protozoan parasites : New challenges in the era of HIV/AIDS organized by National Institute of Cholera and Enteric Diseases, Kolkata, ICMR, India during October 3-5, 2005.	
	17th National Congress of Parasitology, Dibrugarh, Assam, India during October 24-26, 2005.	
	CMC Winter International symposium IV on Molecular Insights into Digestive Disorders at Vellore, India, during December 15-16, 2005.	
	50th Golden Jubilee International Symposium on "Teaching Research and Exploration in Biochemistry : Fifty Years of Journey" in Department of Biochemistry, University of Calcutta at Science College, University of Calcutta, Kolkata during January 6-7, 2006	
	Symposium and Hands on Training Course/Workshop on Genomics and Proteomics organized by Industrial Toxicology Research Centre, CSIR, Lucknow at Lucknow, India during January 30 to February 4, 2006.	
	International Conference on Nano-Bio Interface 2006" organized by Dr. B. C. Guha Centre for Genetic Engineering & Biotechnology, University of Calcutta at Saha Institute of Nuclear Physics, Kolkata, India during March 1-3, 2006.	
	National symposium on recent trends in Parasitological Research organized by Department of Zoology, University of Calcutta, Kolkata, India during March 23-24, 2006.	
Dr. H. Koley	International Conference on free radicals and antioxidants is in health diseases and radiation at Calcutta, India during January 16-18, 2006.	
Rittwika Bhattacharya	XIII International Congress of Virology.[ICV] San Francisco, California, USA. Moscone Convention Center. July 23rd-28th, 2005	Detection and molecular characterization of human picobirnavirus in Kolkata, India
Ganesh C. Sahoo	XIII International Congress of Virology [ICV] San Francisco, California, USA. Moscone Convention Center. July 23rd-28th, 2005	i) Functional diversity of NSP4 protein of Rotaviruses
		ii) Molecular diversity of NSP4 gene among rotaviruses from eastern India

	74th Annual Meeting of Society of Biological Chemists (India). CDRI, Lucknow University, Lucknow, November 7-10, 2005	Antigenic diversity of NSP4 protein of rotaviruses
S. Samajdar	74th Annual Meeting Society of Biological Chemists (India) held at CDRI 2005 at Lucknow, India during November 7-10, 2005.	Poster presented on Diversity of Group A Rotavirus Strains among Children in Eastern India
Mukti K. Nayak	74th Annual Meeting of Society of Biological Chemists (India). CDRI, Lucknow University, Lucknow, November 7-10, 2005	Novel strains of noroviruses causing gastroenteritis in Kolkata

18 Training / Awards received by the Scientists/Fellows/Staff

18. Training / Awards received by the Scientists/Fellows/Staff

Dr. M.K. Chakrabarti

1. Visited Department of Microbiology, Nagasaki University, Nagasaki, Japan as a JICA counterpart trainee from 28th March to 30th July 2005.

Dr. S. Dutta

1. Visited Department of Bacteriology, Kyushu University, Fukuoka, JAPAN from 12th December 2005 to 9th January 2006 (29 days) under RONPAKU (Dissertation Ph.D) fellowship programme of Japanese Society for promotion of Science (JSPS) for JFY 2005-06 and presented a seminar before Ph. D committee as a partial fulfillment for the award of Ph. D degree (Microbiology) under Kyushu University.
2. Awarded with Ph. D (Medical Science) degree under Kyushu University (March 2006), JAPAN.
3. Selected for and successfully completed RONPAKU (Dissertation Ph.D.) fellowship programme of Japanese Society for promotion of Science (JSPS) during 2001-2005, awarded by Indian counterpart agency of JSPS (DST, M/O Science and technology, New Delhi, India, vide ID No. DST- 10114).
4. Invited to become member of the Council of Health Care Advisors, Gerson Lehrman Group, New York, USA in 2004-2006.

19 Other activities of the Scientists

19. Other activities of the Scientists

Dr. M. K. Chakrabarti

1. Chaired a session at The Section of Medical Science including Physiology, 93rd Session of Indian Science Congress during 3-7 January 2006, Hyderabad.
2. Served as Council member, 93rd Session of Indian Science Congress 2005 to 2006.
3. Served as Member of Editorial Board of Indian Journal of Physiology and Allied Sciences and Reviewer of Comparative Medicine & Toxicology Letters.
4. Served as a member of Post Graduate Board of Studies in Physiology of Presidency College and the Departments of Microbiology of Vidyasagar University.
5. Served as Moderator and Member of Board of Examiners of post-graduate Departments of Calcutta, Burdwan, Vidyasagar and Tripura University.
6. Partcipated in different academic activities as Member, Executive committee of the Physiological Society of India.
7. Organised different academic activities as Honorary General Secretary, Prof. N. M. Basu Memorial Committee in Physiological Sciences.
8. Served as a Member, Animal Ethics Committee, Presidency College, Kolkata.

Ph.D. Degree Awarded:

Dr. Arunika Mukhopadhyay has obtained her Ph.D degree on "Oral immunization of rabbit with heat-killed *Shigella flexneri 2a*: study of protection, immune response and antigenic recognition" from the Department of Life Science and Bio-Technology of Jadavpur University under the supervision of Dr. M.K. Chakrabarti in the year 2005.

Dr. S. Dutta

1. Became reviewer of Journal of Health Population and Nutrition, Bangladesh.
2. Became reviewer of Journal of Medical Microbiology, Society for General Microbiology, UK.
3. Became guide of a M.Sc. (Microbiology) student of Rani Durgawati University, Jabbalpur for completion and submission of his M.Sc. dissertation work in June 2005.
4. Involved in teaching and hands-on training programme of medical (MD), undergraduate, post-graduate students of Kolkata University, Vidyasagar University and Jadavpur University, scientists and research scholars, WHO Fellow visited this institute from time to time.

Dr Triveni Krishnan

1. Invited as Honorary lecturer to teach Virology and Microbiology to M.Sc students of University of Calcutta.
2. Invited to act as paper setter / theory examiner and Internal examiner for conducting practical examination in Microbiology for M.Sc students of Bethune College, affiliated to University of Calcutta.

20 List of Publications

20. List of Publications:

1. Acosta CJ, Galindo CM, Ali M, Elyazeed RA, Ochiai RL, Danovaro-Holliday MC, Page AL, Thiem VD, Jin Y, Park JK, Lee H, Puri MK, Ivanoff B, Agtini MD, Soeharno R, Simanjuntak CH, Punjabi NH, Canh do G, Sur D, Nizami Q, Manna B, Bai-qing D, Anh DD, Honghui Y, Bhattacharya SK, Bhutta Z, Trach DD, Xu ZY, Pang T, Donner A, Clemens JD. A multi-country cluster randomized controlled effectiveness evaluation to accelerate the introduction of Vi polysaccharide typhoid vaccine in developing countries in Asia: rationale and design. Trop Med Int Health. 2005; 10: 1219-28.

2. Akter J, Das SC, Ramamurthy T, Ashraf H, Saha D, Faruque ASG, Nair GB and Salam MA. Prevalence and characteristics of *Escherichia coli* isolates harboring Shiga toxin gene (*stx*) from acute diarrhoeal patients in Dhaka, Bangladesh. Trop. Med. Health. 2005; 33:119-26.

3. Bagchi, AK and Sinha AK. Phosphotidylinositol-3 kinase mediated tyrosine kinase up-regulation in immunized mice with 57kDa major antigenic outer-membrane protein of *Shigella dysenterae* type 1. J Med Microbiol 2005; 54: 631-37.

4. Ballal M and Ramamurthy T. Enteroaggregative *Escherichia coli* Diarrhea in Manipal. Indian Pediatr. 2005; 42: 722-23.

5. Banerjee A, Banerjee S, Chowdhury A, Santra A, Chowdhury S, Roychowdhury S, Panda CK, Bhattacharya SK and Chakravarty R. Nucleic acid sequence analysis of basal core promoter/precore/core region of hepatitis B virus isolated from chronic carriers of the virus from Kolkata, eastern India: low frequency of mutation in the precore region. Interviorlogy. 2005; 48: 389-99.

6. Bhanja P, Sengupta S, Yaima Singh N, Sarkar K, Bhattacharya SK and Chakrabarti S. Determination of Gag and Eng subtypes of HIV-1 detected among injecting drug users (IDUs) in Manipur, India: Evidence for intersubtype recombination. Virus Research (Elsevier). 2005; 114: 149-53.

7. Bhardwaj R, Majumdar S, Ganguly NK, Taneja N, Dutta S, Ramamurthy T and Chakraborti A. Characterization of adhesin variants in Indian isolates of enteroaggregative *Escherichia coli.* FEMS Microbiol Lett. 2005; 258: 274-83.

8. Bhattacharya MK, Naik TN, Palit A and Bhattacharya SK. Impact of a harm-reducing programme on soft tissue infections among injecting drug users of Kolkata, India. J Health Popul Nutr. 2006; 24: 121-22.

9. Bhattacharya R, Sahoo G C, Nayak M K, Ghosh S, Dutta P, Bhattacharya M, Mitra U, Gangopadhyay D, Dutta S, Niyogi S K, Saha D R, Naik T N, Bhattacharya S K, Krishnan T. Molecular epidemiology of human astrovirus infections in Kolkata, India. Infection, Genetics and Evolution 2006; 6: 425-35.

10. Bhattacharya T, Chatterjee S, Maiti D, Bhadra RK, Takeda Y, Nair GB and Nandy RK. Molecular analysis of the *rstR* and *orfU* genes of the CTX prophages integrated in the small chromosomes of environmental *Vibrio cholerae* non-O1, non-O139 strains. Environmental Microbiology. 2006; 8: 526-34.

11. Chakraborty R, Chakraborty S, De K, Sinha S, Mukhopadhyay AK, Khanam J, Ramamurty T, Takeda Y, Bhattacharya SK and Nair GB. Cytotoxin and cell vacuolating activity of Vibrio fluvialis isolated from paediatric patients with diarrhoea. J Med Microbiol 2005; 54: 707-16.

12. Chattopadhyay D, Arunachalam G, Ghosh L, Rajendran K, Mandal AB and Bhattacharya SK. Antipyretic activity of Alstonia macrophylla Wall ex A.DC: an ethnomedicine of Andaman Islands. J Pharm Pharm Sci 2005; 8: 558-64.

13. Chowdhury A, Santra A, Bhattacharjee K, Ghatak S, Saha D R, Dhali G K. Mitochondrial oxidative stress and permeability transition in Isoniazid and Rifampicin induced liver injury in Mice. J Of Hepatology 2006; 45:117-26.

14. Chowdhury A, Santra A, Chakravorty R. Banerji A, Pal S, Dhali GK, Datta S, Banerji S, Manna B, Roy Chowdhury S, Bhattacharya SK. and Guha Mazumder D. Community-based epidemiology of hepatitis B virus infection in West Bengal, India: Prevalence of hepatitis B e antigen-negative infection and associated viral variants. J Gastroenterol Hepatol. 2005; 20: 1712-720.

15. Chaudhuri S, Das S, Chowdhury A, Santra A, Bhattacharya SK and Naik TN. Molecular epidemiology of HCV infection among acute and chronic liver disease patients in Kolkata, India. J Clin Virol. 2005; 32: 38-46.

16. Das SC, Khan A, Panja P, Datta S, Sikdar A, Yamasaki S, Takeda Y, Bhattacharya SK, Ramamurthy T, and Nair GB. Dairy farm investigation on Shiga toxin-producing *Escherichia coli* (STEC) in Kolkata, India with emphasis on molecular characterization. Epidemiol Infect 2005; 133: 617-26.

17. Datta P, Mallik P, Ghosh AN and Chakravorthy M. Temperature sensitive mutation in the 38 kDa minor structural protein gene of phage MB78 interferes with phage morphogenesis.Virus Genes. 2005; 30: 197-207.

18. Datta S, Chattopadhyay S, Chowdhury A, Santra A, Saha DR, Ramamurthy T, Bhattacharya SK, Berg DE, Nair GB and Mukhopadhyay AK. Diagnosis and genotyping of *Helicobacter pylori* by polymerase chain reaction of bacterial DNA from gastric juice. J Gastroenterol Hepatol 2005; 20: 1253-259.

19. Datta S, Chattopadhyay S, Patra R, De R, Ramamurthy T, Hembram J, Chowdhury A, Bhattacharya SK, Berg DE, Nair GB and Mukhopadhyay AK. Most *Helicobacter pylori* strains of Kolkata in India are resistant to metronidazole but susceptible to other drugs commonly used for eradication and ulcer therapy. Aliment Pharmacol Ther 2005; 22: 51-57.

20. Debnath A, Akbar Md., Mazumder A, Kumar S and Das P. *Entamoeba histolytica* : characterization of human collagen type I and Ca2+ activated differentially expressed genes. Exp Parasitol 2005; 110: 214-19.

21. Dutta S, Iida K-I, Kawamura Y, Ezaki T, Nair GB and Yoshida S-I. Alteration in the GyrA subunit of DNA gyrase and the ParC subunit of topoisomerase IV in Quinolone Resistant *Shigella dysenteriae* serotype 1 clinical isolates from Kolkata, India. Antimicro Agent Chemother. 2005; 49: 1660-661.

22. Dutta S, Sur D, Manna B, Deen J, Clemens J and Bhattacharya SK. Roll-back of *Salmonella enterica* serotype *Typhi* resistance against chloramphenicol and other antimicrobials in Kolkata, India. Antimicrobial Agents Chemother. 2005; 49: 1662-663.

23. Faruque SM, Bin Naser I, Fujihara K, Diraphat P, Chowdhury N, Kamruzzaman M, Qadri F, Yamasaki S, Ghosh AN and Mekalanos JJ. Genomic sequence and receptor for the *Vibrio cholerae* phage KSF-1phi: evolutionary divergence among filamentous vibriophages mediating lateral gene transfer. J Bactriol 2005; 187: 4095-103.

24. Faruque SM, Naser IB, Islam MJ, Faruque AS, Ghosh AN, Nair GB, Sack DA and Mekalanos JJ. Seasonal epidemics of cholera inversely correlate with the prevalence of environmental cholera phages. Proc Natl Acad Sci USA. 2005; 102: 1702-707.

25. Gangopadhyay DN, Chanda M, Sarkar K., *Niyogi SK*, Chakraborty S, Saha MK, Manna B, Jana S, Ray P, Bhattacharya SK and Detels R. Evaluation of sexually transmitted disease / Human Immunodeficiency Virus intervention programs for sex workers in Calcutta, India. Sexually Transmitted Diseases 2005; 32: 680-84.

26. Ghosh A. Saha DR, Hoque KM, Asakuna M, Yamazaki S, Koley H, Das SS, Chakrovorty M and Pal A. Enterotoxigenicity of mature 45-kilodalton and processed 35 kilo Dalton forms of Haemagglutinin protease purified from a cholera toxin gene negative *Vibrio cholera* Non-O1Non- O139 strain. Inf Immun 2006; 74: 2937-946.

27. Ghosh S, Varghese V, Samajdar S, Bhattacharya SK, Kobayashi N and Naik TN. Molecular characterization of a porcine Group A rotavirus strain with G12 genotype specificity. Arch Virol 2006; (Epub ahead of print).

28. Gupta DD, Saha S and Chakrabarti MK. Involvement of protein kinase C in the mechanism of action of Escherichia coli heat-stable enterotoxin (STa) in a human colonic carcinoma cell line, COLO-205. Toxicol Appl Pharmacol 2005; 206: 9-16.

29. Hens DK, Ghosh AN and Kumar R. A new small temperate DNA phage BcP15 isolated from Burkholderia cepacia DR11. Arch Virol 2005; 150: 2421-428.

30. Hens DK, Niyogi SK and Kumar R. Epidemic strain *Shigella dysenteriae* type 1 Dt66 encodes several drug resistances by chromosome. Arch Med Res 2005; 36: 399-403.

31. Ishino M, Mise K, Takemura H, Ahmed MU, Alam MM, Naik TN and Kobayashi N. Comparison of NSP4 protein between group A and B human rotaviruses: detection of novel diarrhea-causing sequences in group B NSP4. Arch Virol 2006; 151: 173-82.

32. Islam MS, Rahman MZ, Khan SI, Mahmud ZH, Ramamurthy T, Nair GB, Sack RB, Sack DA. Organization of the CTX Prophage in Environmental Isolates of *Vibrio mimicus*. Microbiol Immunol 2005; 49: 779-84.

33. Kang G, Kelkar SD, Chitambar SD, Ray P and Naik T. Epidemiological profile of rotaviral infection in India: challenges for the 21st century. J Infect Dis 2005; 192 Suppl 1: S120-126.

34. Kumar V, Bimal S, Kesari S, Kumar AJ, Bagchi AK, Akbar MA, Kishore K, Bhattacharya SK and Das P. Evaluation of a dot-immunoblot assay for detecting leishmanial antigen in naturally infected *Phlebotomus argentipes* (Diptera: Psychodidae). Annals Trop Med Parasitol 2005; 99: 371-76.

35. Lal R, Chakrabarti S and Chungfu Young. Impact of Genetic diversity of HIV-1 on diagnosis, antiretroviral therapy and vaccine

development Indian J Med Res 2005; 121: 287-314.

36. Mandal A, Chakraborti T, Choudhury R, Ghosh B, Ghosh AN, Das S and Chakraborti S. Role of MMP-2 in inhibiting Na+ dependent Ca2+ uptake by H2O2 in microsomes isolated from pulmonary smooth muscle. Mol Cell Biochem 2005; 270: 79-87.

37. Manna B, Niyogi SK, Bhattacharya MK, Sur D and Bhattacharya SK. Observations from diarrhea surveillance support the use of cholera vaccine in endemic area. Int J Infect Dis 2005; 9: 117-119.

38. Nandi B, Nandy RK, Sarkar A, Ghose AC. Structural features, properties and regulation of the outer-membrane protein W (OmpW) of *Vibrio cholerae*. Microbiology 2005; 151: 2975-86.

39. Niyogi SK. Shigellosis. J Microbiol 2005; 43: 133-143.

40. Ochiai RL, Wang XY, Seidlein LV, Yang J, Bhutta ZA, Bhattacharya SK, Agtini M, Deen JL, Wain J, Kim DR, Ali M, Acosta CJ, Jodar L and Clemens JD. *Salmoella paratyphi* A rates, Asia. Emerg Infect Dis 2005; 11: 1764-966.

41. Palit A, Sur D, Mitra Dhar K, Saha MR. Asymptomatic cryptosporidiosis in a periurban slum setting in Kolkata, India – a pilot study. Jpn J Infect Dis 2005; 58: 110-11.

42. Palit A, Bhattacharya SK and Kundu SN. Host preference of *Phlebotomus argentipes* and *Phlebotomus papatasi* in different biotopes of West Bengal, India. Int J Env Hlth Res 2005; 15: 449-454.

43. Pandey K, Sinha PK, Das VNR, Kumar N, Hassan SM, Verma N, Lal CS, Bimal S, Das P and Bhattacharya SK. HIV-1 infection, visceral leishmaniasis, Koch's chest and tuberculous meningitis in the same patient – a case report. Annals Trop Med Parasitol 2005; 99: 807-11.

44. Pandey K, Sinha PK, Das VNR, Sur D, Kumar N and Bhattacharya SK. Neurocysticercosis in a patient with Visceral Leishmaniasis co-infected with HIV: A case report. Infect Dis Clin Practice 2005; 13: 144-45.

45. Pandey K, Sinha PK, Das VNR, Kumar N, Verma N, Lal CS, Vimal S, Sur D and Bhattacharya SK. Nexus of infection with human immunodeficiency virus, pulmonary tuberculosis and visceral leishmaniasis: a case report from Bihar, India. Am J Trop Med Hyg 2005; 72: 30-2.

46. Pazhani GP, Ramamurthy T, Mitra U, Bhattacharya SK and Niyogi SK. Species diversity and antimicrobial resistance of Shigella spp. Isolated between 2001 and 2004 from hospitalized children with diarrhoea in Kolkata (Calcutta), India. Epidemiol Infect 2005; 133: 1089-095.

47. Qadri F, Svennerholm AM, Shamsuzzaman S, Bhuiyan TR, Harris JB, Ghosh AN, Nair GB, Weintraub A, Faruque SM, Ryan ET, Sack DA and Calderwood SB. Reduction in capsular content and enhanced bacterial susceptibility to serum killing of Vibrio cholerae O139 associated with the 2002 cholera epidemic in Bangladesh. Infect Immun 2005; 73: 6577-583.

48. Rajendran K. Cluster Analysis in Bio-Medical Research. Newsletter from SPSS South Asia. Issue 2: 2005-2006, pp 8-10.

49. 49. Ranjan A, Sur D, Singh VP, Siddique NA, Manna B, Lal CS, Sinha PK, Kishore K and Bhattacharya SK.Risk factors for Indian kala-azar. Am J Trop Med Hyg 2005; 73: 74-8.

50. Roy S, Dutta B, Ghosh AR, Sugunan AP, Nandy RK, Bhattacharya SK and Sehgal SC. Molecular tracking of the lineage of strains of *Vibrio cholerae* O1 biotype El Tor associated with a cholera outbreak in Andaman and Nicobar Islands, India. Trop Med Inter Hlth 2005; 6: 604-11.

51. Saha DR, Pal A, Rajendran K and Dutta P. Histopathological changes in experimental cholera with a non toxigenic non- O1 non-O139 *Vibrio cholerae* strain isolated from Kolkata, India. Indian J Exp Biol 2006; 44: 221-27.

52. Sarkar K, Bal B, Mukherjee R, Chakraborty S, Niyogi SK, Saha MK and Bhattacharya SK. Epidemic of HIV Coupled with hepatitis C Virus among Injecting Drug Users of Himalayan West Bengal, Eastern India, Bordering Nepal, Bhutan and Bangladesh. Substance Use & Misuse. 2005, 41: 341-52.

53. Sarkar K, Bal B, Mukherjee R, Niyogi SK, Saha MK and Bhattacharya SK. Cross border HIV epidemic among IDUs of Himalayan West Bengal, eastern India, bordering Nepal, Bhutan & Bangladesh. Eur J Epidemiol 2005; 20: 373-74.

54. Sarkar K, Bal B, Mukherjee R, Niyogi SK, Saha MK and Bhattacharya SK. Epidemiology of HIV infection among brothel based sex workers of Kolkata, India. J. Hlth Pop Nutr 2005; 23: 231-35.

55. Sarkar K, Bal B, Mukherjee R, Saha MK, Chakraborty S, Niyogi SK and Bhattacharya SK. Young age is a risk factor for HIV among female sex workers-An experience from India. Journal of Infection 2005 (Epub ahead of print).

56. Sen A and Ghosh AN. New *Vibrio cholerae* O1 biotype ElTor bacteriophages. Virol J 2005; 2: 28.

57. Sen A and Ghosh AN. Physicochemical characterization of vibriophage N5. Virol J 2005; 2: 27.

58. Sen A, Nandi RK and Ghosh AN. Ion-swimming speed variation of *Vibrio cholerae* cells. J Biosci 2005; 30: 465-67.

59. Sengupta S, Jana S, Roy P, Sarkar K, Bhattacharya SK and Chakrabarti S. Phylogenetic analysis of the p24-p7 region of the human immunodeficiency virus type 1 *gag* gene to determine subtype distribution among Female sex workers in Calcutta, India. J Clin Microbiol 2005; 43: 5787-791.

60. Sengupta S, Jana S, Sarkar K, Bhattacharya SK and Chakrabarti S. Determination of Gag Subtypes of HIV type 1 detected among female sex workers in Calcutta, India. AIDS Res Hum Retroviruses 2005; 21: 806-9.

61. Sengupta S, Khetawat D, Jana S, Sarkar K, Bhattacharya SK and Chakrabarti S. Polymorphism of HIV-1 gag (p17) gene from female sex workers in Calcutta, India. Arch Virol 2005; 150: 2117-124.

62. Shammas MA, Koley H, Batchu RB, Bertheau RC, Protopopov A, Munshi NC and Goyal RK. Telomerase inhibition by siRNA causes senescence and apoptosis in Barrett's adenocarcinoma cells: mechanism and therapeutic potential. Mol Cancer 2005; 15: 4: 24.

63. Sinha AK and Bagchi AK. Cytokine release induced by killed bacteria associated with anti-IFN-antibody in *Shigella* infection. *CYTOKINE* 2005; 31: 87-93.

64. Sinha PK, Pandey K and Bhattacharya SK. Diagnosis & management of leishmania/HIV co-infection. Indian J Med Res 2005; 121: 407-14.

65. Sinha PK, Ranjan A, Singh VP, Das VN,

Pandey K, Kumar N, Verma N, Lal CS, Sur D, Manna B and Bhattacharya SK..*Visceral leishmaniasis* (kala-azar)-the Bihar (India) perspective. J Infect 2006; 53: 60-64.

66. Sur D, Deen JL, Manna B, Niyogi SK, Deb AK, Kanungo S, Sarkar BL, Kim DR, Danovaro-Holliday C, Holliday K, Gupta VK, Ali M, von Seidlein L, Clemens JD and Bhattacharya SK. The burden of cholera in the slums of Kolkata, India: Data from a prospective, community-based study. Archives of Disease in Childhood. 2005; Arch Dis Child 2005; 90: 1175-81.

67. Sur D, Saha DR, Manna B, Rajendran K and Bhattacharya SK. Periodic deworming with Albendazole and its impact on growth status and diarrhoeal incidence among children in an urban slum of India. Trans Roy Soc Trop Med Hyg 2005; 99: 261-67.

68. Sur D, Sarkar BL, Manna B, Deen J, Datta S, Niyogi SK, Ghosh AN, Deb A, Kanungo S, Palit A and Bhattacharya SK. Epidemiological, microbiological & electron microscopic study of a cholera outbreak in a Kolkata slum community. Indian J Med Res 2006; 123: 31-6.

69. 69. Sur D, von Seidlein L, Manna B, Dutta S, Deb AK, Sarkar BL, Kanungo S, Deen JL, Ali M, Kim DR, Gupta VK, Ochiai RL, Tsuzuki A, Acosta CJ, Clemens JD and Bhattacharya SK.The malaria and typhoid fever burden in the slums of Kolkata, India: data from a prospective community-based study. Trans R Soc Trop Med Hyg. 2006 Jan 18; [Epub ahead of print].

70. Tripathy SP, Kulkarni SS, Jadhav SD, Agnihotri KD, Jere AJ, Kurle SN, Bhattacharya SK, Singh K, Tripathy SP and Paranjape RS. Subtype B and subtype C HIV type 1 recombinants in the northeastern state of Manipur, India. AIDS Res Hum Retroviruses. 2005; 21: 152-57.

71. Varghese V, Ghosh S, Das S, Bhattacharya SK, Krishnan T, Karmakar P, Kobayashi N and Naik TK. Characterization of VP1, VP2 and VP3 gene segments of a human rotavirus closely related to porcine strains. Virus Genes. 2006; 32: 241–47.

72. Vimal S, Das VNR, Sinha PK, Gupta AK, Verma N, Ranjan A, Singh SK, Sen A, Bhattacharya SK, and Das P. Usefulness of the direct agglutination test in the early detection of subclinical *Leishmania donovani* infection: a community-based study. Annal Trop Med Parasitol 2005; 99: 743-49.

73. Vimal S, Singh SK, Das VNR, Sinha PK, Gupta AK, Bhattacharya SK and Das P. *Leishmania donovani*: Effect of therapy on expression of CD2 antigen and secretion of macrophage migration inhibition factor by T-cells in patients with visceral leishmaniasis. Exp Parasitol 2005; 111: 130-32.

74. Vimal S, Singh SK, Pandey K, Sinha PK, Das PK, Schallig H, Das P and Bhattacharya SK. Comparative evaluation of aqueous and freeze dried antigen for sero-diagnosis of VL and PKDL cases by DAT. Am J Immun 2005; 2: 74-8.

Book chapter

1. Biswas T and Ray A. Activation of peritoneal macrophage and B cell subsets by porin of *Shigella dysenteriae* type 1 for mucosal immune response - In BA Veskler (*ed*) Progress in Immunology Research, Nova Science Publishers Inc., New York. 2005; Pp. 231-247.

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