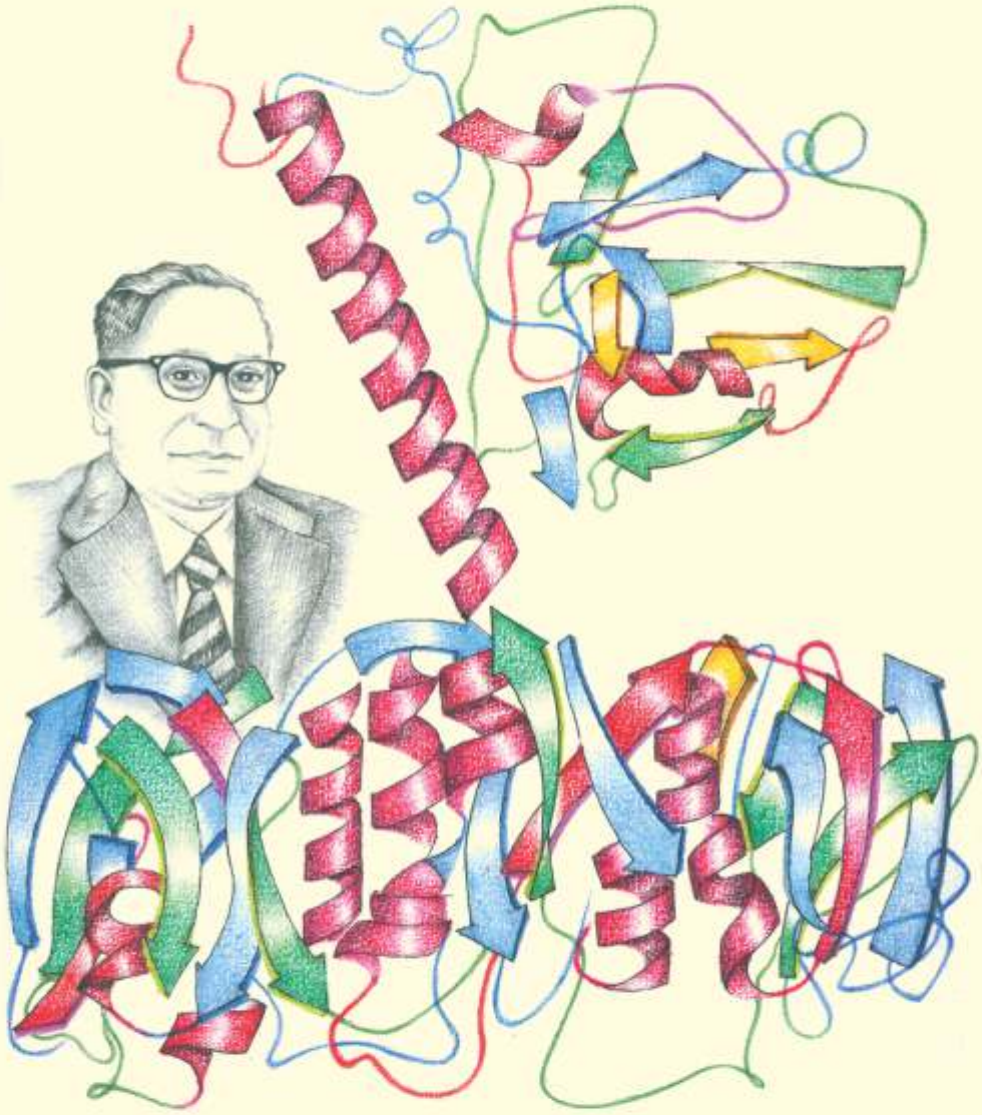
2008-2009

वार्षिक रीपोर्ट

Annual Report

50 Years of Cholera Toxin



राष्ट्रीय कॉलरा और आंत्र रोग संस्थान

(भारतीय आयुर्विज्ञान अनुसंधान परिषद्)

NATIONAL INSTITUTE OF CHOLERA AND ENTERIC DISEASES

(INDIAN COUNCIL OF MEDICAL RESEARCH)

Annual Report
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P-33, C.I.T. Road, Scheme-XM, Beliaghata, Kolkata - 700 010

डॉ विश्व मोहन कटोच
एम डी, एफ एन ए एससी, एफ ए एम एस, एफ ए एससी, एफ एन ए
सचिव, भारत सरकार
(स्वास्थ्य अनुसंधान विभाग)
स्वास्थ्य एवं परिवार कल्याण मंत्रालय एवं
महानिदेशक, आई सी एम आर
Dr. Vishwa Mohan Katoch
MD, FNASc, FAMS, FASc, FNA
Secretary to the Government of India
(Department of Health Research)
Ministry of Health & Family Welfare &
Director-General, ICMR

भारतीय आयुर्विज्ञान अनुसंधान परिषद
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वी. रामलिंगस्वामी भवन, अंसारी नगर
नई दिल्ली - 110 029 (भारत)

Indian Council of Medical Research
(Department of Health Research)
Ministry of Health & Family Welfare
V. Ramalingaswami Bhawan, Ansari Nagar
New Delhi - 110 029 (INDIA)

MESSAGE

I am happy to record my appreciation of the very significant contributions made by the National Institute of Cholera & Enteric Diseases (NICED), Kolkata in applied and basic research on diarrheal diseases. So many publications from the Institute in leading international journals and its involvement in major national programs on control of diarrheal diseases and on surveillance of AIDS bear testimony to the role played by the Institute in its area.

I, on behalf of the Council, extend my support and best wishes for Institute in its future endeavors.

(V.M.Katoch)

Tele. : (Off.) +91-11-26588204, 26589620; Fax (Off.) : +91-11-26588662, E-mail : secydhr@icmr.org.in; dg@icmr.org.in

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संदेश

मुझे अतिसारीय रोगों पर व्यावहारिक एवं मौलिक अनुसंधान के क्षेत्र में राष्ट्रीय हैज़ा तथा आन्त्ररोग संस्थान, कोलकाता द्वारा दिए गए महत्वपूर्ण योगदानों की सराहना करते हुए प्रसन्नता हो रही है। वित्तीय सहायता प्रदान करने की प्रमुख अंतर्राष्ट्रीय एजेंसियों के माध्यम से संचालित इसकी कई शोध परियोजनाओं के परिणामस्वरूप बड़ी संख्या में शोध पत्रों का प्रकाशन और अतिसारीय रोगों के नियंत्रण पर प्रमुख राष्ट्रीय कार्यक्रमों एवं एड्स की निगरानी पर इसकी संबद्धता इस क्षेत्र में इस संस्थान की भूमिका को प्रमाणित करता है।

मैं परिषद की ओर से संस्थान को इसके भावी प्रयासों को प्रोत्साहित करते हुए अपनी शुभ कामनाएं देता हूं।

Paumisa

(विश्व मोहन कटोच)

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From the Director's Desk

G. BALAKRISH NAIR
PhD, FNA, FNASc, FAAM
Director

How many outbreaks of cholera must occur before we have a permanent, affordable and effective solution to reduce or eliminate these outbreaks? This has been a disturbing question that has faced the Institute and the country. We have directed our research into such realistic areas for the past several years. There is light at the end of the tunnel. After approximately a 30-year hiatus, we are witnessing the licensure of an oral inactivated cholera vaccine in India which had its genesis in Sweden, was improvised and used in Vietnam and fine tuned at the International Vaccine Institute (IVI) in Seoul, Korea. The improved vaccine was field trialed in Kolkata urban slums supported by IVI through the Bill and Melinda Gates Foundation and the technology of manufacture was transferred from IVI to Shantha Biotechniques Ltd in Hyderabad. This is the portrait of the future; multiple players working in synchrony to alleviate misery and to make success happen. The future will show how this vaccine will improve the lives of the impoverished due to cholera and other diarrhoeal infections. For India and for NICED, 2009 has therefore been the cholera vaccine year and this remarkably coincides with the fiftieth year of the discovery of cholera toxin by Dr. S.N. De in Kolkata.

There is great enthusiasm at NICED because we now have a public tool to address endemic and epidemic cholera. Not only would we mitigate the effects of the ongoing outbreaks but we will also be able to prevent future outbreaks in the given locale which we were previously helpless in providing. This is certainly not the end of our efforts on cholera vaccines but is in fact the beginning. Our research to improve on the current vaccine is a continuing agenda. The next on the horizon is a live oral cholera vaccine developed in India with support from the Department of Biotechnology which will be going into Phase III trial very soon. The vaccine is also GMP produced by Shanta Biotechnques. Our vaccine efforts have also expanded to typhoid, rotavirus, enterotoxigenic *Escherichia coli* and shigellosis. I must acknowledge here the extraordinary foresight of Dr. S.K. Bhattacharya, the previous Director of NICED and Dr. N.K. Ganguly, the former Director General of ICMR, who initiated these vaccine trials some years ago and in reality changed the visage of NICED both in competence and in capacity.

The financial year 2008-2009 was significant from basic science research viewpoint also. To mention a few of the newer areas of basic research that we have explored and are making a dent are the colonization, antibiotic resistance and role in sepsis of neonatal gut flora, molecular understanding of ETEC colonization factors, mechanism of immunomodulatory functions of cholera toxin, pro-inflammatory function of *Vibrio cholerae* flagellin and their role in reactogenecity and immune responses, forays into metagenomic studies and basic understanding of the pathogenecity of rotavirus infections, interesting insights into viable but noncultural bacteria and some substantial molecular progress into hospitalized diarrhea stool samples that do not yield a known etiology. Another major front that has been opened with help from the south east Asian regional office of WHO is climate change and diarrhea particularly cholera. Our new Division, the Data Management division has started functioning and we intend to expand the Bioinformatic unit from a service oriented unit to a division as soon as we have additional staff Scientist positions. Other areas which have been a bit slower to take off are the Enteric pathogen repository and the multicenteric diarrhea surveillance for technical reasons. I have personally learned in the past two years is not to beat the pace; sometimes things fall in place with time. While as a group we plan the future of NICED, we are also conscious that plans do not always work because of other pressing needs of the country. In 2009, NICED was called on several emergency situations to investigate cholera outbreaks, to contribute to avian influenza investigations and more recently the H1N1 episode when NICED was designated as the Eastern zone diagnostic centre for a period of time. Our bacteriologists, epidemiologists and virologists all rose to the occasion and participated in these emergency situations with great patriotism.

We have expanded the scope of our vaccine trails by the development of a new Immunomonitoring laboratory in collaboration with IVI. The laboratory is in the process of being certified by WHO as a Good Clinical Laboratory Practices laboratory allowing us to perform immunoassays of vaccine trial samples which will meet international regulatory requirements. We are also in the process of developing modern laboratory infrastructures. Among the three building that we have now, our long term plans are to develop one as a Centre of excellence in basic research on enteric diseases, the other as a facility to practice the best of epidemiological research including vaccine trials and the third as a building which will be dedicated to training and education.

Much of what is happening and much of what will happen is because of the overwhelming support of Dr. Viswa Mohan Katoch, the Director General of ICMR and Secretary of the Department of Health Research, Government of India and from the staff of the Indian Council of Medical Research. I must also thank the Scientists and Staff of NICED for their wonderful teamwork and their dedicated research. It is a team which makes an Institute like this click.

निदेशक की मेज से

और कॉलरा के कितने प्रकोप होने चाहिए, जिससे पहले हम इस रोग को खत्म करने या कम करने का स्थाई, सस्ता और प्रभावी समाधान खोज पाएंगे? यह संस्थान और देश के लिए एक चिन्ताजनक प्रश्न है, इसलिये पिछले कुछ वर्षो से हमने संस्थान के अनुसंधान की दिशा को इस सच्चाई को जानने के लिए मोड़ दिया है। सुराग के अन्त में प्रकाश होता है। लग-भग तीस वर्षो के अंतराल के बाद हम भारत में एक मौखिकनिष्क्रिय कॉलरा के टीके को देरव रहे हैं। जिस की खोज स्वीडन में हुई और इसमें सुधार के वाद वियतनाम में इस का प्रयोग किया गया। इंटरनेशनल वैक्सीन संस्थान (आई वी आई) सियोल कोरिया में इसके बाद फिर सुधार किया गया। आई वी आई के सहयोग से बिल और मेलिन्डा गेटस फाउडेशन के माध्यम से कोलकता की मलिन बस्तियों में वैकसीन ट्रायल की गयी। निर्माण की तकनीक को आई. वी. आई. से शान्ता बाईयोटेकनिक लिमिटेड हैदराबाद को स्थांनतरित कर दिया गया। यह एक भविष्य की चित्र है, कि एक दुखद स्थिति को अन्त करने के लिए इस सिद्धान्तनीत में वहुत सारे वैज्ञानिक मिल कर काम कर रहे हैं, जिससे सफलता हासिल हो सके। भविष्य यह बताएगा कि यह वैकसीन कॉलरा और आन्त्र रोग संक्रमण से प्रभावित हुए जीवन में कैसे सुधार लाएगी। भारत और एन. आई. सी. ई. डी० के लिए वर्ष - 2009 कॉलरा वैकसीन वर्ष रहा है और उल्लेखनीय है - कि कोलकता में डा० एस. एन. दे द्वारा कॉलरा टाक्सीन की खोज का यह पचासवाँ वर्ष भी रहा है।

नाइसेड में बहुत उत्साह है, क्यों कि इस अन्तहीन और महामारी कॉलरा से निपटने के लिए हमारे पास एक साधन है। इससे महामारी को कम करने में ही नही बल्कि इसकी रोकथाम में भी हम सफल होंगे जिस के समाधान में हम पहले असहाय थे। कॉलरा वैकसीन में हमारा यह अंतिम प्रयास नहीं है, बल्कि यह एक शुरूआत है। इस वर्तमान वैकसीन में सुधार का अनुसंधान हमारी अनवरत कार्य सूची है। बायोटेकनालाजी विभाग के सहयोग से भारत में विकसित लाइव औरल कॉलरा वैकसीन हमारा अगला प्रयास है जिस का प्रयोग तीसरे चरण में शीघ्रही किया जायगा। शान्ता बायोटेकनीक द्वारा उत्पादित वैकसीन जी एम पी भी है। हमारा टायॉफाइड, रोटावायरस, इन्टरोटाक्सीजेनिक ईशरिचिया कोली और शिगलोसिस की वैकसीन बनाना लक्ष्य है। हम डा० एस. के. भट्टाचार्या, पूर्व निदेशक नाइसेड और डा० एन. के. गांगुली, आई. सी. एम. आर. पूर्व महानिदेशक के प्रति उन की असाधारण दूर दृष्टि के कृतज्ञ है। जिन्होंने इस वैकसीन की कुछ वर्षों पहले प्रयोग करने की शुरूआत की और नाइसेड के व्याक्तित्व को सामर्थ्य और क्षमता दोंनो क्षेत्रो में वास्तविक रूप में बदल दिया है।

विगत वर्ष 2008-09 बुनियादी विज्ञान अनुसंधान की दृष्टि से महत्वपूर्ण था, बुनियादी अनुसंधान के कुछ नए क्षेत्र उल्लेखनीय है जैसे कोलोनाइजेशन, एंटीबायटिक प्रतिरोध, सेपसिस में गट फ्लोरा का रोल, ईटेक के कोलोनाइजेशन फैक्टर की मौल्युकोलर भूमिका, कॉलरा टाकसिन की इम्मुयनोमोडुलेटरी भूमिका एवं रोटावाईरस की पैथोजेनिसेटी, वाइएबल किन्तु नान कल्चरल बैक्टेरिया इत्यादि की भूमिका। इससे अतिरिक्त WHO दक्षिण पुर्वो क्षेत्रीय, के सहयोग से जलवायु परिवर्तन के साथ कॉलरा के संबंध का अनुसंधान किया जा रहा है। नए प्रभाग जैसे बॉयोइनफारमेटिक एवं डाटा मैनेजमैन्ट इत्यादि ने कार्य आरम्भ कर दिया है। कुछ नए प्रभाग जैसे एंटरिक पैथोजन रिपोसिटरी एवं बहुक्षेत्रिय डायरिया सर्वेलेन्स, तकनिकी कारणो से शुरू नही पाए पर हमारी भविष्य योजना में है। 2007 मे नाईसेड को कई आपातकालिन परिस्थियों को संभालना पड़ा जैसे कि कॉलरा आउटब्रेक, पैनडिमिक HINI वाइरस की पूर्वी भारत में डाएगनोसिस। हमारे सब वैज्ञानिको ने मिल कर इन स्थितियों का सामना किया।

वर्ष 2009 में हमने, आई वी आई के साथ मिलकर इम्युनोमानिटंरिग लैबोरेटरी का विकास करके वैकसीन के प्रयोग के क्षेत्र का विस्तर किया है। यह प्रयोगशाला WHO (डबलु एच ओ) द्वारा गुड क्लिनिकल लैबोरेटरी प्रैकटिस की प्रयोग शाला के रूप में प्रमाण पत्र पाने की प्रक्रिया में है। यह हमें वैकसीन ट्रायल सैम्पलों के इम्युनोएसेस करने की अनुमति देगा। जिससे

हम अन्तराष्ट्रीय नियमों को पूरा कर सकगें। हम आधुनिक प्रयोगशाला को विकसित करने की प्रक्रिया में हैं। इन तीन भवनों में जो हमारे पास हैं - हमारी लंबी योजनाओं में से एक को आंन्त्र रोगों पर अनुसंधान का उतकृष्ट केन्द्र बनाना है, दूसरे को इपीडेमोलॉजिकल अनुसंधान जिसमें वैकसीन ट्रायल भी शामिल है, तीसरे भवन को प्रक्षिणन और शिक्षा केन्द्र बनाना है।

बहुत कुछ जो घटित हुआ है या जो घटित होगा, यह सब भारत सरकार स्वास्थ्य अनुसंधान के सचिव तथा भारतीय आर्युविज्ञान अनुसंधान परिषद के महानिदेशक डा० विश्वमोहन कटोच और आई. सी. एम. आर. के कर्मचारियों के भारी समर्थन के कारण हुआ है।

मैं नाइसेड के वैज्ञानिको और कर्मचारियों को भी उन की आर्श्चयजनक निष्ठा के लिए धन्यवाद देता हूँ।

जी. बालाकृष नायर

पी एच. डी., एफ.एन.ए., एफ.एन.ए.एस.सी., एफ.ए.ए.एम

निदेशक

NICED

RESEARCH

Bacteriology

Biochemistry

Clinical Medicine

Data Management

Epidemiology

Electron Microscopy

Immunology

Pathophysiology

Parasitology

Virology

SERVICES

Antisera Supply

Bioinformatics Centre

Clinical Laboratory

Epidemic Investigation

Vibrio Phage Laboratory

Library

TRAINING

Clinical Management

Laboratory Diagnosis

Molecular Epidemiology

Research & Training
on diarrheal diseases
(WHO collaborative centre)

ADMINISTRATION

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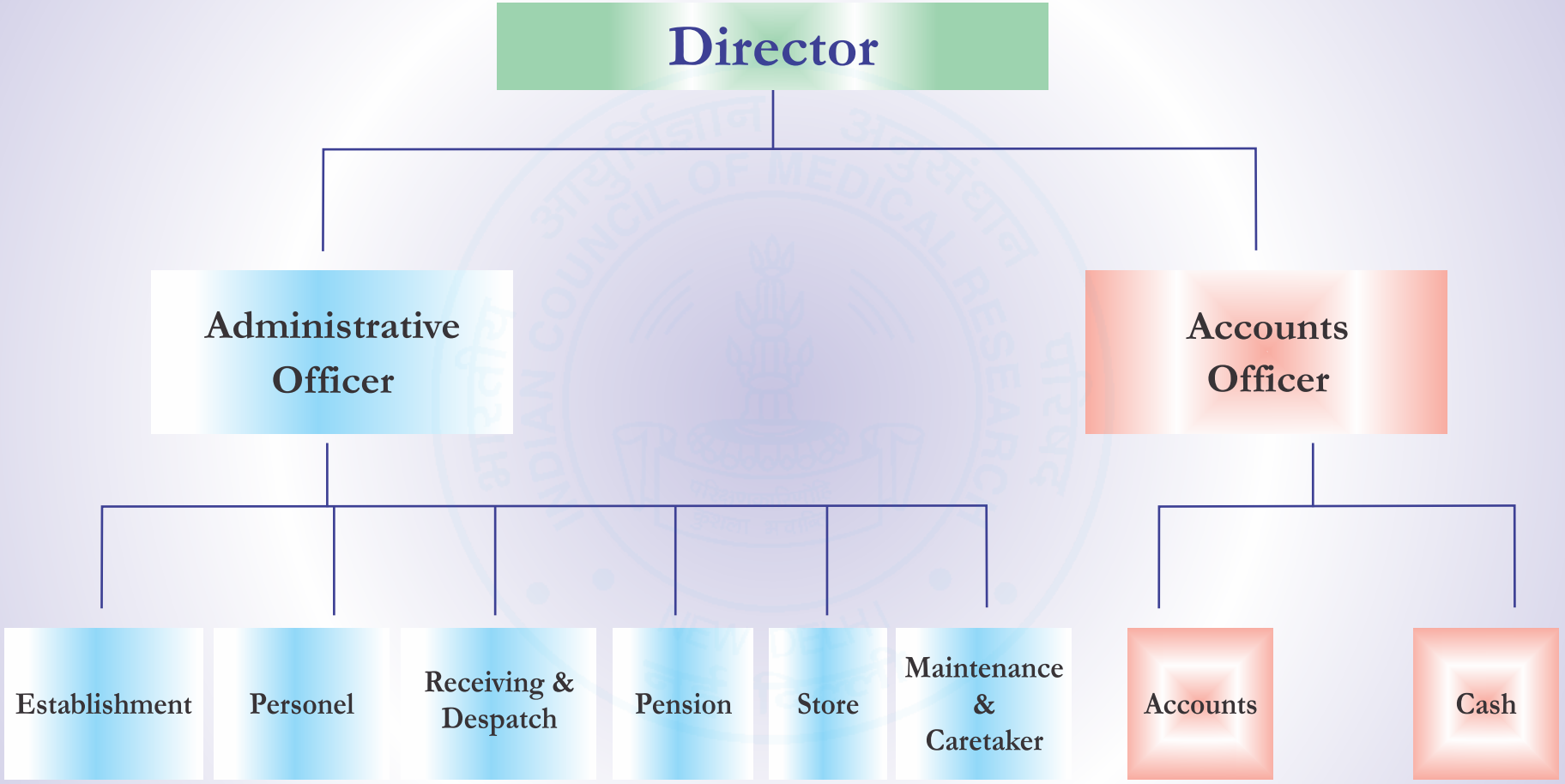
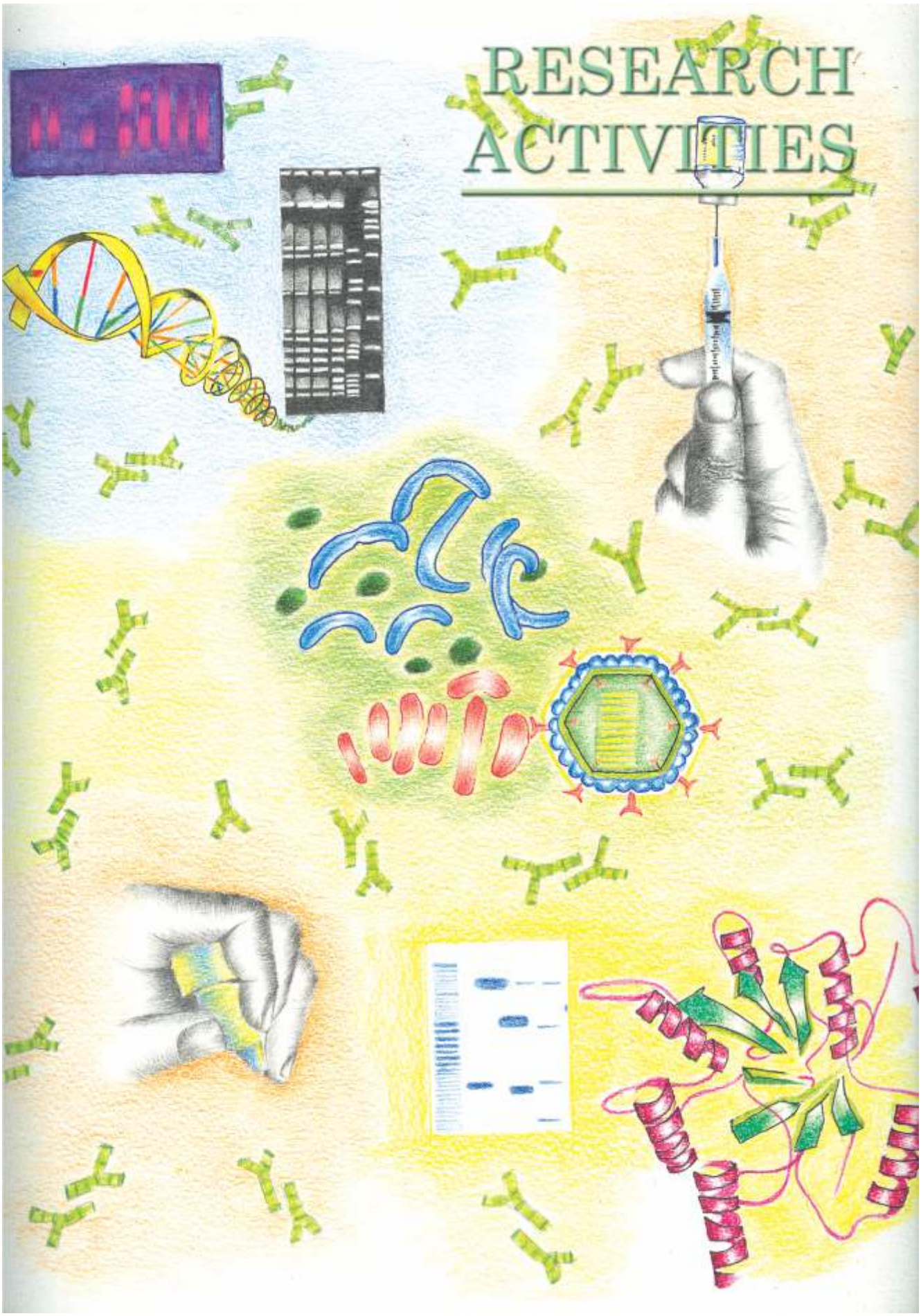
ADMINISTRATION



RESEARCH ACTIVITIES



BACTERIOLOGY

Research at the Division of Bacteriology involves characterization of enteric bacteria including *Vibrio cholerae, V. parahaemolyticus, Salmonella* spp and *Shigella* spp. isolated from hospital and community surveillance todays by applying molecular genetic and classical microbiological techniques. The Division provides referral services for identification and characterization of different enteric bacteria and also laboratory support during investigation of outbreaks/epidemics of diarrhoeal diseases in West Bengal and other parts of the country. In the recent past, the Division has focused on in-depth analysis of novel serotypes and virulence genes relevant to changes in drug resistance pattern, transmission characteristics and clinical features of the recent isolates. Data on clonality of El Tor hybrid strains from Indian and other Asian countries will be shared with members of the PulseNet Asia-Pacific. Facilities for molecular methods will be established for the rapid identification of enteric pathogens from stool specimens that were negative by conventional assay systems.

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T. Ramamurthy, Scientist F
S. Dutta, Scientist E
A. Palit, Scientist E
B.L. Sarkar, Scientist E
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A.K. Mukhopadhyay, Scientist C
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1. Molecular characterization, toxin production and antimicrobial susceptibly of *Bacillus cereus* isolated from acute diarrheal patients in Kolkata

Investigators: M. Banerjee and T. Ramamurthy

Bacillus cereus is one of the pathogens responsible for human diarrhoea, mainly due to consumption of contaminated food. Systematic surveillance and molecular characterization on *B. cereus* isolated from acute diarrhoeal patients has not been reported in India. In order to understand the importance of this pathogen as a causative agent of diarrhoea, a study was conducted using 1536 stool specimens consecutively collected for two years (October 2006 through September 2008) from the Infectious Disease Hospital and B. C. Roy Children Hospital in Kolkata. Fifty-five samples were positive for *B. cereus*, which is 3.5% of the total sample screened. *B. cereus* related acute diarrhoea was detected more in pediatric and young age groups. Forty three percent of confirmed patient belonged to 1-15y age group.

The three genes, *hblA, hblC* and *hblD* encoding the enterotoxin HBL complex were detected in 28 isolates (51.9%). Eight (14.8%) isolates possessed two of the three *hbl* genes and three (5.6%) had only one gene coding the HBL complex. Fifteen (27.8%) *B. cereus* isolates had none of HBL complex. All the three genes *nheA, nheB and nheC*, encoding the nonhemolytic enterotoxin of NHE complex were detected in 48 (88.9%) isolates. Five (9.3%) isolates harbored two *nhe* genes, whereas only one (1.9%) isolates lacked all three genes of NHE complex. The non-hemolytic enterotoxin (NHE) genes *nheA, nheB,* and *nheC* (98%, 96% and 91% respectively) are frequently detected than hemolytic enterotoxin (HBL) genes, *hblA, hblC* and *hblD* (65%, 59% and 67%, respectively). Haemolysin assay performed with culture supernatant of *B. cereus* showed that the majority (78%) of the *B. cereus* isolates exhibited hemolysis. Except for two isolates, expression of haemolysis is associated with presence of any of the *hbl* genes. Haemolytic activity was not detected in 13 isolates, which did not harbour any *hbl* genes.

Qualitative tests on *B. cereus* enterotoxin production using BCET-RPLA kit of 54 isolates showed that 36 (67%) of the isolates were able to produce BCET on BHIG in a varied concentration ranging from 8 to ≥256 ng/ml. Of these enterotoxigenic isolates, 3% produced 4 ng/ml, 6% produced 8 ng/ml, 19% produced 16 ng/ml, 14% produced 32 ng/ml, 16.5% produced 64 ng/ml, 24% produced 128 ng/ml and 16.5% produced ≥256 ng/ml. There appears to be a correlation between presence of *hbl* genes and expression of BCET. Of the 18 isolates that were negative in the BCET, 15 did not harbour any *hbl* genes and in 3 *hblC* was absent. There was no correlation between combination of *hbl* genes and an activity of expressed BCET. All the isolates were susceptible for amikacin, ciprofloxacin, gentamicin, and imipenem. Majority of the isolates were also susceptible for ofloxacin and azithromycin. Amoxyclav and cephalosporins resistance was seen in majority of the isolates.

Twenty isolates were selected for PFGE analysis based on the PCR results, which represents different combination of *hbl* and *nhe* genes. The fingerprints generated by macrorestriction with *Sma*I comprised approximately 20-25 bands of approximately 5-500 Kb, whereas with *Not*I approximately 5-13 bands of approximately 50-700 Kb found. In the present study, PFGE banding patterns of two isolates were identical with both the enzymes tested. These isolates also exhibited identical virulence gene profiles. Community based studies are needed to understand the diarrheal disease burden of this pathogen in this region.

2. Molecular characterization of *Salmonella enterica* serovar Typhi isolated from blood of clinically suspected typhoid fever cases in children in Kolkata

Investigator: Shanta Dutta

Typhoid fever is an important cause of morbidity & mortality in many developing countries of the tropical part of the world especially among the children. *Salmonella enterica* serovar Typhi (*S*. Typhi) is the etiological agent of typhoid fever. It is estimated that annual global incidence is 16 million cases with 600000 deaths.

The diagnosis of typhoid fever and paratyphoid fever is classically done by isolation of the organism using standard blood culture. It requires about a week for obtaining the result. Serology-based tests like The Widal, Typhidot and Tubex kit tests are used for rapid detection of the disease. However, they are neither specific nor sensitive and do not fulfill the criteria of an ideal diagnostic test. PCR is the most common molecular method employed to overcome these problems and any of the genes encoding *flic-d*, Vi capsular antigen and 16s rRNA are generally amplified for early diagnosis of typhoid fever. Here, we wanted to validate if the PCR method may be used for diagnosis of clinically suspected typhoid fever cases. The diagnosis is confirmed by isolation of the organism from blood samples of the patients. To do this, we standardized nested PCR for *flic d* gene of *Salmonella typhi* in the blood of patients with clinically suspected typhoid fever and attending Dr. B. C. Roy Memorial Hospital for Children. We also performed molecular characterization of *S. typhi* clinical isolates from typhoid cases children with reference to antimicrobial resistance profiles, virulence gene profile and molecular subtyping.

A total of 25 blood samples were collected from the patients with clinically suspected typhoid fever and attending OPD of Govt. Hospitals. The samples were subjected to microbiological culture, the Widal serological test and molecular methods like nested PCR for diagnosis of typhoid fever. Five ml of collected blood were inoculated into Bactec Bottle for isolation of *S*. Typhi. Sera were separated from clotted blood and used for Widal serological tube test using available Widal kit. DNA were extracted from citrated blood samples using extraction kit for performing PCR to amplify *fliC d* gene using suitable published primers. The results were compiled, data were entered into the computer and analysed using SPSS software. Out of 25 blood samples tested so far 23 were positive by PCR, 21 samples showed Widal TO and TH titres of $\geq$ 80, only three samples were positive for S. Typhi isolates, indicating high sensitivity for PCR and Widal as compared to conventional culture methods. The study is in progress.

We have included a number of *Salmonella* strains isolated from Kolkata in a typhoid vaccine trial study, for molecular typing and transmission analysis. PFGE and SNP typing were carried out using standard protocol. A total of 188 isolates were subjected to SNP analysis and the following haplotypes were obtained: H58 (139 isolates), H42 (33 isolates), H50 (9 isolates), H85 (3 isolates), H16 (2 isolates), H8 (1 isolate), pre H58 (3 isolates) with predominance of A, B and G subtypes of H58. The PFGE profile (Figure) shows circulation of more than one clone in Kolkata. Single pulsotype (P1) was predominant (30%) among the isolates. The study is in progress.

3. O1 El Tor *Vibrio cholerae* with classical B subunit gene (ctxB) of cholera toxin (CT)

Investigator : R.K. Nandy

Co-Investigators : B. L. Sarkar, A.K. Mukhopadhyay and G.B. Nair

Recently, genetic markers have been used in addition to the phenotypic traits for biotype assignment of *Vibrio cholerae*. Screening of strains for the detection of biotype specific *ctxB* alleles led to the detection of classical biotype specific *ctxB* allele among *V. cholerae* O1 strains that typically exhibited El Tor phenotypes and these strains were named as O1 El Tor variant strains. Existence of such O1 El Tor variant strains has recently been reported from many countries of Asia and Africa including India, Bangladesh, Mozambique, Vietnam, Hong Kong, Japan, and Zambia. The present study was undertaken to understand the changing traits of *ctxB* alleles among *V. cholerae* strains isolated in Chennai, India since 1980.

Screening of *V. cholerae* O1 El Tor strains isolated in Chennai, India revealed temporal shifts of *ctxB* alleles over 1980-2008. Strains with both classical and El Tor alleles of *ctxB* were identified until 1991and these strains were named as *V. cholerae* O1 El Tor variant-I. In fact, O1 El Tor variant-I strains were detected as sole type during 1980 to 1984. The O1 El Tor variant-II strains that possessed only classical *ctxB* have started appearing in 1992 and caused complete replacement of the other type since 1993, the period when emergence of O139 took place in Chennai. Prototype El Tor strains was identified between 1985 and 1992. These results demonstrated that over the decades, predominance of O1 El Tor variants are more usual in Chennai, an area known to be cholera endemic in the Indian subcontinent.

Identification of these strains capable of expressing toxin characteristic of the classical biotype raised wide interest to understand its epidemiological implication. Recent events of more severe cholera episodes with high case fatality rate in certain East African countries as well as remote areas of Asia has been ascribed to the O1 variant strains that typically have El Tor background and express toxin of classical biotype.

4. Detection, distribution and expression of virulence factor(s) among clinical *V. cholerae* non-O1, non-O139 strains

Investigator : R.K. Nandy

Co-Investigator : T. Ramamurthy

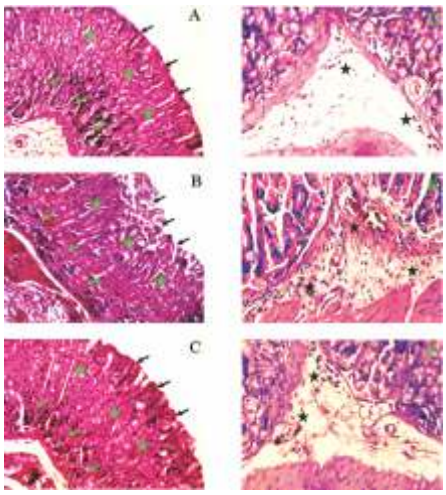
V. cholerae strains belonging to non-O1, non-O139 serogroups are known to cause sporadic cases of gastroenteritis. This study was carried out with 54 non-O1, non-O139 *V. cholerae* strains that were isolated from hospitalized diarrheal cases at Kolkata. All but one were non-toxigenic in nature but had the potential to cause watery diarrhea. Interestingly, CTX prophage present in the toxigenic non-O1, non-O139 showed its lineage different from its counterpart present in O1 or O139 strains. Studies also demonstrated that the toxigenic non-O1, non-O139 strain was capable to produce cholera toxin (CT), though the amount was about 10 folds less to that of the epidemic strains when tested *in vitro* and the amount varied with cultural conditions. PCR based screening was employed targeting a number of other virulence genetic elements, including *hlyA*, *rtxA*, TTSS, VSP-I, and VSP-II. Most of the 54 non-O1, non-O139 strains possessed *hlyA* (87%) and *rtxA* (81.5%). The prevalence of the RTX cluster in most of the non-O1, non-O139 strains isolated is consistent with the hypothesis that RTX contributes to virulence in non-toxigenic *V. cholerae*. Among the other important virulence factors 31.5% strains possessed TTSS gene cluster. Only one non-

O1, non-O139 strain was PCR positive for both VSP-I and VSP-II genes, while the rest of the non-O1, non-O139 strains were negative for both VSP-I and VSP-II which is consistent with previously published reports. None of the non-O1, non-O139 strains were PCR positive for nonagglutinable *V. cholerae* heat-stable enterotoxin.

Functional expression of virulence phenotypes was assayed by using a panel of 13 clinical non-O1, non-O139 *V. cholerae* strains. These strains caused hemolysis of rabbit erythrocytes, secreted cytotoxic factors in the culture-free supernatant, produced biofilm, and were motile; however, interesting patterns could be detected. For example, a tendency to have a higher hemolytic titer and higher cytotoxic activity was evident in strains that carried the TTSS gene cluster. The strains that possess the TTSS cluster also appeared to be more motile than those lacking the TTSS, suggesting a role for this secretion system in human infections by non-O1, non-O139 strains. No clear correlation could be obtained between the presence of specific virulence genes and biofilm formation. The culture filtrate obtained from these strains induced cell rounding in HeLa cells, which can be reversed by replacing the culture filtrate with fresh medium. Interestingly, the strains that lacked *ctxA*, *hlyA*, and TTSS and strains that lacked *rtxA* were capable of inducing cell rounding. Certain interesting characteristics were evident, including the identification of single strain carrying *ctxA*. Most strains isolated also contained RTX and/or HlyA, but two of the strains identified in this study contained none of these defined factors, indicating that additional virulence factors associated with human disease remain to be identified. The continuous development of genetic diversity in non-O1, non-O139 strains within the environment and the association of *V. cholerae* non-O1, non-O139 strains of several serogroups with clinical diarrheal cases are likely to complicate the development of an effective cholera vaccine.

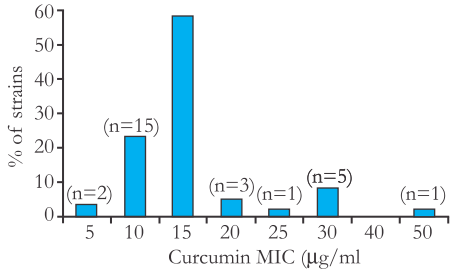
5. Antimicrobial activity of curcumin against Indian *Helicobacter pylori* and also during mice infection

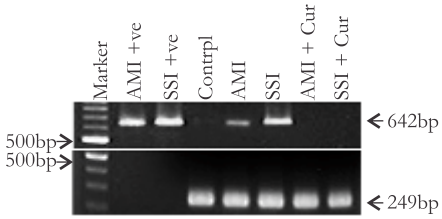
Investigator: Asish K. Mukhopadhyay



Helicobacter pylori is of growing concern today because of its crucial role in the pathogenesis of chronic gastritis, peptic ulcer diseases and in the multi-step carcinogenic process of gastric cancer. The rapidly emerging drug resistance in *H. pylori* strains during treatment with various antibiotics is a major obstacle for successful eradication therapies. Because of the prevalence of antibiotic-resistant *H. pylori* strains, there is an increasing search for safe and effective non-antibiotic compounds that inhibit *H. pylori* growth. In Indian traditional medical system, a number of plants and plant products are known to possess potent medicinal properties, suggesting their usefulness in the treatment. Several studies have shown that curcumin (diferuloylmethane), the most active constituent of the perennial herb *Curcuma longa* (commonly known as turmeric), possesses antibacterial potential. This prompted us to explore its antimicrobial potential on Indian *H. pylori* strains that are geographically distinct from East Asian and Western strains. Moreover, a majority of the Indian population harbors *H. pylori* and quite a number of them suffer from *H. pylori* associated gastrointestinal diseases.

Our study have primarily shown that curcumin potentially inhibited the growth of all the *H. pylori* strains tested *in vitro* that were isolated from infected patients suffering from gastrointestinal disorders (Figure 1). It is noteworthy, that a majority of these strains were metronidazole resistant





with a MIC ranging from 16µg/ml- >64µg/ml. So, our results suggest that curcumin acts through mechanisms distinctly different from the mode of action of these antibiotics for inhibition of *H. pylori* growth.

The mouse model of *H. pylori* infection has been widely used to investigate host responses to *H. pylori* infection as well as eradication studies. Our study has shown that curcumin treatment completely eradicated *H. pylori* from infected mouse stomach (Figure 2). This eradication by curcumin was irrespective of the bacterial genotype that is independent of the presence of *cag*PAI. These data are of immense importance in the perspective of development of alternative therapy against *H. pylori* infection since studies on high doses of curcumin in animals and human have confirmed a lack of any toxic side effects. Histological analysis clearly showed that the gastric damage induced by *H. pylori* infection was almost completely restored by curcumin thus highlighting its potential as an alternative therapy against *H. pylori* infection (Figure 3). In conclusion, all these observations not only indicate the therapeutic potential of curcumin against *H. pylori* infections but also highlight the anti-inflammatory effect of curcumin, although further studies are required to extrapolate its effect on humans.

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

Investigator: B. L. Sarkar

During the period under study, a total of 555 strains of *V. cholerae* were received from different parts of the country for serotyping, biotyping and phage typing. Of these, 493 (88.83%) representative strains confirmed as *V. cholerae* O1 biotype ElTor were included in phage typing study. The largest number of strains was received from Maharashtra state. Majority of the strains belonged to Inaba (76%). For the last couple of years, Ogawa was the dominant serogroup. A total of 59 (11.97%) strains were found to be untypeable with the conventional scheme of Basu and Mukerjee. These strains were grouped under type 2 with Basu and Mukerjee scheme. 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 (81.95%) followed by type 26 (2.23%), type 24 (0.61%), type 11 (0.61%) respectively. It has been observed that type 27 was the predominant phage type circulating in this country.

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

Investigators: B. L. Sarkar and R. K. Nandy

N-4 Φ, a lytic bacteriophage of ElTor O1 typing scheme was selected for complete nucleotide sequencing. Genomic DNA was subjected to enzymatic digestion with a twenty eight restriction enzymes. The size of the DNA was 40 kb as estimated with *Hin*dIII and E*co*RV. The phage DNA was randomly sheared using an ultrasonic disintegrator and was controlled in such a way to generate fragments ranging from 0.2-1kb with most between 500-600bp regions. 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. A total of 470 transformants were arbitrarily picked up for further study. Purified plasmid DNA was isolated form randomly selected 228 clones and tested for the presence of insert. Results showed that among these 228 clones, contained inserts with sizes ranging between 500 and 600bp. Here, universal M13 primers were used as sequencing primer. A total of 228 clone sequences and subsequently 456 nucleotide sequence data with both forward and reverse primers were used to obtain whole genome sequence of phage N4. The nucleotide sequences were assembled using Sequencher 4.1 software package, creating a genome scaffold. Trimmed and edited clone sequences were assembled into three contigs. Three gaps were formed between these contigs which were connected by primer walking. Primers designed to extend the ends of contigs from one end yielded a sequence identical to the other end of the contig. Six primers were required to cover up these gaps and designated as walking primers. Primer walking using these six primers facilitated to complete the whole genome and a draft sequence of the phage N4 genome was made. This draft sequence was transformed into final after reconfirmation and exposed a fact that the vibriophage N4 has a circular, double stranded chromosome with 38,497 bp in size. 47 ORFs were identified as distributed throughout the phage genome and predicted as coding regions. Only to a minority of the putative ORFs, a putative function could be assigned. Out of 47 ORFs of the N4 genome, 30 were supposed to predict their function by homology search but 17 ORFs were found with no significant homology with the database. These ORFs with unknown function were clustered as unclassified. The vibriophage N4 genome was split into four provisional clusters of genes with related functions. One cluster was supposed to support DNA replication, the others encode structural, regulatory, and lytic functions.

8. Studies on the effect of Vibrio *cholerae* phages on *V. cholerae* in RITARD model

Investigators: B. L. Sarkar, H. Koley and D. R. Saha

Vibrio cholerae O1 strain MAK 757 and the cocktail of five *V. cholerae* O1 phages were challenged in Rabbit Ileal Loop (RIL) model. Two sets of rabbits were used for this purpose, one was infected with only *V. cholerae* MAK 757 strain and in another one MAK 757 and a cocktail of respective vibriophages were used. In both the rabbits, diarrhoeagenic inflammation was observed but it was found lesser in the rabbit where vibriophages were challenged.

After that, similar experiment was carried out in RITARD model. In one set (control), the rabbit was infected with only *V. cholerae* MAK 757 strain. In another set, rabbit was challenged with MAK 757 with cocktail phage of *V. cholerae* O1. This study concerns the feasibility of possible exploitation of bacteriophages as a biocontrol agent to eliminate the pathogen *V. cholerae* in the gut. Control rabbit challenged with 10^9 CFU/ml MAK 757 developed Grade II to IV diarrhoea but the phage treated one which received 10^9 CFU/ml MAK 757 and 10^8 PFU/ml cocktail phages, produced only Grade II diarrhoea. Phage-treated rabbit euthenized 24h post infection had 100-fold less infectious cells (1.3×10^9 CFU/ml) compares to the untreated control. It also showed the presence of 100 fold increase in phage titre (0.9×10^{10} PFU/ml) compared to the initially administered dose. On the other hand, histological results revealed that in control rabbit (MAK 757 treated) vili lost its normal shape and that it displayed more inflammatory cellular infiltration in lamina propia. In experimental rabbit (phage-treated)

on the other hand vili of the intestinal mucosal almost appeared normal. The studies are underway to confirm that cholera phage can be the alternate to antibiotic as phage therapy.

9. Identifying environmental risk factors for endemic diarrheal diseases in West Bengal, India: a remote sensing geographic information system (GIS) approach.

Investigator: A. Palit

The results obtained from both OLS (Ordinary least squares) and spatial autoregressive (SAR) lag model suggest that water environment variable has association with the higher incidence rate for diarrhea, cholera or typhoid, which can only be clearly understood subject to selection of higher numbers of points of sources. The OLS model shows one community of population in the area had lower typhoid incidence rate. The SAR model shows higher percent of the particular community population in the area or the higher percent of people using boil/filter water in the area had lower diarrhea incidence rate. SAR model, likewise ORS also shows higher percent of a particular community population in the area had lower typhoid incidence rateAccording to the results obtained, we conclude that in the areas under Kolkata Municipal Corporation, drinking water quality is a matter of concern due to both problems of public supply infrastructure and inadequate household reservoir protection or sanitary installations. Bacterial contamination is of particular concern in public eateries as well as in the low socio-economic domiciles.

The effects of external factors on water quality were found to be complex. We suggest that sampling frequency of water quality (2%, on average sampled about six times during one year) should be increased to develop highly significant empirical models.

GIS methods can be efficiently applied to complement and extend results obtained from logistic regression analysis: spatial data analysis can provide additional relevant inputs for statistical analysis (in our case, altitude and distance between domiciles and treatment station). Spatial extrapolation can significantly improve visualization of health risks, supporting environmental and infrastructure planning and health care measures.

10. Evaluation of the Impact of Climate Change on the Occurrence of Diarrhoeal Diseases with Emphasis on Cholera (Preparation of a generic protocol for WHO_APW).

Investigator: S. Kanungo, A. Deb, A. Palit and G.B. Nair

The protocol has been prepared for WHO-SEARO as an Agreement for Performance of Work between SEARO and National Institute of Cholera and Enteric Diseases, Kolkata.

NICED gratefully acknowledges the support of SEARO and Dr. Jai P. Narain, Director, Dept. of Communicable Diseases, SEARO, WHO. The NICED Climate-Diarrhoea Team consisted of Suman Kanungo, Alok K. Deb, Anup Palit and G.B. Nair. The protocol has been prepared for WHO-SEARO as an Agreement for Performance of Work between SEARO and National Institute of Cholera and Enteric Diseases, Kolkata.

The said generic protocol has been developed and placed under expert review committee for their suggestions and comments, modified accordingly and subsequently submitted to WHO-SEARO, New Delhi. The protocol is currently under evaluation.

11. Analysis of environmental samples with special reference to potable water to understand transmission route of diarrhoeal pathogens.

Investigator: A. Palit

Investigation and evaluation of the association between the quality of potable water and diarrheal episodes is a continued activity for the laboratory. So far, in an one and half year study within Kolkata Municipal Corporation, Kolkata, correlation of diarrheal episodes with the source and quality of potable water was monitored weekly in selected diarrhea prone foci. Out of 517 water samples collected and analyzed, stored water (for washing) showed a higher prevalence of fecal coli forms (58%) ($p<0.00000001$) in comparison to stored (for drinking) water samples (28%) and tap/tube well water source (8%) respectively. Among the samples from different sources, water stored for washing had highest level of non-permissible/unsatisfactory range of physicochemical parameters. Fecal contamination levels in household water containers were generally high though pH, TDS etc. were within permissible range. Two-third of the samples of water stored for washing samples failed to reach the satisfactory level of residual chlorine level. In this circumstance, it is difficult to correlate the quality of storage water with incidence of diarrhea.

Microbial analysis and examination of samples of potable water sources from different parts of West Bengal and other states of India during diarrhea or cholera outbreak is routinely done in the laboratory. The results are communicated to the appropriate Govt. agencies.

12. Neonatal gut flora: colonization, role in sepsis and antibiotic resistance

Investigator: Sulagna Basu

The first step to most nosocomial infections is colonization which takes place just after birth and can be affected by the hygiene conditions during labor and delivery, environment, the life support systems, post-natal care and feeding. The favored niche for colonization is the gastrointestinal tract and among the hundreds of bacteria that colonize the GI tract there are potential pathogens. The neonate is vulnerable to these pathogens because an immature host defense system and immature gut barrier is unable to retain these bacteria within the lumen of the gut. When such viable bacteria cross the gut barrier via the mesenteric lymph node and reach the blood stream it can cause sepsis. This process of the passage of bacteria from the gut to the blood is called bacterial translocation.

We studied the neonatal flora with special reference to Gram negative rods and tried to evaluate whether the flora plays a role in sepsis in neonates in a tertiary care hospital in India. The flora evaluated revealed great diversity and the most predominant Gram negative rod isolated was *Klebsiella pneumonia* followed by *E.coli*. *Klebsiella pneumoniae* was the most frequently isolated organism from the blood of the septecemic babies. The other Gram negative organisms that caused septicemia in the neonates were *E.coli*, *Acinetobacter baumannii*, *Burkholderia cepacia* and *Enterobacter cloacae*.

Assessment of the gut flora and the isolates in the blood showed that in quite a number of babies the organism in the blood was also isolated from the gut. In such cases the pulsotypes (pulsed field gel electrophoresis pattern) and antibiotic sensitivity pattern of the organisms were studied. Bacterial translocation was considered likely when organisms of indistinguishable pulsotypes were isolated from the blood and also the gut

of the neonates and had a similar antibiotic sensitivity pattern. Translocation was the most likely cause of sepsis in 50% of neonates in whom a Gram negative organism was isolated from the blood.

In neonatal septicemia another emerging problem is antibiotic resistance. Till date antibiotics remain the only option for treatment of the newborns with infection and antimicrobial resistance is a reason for great concern. Clinically, the most widely used family of antimicrobial agents are the ß-lactams, and the most significant mechanism of resistance to the ß-lactam agents is the production of enzymes that inactivate them, namely ß-lactamases. ESBLs, so named because of their increased spectrum of activity, confer resistance to third- and fourth generation cephalosporins and monobactams. They are usually derived from earlier, narrow-spectrum β-lactamases and differ from the parent enzyme by a few point mutations, which confer an extended spectrum of activity.

An assessment was made of the changing pattern of antibiotic resistance both by phenotypic and genotypic methods with reference to ESBLs in *Klebsiella pneumoniae* and *Escherichia coli* from neonates. The study showed that more than 90% of the isolates colonized in the gut of the babies are ESBL producers and about 78% that caused infections were also ESBL producers. Furthermore, it illustrated that there is a complex interplay of genes that are involved in ESBL-mediated resistance in neonates.

13. Studies on colonization ability of tcp^{-ve} *Vibrio cholerae* strains in animal model.

Investigator: Hemanta Koley

The colonization of the small bowel and the action of enterotoxin on the mucosal cells leads to marked changes in the biochemical and physiological functions. But the mechanisms whereby *V.cholerae* reach to the mucosal surface and effectively colonize this area are poorly understood. A number of possible mechanisms have been postulated through studies conducted in animal models. The possible mechanisms, directly or indirectly, depend on several factors produced by *V.cholerae* and have been implicated as important agents aiding the process of colonization. Among others, the known virulence factors of *V.cholerae* particularly the O1 serogroup, are cholera toxin, hemagglutinin, outer membrane proteins, toxin co-regulated pili, core encoded pilin, other pili and lipopolysaccharide and in the new serogroup of *V.cholerae* O139, the capsular polysaccharide. The process of colonization can largely be understood by conducting investigations using different animal models because the complex interactions between two dynamic living systems are impossible to duplicate *in vitro*. Most pathogenic O1, O139, and non-O1, non-O139 strains are ctx$^-$ tcp$^-$ but can still cause diarrhea. In this study, we are working on to find out the new factor responsible for diarrhoea some times lethal diarrhoea in different animal models.

14. Molecular characterization of multi-drug resistant *Shigella spp.* isolated from epidemic and endemic cases of Shigellosis in India

Investigator: T. Ramamurthy

Shigellosis remains an important public health problem with *Shigella sonnei* in Europe and US, and *S. flexneri* in Asian and African countries being of epidemiological importance. Antimicrobial therapy is advocated for shigellosis to shorten the duration of illness. However, in Asia and Africa, antimicrobial resistance is emerging among *Shigella* spp. and the treatment options is becoming limited. This study shows the prevailing mechanisms

of antibiotic resistance and clonal relatedness of *Shigella* strains isolated from epidemic and endemic cases of shigellosis in different parts of India.

Sixty strains of *Shigella* spp. (20 *S. dysenteriae*, 16 *S. flexneri*, 7 *S. boydii* and 17 *S. sonnei*) isolated from dysentery outbreaks from different parts of India and sporadic hospitalized cases of shigellosis in Kolkata and Goa were included in this study. Strains were confirmed as *Shigella* spp. by standard biochemical tests and serotyped using commercially available antisera. Table 1 shows the identified serotypes of the 60 *Shigella* strains, their antimicrobial resistance profiles and resistance genes. *S. dysenteriae* type 1 strains (n=17) were uniformly resistant to all the tested antimicrobials, except for azithromycin and ceftriaxone. *S. dysenteriae* type 1 strains HU8 and BCH518 isolated during 1988 and 1995 from a dysentery outbreak and sporadic infections respectively, had similar resistance profiles. The two *S. dysenteriae* type 5 strains were susceptible to ampicillin, fluoroquinolone, azithromycin and ceftriaxone and had a resistance profile of CoTCNaS. Except for two strains (NK2685 and NK2683), all the tested *S. flexneri* strains (n=16) were resistant to cotrimoxazole, tetracycline and streptomycin. Three strains of *S. boydii* serotype 12 and the majority (94%) of the *S. sonnei* strains had an identical resistance pattern (CoTNaS). The MIC for azithromycin resistant *S. flexneri* type 3b (NK2788) and *S. boydii* type 1 (G24371) was 192 and 128 g/ml, respectively. None of the other *Shigella* strains proved to be resistant to azithromycin and ceftriaxone.

Fluoroquinolone resistance and resistance mechanisms

Ciprofloxacin, norfloxacin and ofloxacin are broad-spectrum fluoroquinolone agents that have excellent activity against most enteric pathogens. In this study, 30% of the *Shigella* strains were resistant to fluoroquinolnes and a *S. boydii* serotype 1 strain (G24371) was resistant to each of the four compounds tested (Table 2). Due to the unrestricted use of fluoroquinolones in Kolkata for the treatment of diarrhoea and other infectious diseases, resistance to these drugs has been reported among enteric pathogens. *S. dysenteriae* type 1 strains isolated from sporadic cases of dysentery from Calcutta (BCH518, NK2678 and H16576), Goa (12567) and outbreak cases from Kolkata, Aizal, and Chandigarh (D2, 21, AZ11, and 115, respectively) were tested for mutations in the *gyrA* and *par*C genes. All fluoroquinolone resistant strains had a uniform mutation in GyrA at position 83, (replacement of serine with leucine) and the majority of strains had a second mutation at position 87 with replacement of aspartic acid either with glycine or asparagine (Table 2). However, the *S. dysenteriae* type 1 strain BCH518, isolated during 1995 had a single mutation in the GyrA at position 83, but strain HU8 isolated in Tripura during 1988 had no mutation in *gyrA* and *parC* and showed reduced susceptibility to nalidixic acid although susceptible to fluoroquinolones (Table 2). However, *S. flexneri* (NK2788), *S. boydii* (G24371) and a *S. dysenteriae* type 1 strain from the Aizwal outbreak showed amino acid replacement at position 87 (D→N). All the fluoroquinolone resistant strains had a single mutation in ParC at position 80 (replacement of serine with isoleucine). In a nalidixic acid resistant *S. sonnei* (NK2017), a mutation was identified at position 83 (replacement of serine with leucine). Fluoroquinolone resistant strains of *S. boydii* (G24371), *S. flexneri* (NK2788) and a representative *S. dysenteriae* type 1 (12567) strongly exhibited fluoroquinolone efflux (Table 3). The steady sate accumulation of norfloxacin and ciprofloxacin was 2 to 4 fold lower in the resistant strains compared to that in the case of sensitive strains, C152 (Table 3). This suggests that the lower accumulation of fluoroquinolones can also account for the resistance of these strains. After the disruption of the efflux pump with the proton motive force uncoupler, m-

chlorophenylhydrazone (CCCP), the accumulation was almost at the same level in all the tested strains. This clearly suggests the role of efflux pumps as one of the responsible factors for the development of resistance.

Plasmid-mediated quinolone resistance due to DNA gyrase protection by a protein from the pentapeptide repeat family called Qnr has recently been described in many clinical isolates of several species. In this study, none of the strains harboured the *qnr* or its alleles. A novel ciprofloxacin-modifying enzyme (aminoglycoside acetyltransferase) encoding gene *aac (6´)-1b-cr* was found in members of the *Enterobacteriaceae*, resistant to fluoroquinolones. We have identified the *aac(6')-1b-cr* gene in *S. boydii* type 1 (G24371) and *S. flexneri* 3b (NK 2788) strains and to our knowledge, this gene has not been reported previously in these *Shigella* species.

Resistance to other antimicrobials and resistance genes

All the *S. dysenteriae* type 1 strains harboured bla_{oxa-1}, catA1, tet(B), and strA genes, encoding resistance for ampicillin, chloramphenicol, tetracycline, streptomycin, respectively (Table 1). The, tet(B) gene was more common (90%) than tet(A) (10%) in S. dysenteriae. Irrespective of serotypes, S. flexneri strains harboured tetB as well as the bla_{oxa-1} genes and in strain NK2788, bla_{oxa-1}, bla_{TEM-1} and tetA genes were detected (Table 1). In this study, $bla_{CTX-M-3}$ was found in a *S. boydii* type 1 strain (G24371) and to our knowledge, this is the first report of this enzyme in this serotype. The majority of S. sonnei strains harboured strA (88%) and tetA (76%) genes rather than aadA1 and tetB (6% each). Genes encoding resistance for kanamycin (aph1a), gentamicin (aadB) and tetracycline (tetC, tetD, tetE and tetY) were not found (data not shown). We found 97% and 3 % of ampicillin resistant *Shigella* strains harbouring bla_{oxa-1} and bla_{TEM-1} genes, respectively. Presence of the chloramphenicol resistance gene catAI that encodes for chloramphenicol o-acetyl-transferase, was confirmed in S. flexneri strains either with strA or aadA1 genes or both (Table 1).

PFGE analysis: It was necessary to show whether the frequency of antimicrobial resistance and its determinants was due to the widespread occurrence of specific clones. *Two S. dysenteriae* type 5 (NK2440 and NK2454) had identical *Xba*I restriction patterns by PFGE (Fig. 1), but were different from serotype 1 strains from our previous finding. Sixteen strains representing different serotypes of *S. flexneri* showed extensive variation in PFGE profile (Fig 2) but six strains of type 2a wre identical and closest to two strains of serotype 2b. The remainders were distinct in DNA profile. Of the seven *S. boydii* strains, 3 belonging to serotype 12 were closely related in DNA profile while the remainders were distinct (Fig. 3). Eleven of the 17 *S. sonnei* strains were identical in DNA pattern (Fig. 4). Although PFGE has proved to be a powerful tool for the discrimination of strains and identification of clonal lineages in several bacterial species, in some *S. boydii* serotypes it may not be as indicative of absolute strain relatedness. The other serotypes were genetically heterogeneous. The population structure of this species therefore warrants further investigation with complementary molecular tools such as multilocus sequence typing.

HONOURS AND ACHIEVEMENTS

G. B. Nair: Awarded J.B. Chatterjee Gold Medal at the J.B. Chatterjee Memorial Oration to commemorate the 37th Death Anniversary of Late Prof. J.B. Chatterjee at the Calcutta School of Tropical Medicine, Kolkata on February 28, 2009.

Elected to Fellowship in the American Academy of Microbiology. Fellowship will be awarded at the 109th ASM General Meeting in Philadelphia on Wednesday May 20, 2009.

Nominated as the Chair of the WHO Global Laboratory Directory for cholera and other diarrhoeal diseases known as CHOLDInet at WHO SEAR sub-unit office, Bangkok, Thailand during March 26-28, 2009.

PRESENTATIONS & VISITS

G. B. Nair: Attended Diarrhoeal Disease Advisory Committee Meeting as a Member at the WHO, Geneva, Switzerland during October 5-10, 2008.

Delivered a talk on "Molecular basis of the emergence of a more severe form of cholera" at the 7th Winter Symposium 2009 Integrating Basic Sciences into Public Health at CMC, Vellore during January 22-24, 2009.

Attended Research Day (February 5, 2009) celebration of the University of Florida, Gainesville, USA at the Emerging Pathogens Institute during February 4-8, 2009.

Delivered a talk on "A New Strain of Cholera and it's Global Impact" at the 'J.B. Chatterjee Memorial Oration' at the Calcutta School of Tropical Medicine, Kolkata on February 28, 2009.

Attended Manipal University Academic Senate meeting at Mangalore on February 3, 2009 and delivered a talk on "The Global Impact of the New Strain of Cholera" at 'Dr. J.V. Bhat Memorial Oration' at Dr. TMA Pai Planetarium, Manipal on March 3, 2009.

Attended the WHO/IHR Informal Consultation Series on "Concept and design of laboratory networks" and "Laboratory network for cholera and diarrhoeal diseases" at WHO SEAR sub-unit office, Bangkok, Thailand during March 26-28, 2009.

S. K. Niyogi: Participated in the 2nd India Probiotic Symposium-Evidence based health benefits of probiotics held at Hotel Intercontinental, Nehru Place, New Delhi during November 7-8, 2008.

Visited the Laboratory of Prevention of International Epidemics, Department of Veterinary Sciences, Osaka Prefecture University, Osaka, Japan in connection with the ongoing research works on Molecular analysis of Shigella spp including antimicrobial resistances during December 15 – 20, 2008.

Participated in the Symposium "Health Challenges of Orissa" on the occasion of Foundation Day Celebration 2009 held at RMRC, Bhubaneswar on March 30, 2009.

T. Ramamurthy: Attended the Global Enteric Multicentric Study (GEMS) project meeting held at Seattle, USA during September 10-12, 2008.

Presented a poster entitled "Diarrhoeal disease surveillance and aetiology of diarrhoea at the Infectious Diseases Hospital, Kolkata (Authors)" at the Forum of the Network of Research Centers of Infectious Diseases held at the National Institute of Hygiene and Epidemiology, Hanoi, Vietnam on October 6, 2008.

Presented a talk entitled "The changing genome of *Vibrio cholerae* O1 and "Vibrio parahaemolyticus pandemic (Authors)" at the 2008 Annual Scientific Meeting of the Australian Society for Microbiology held at Melbourne, Australia during November 6-10, 2008.

Attended short-term training on Molecular Techniques and Bioinformatics Tools in Biological Research as a Resource Person and delivered a talk on "Molecular diagnosis of diarrhoeal diseases" on December 4, 2008 at Faculty of Veterinary Science, Assam Agriculture University, Guwahati.

Visited Osaka Prefecture University, Sakai, Osaka, Japan to attend the discussion meeting with Prof. S. Yamasaki during December 15-20, 2008.

S. Dutta: Delivered lecture on "Rapid diagnostic tests for enteric fever-the view from India" at the CME

jointly organized by Health Protection Agency, UK; Safdarjung Hospital and Vardhman Mahavir Medical College, New Delhi; BLK Hospital, New Delhi on "Antimicrobial resistance in infectious diseases" and "Recent advances in diagnosis of Enteric fever", held at India Habitat Centre, New Delhi on November 20-21, 2008.

R.K. Nandy: Accepted for poster presentation entitled "Genesis of Vibrio cholerae O1 clinical strains that carry both classical and El Tor types of CTX prophages segregated into two chromosomes (Authors)" at the 43rd United Sates-Japan Conference on Cholera and Other Bacterial Enteric Infections held in Kyushu University, Fukuoka, Japan during November 17-19, 2008.

S. Basu: Preseted a poster entitled "Role of gut microflora in neonatal sepsis (Sulagna Basu, Parijat Das, Arun K. Singh, Sudipta Dasgupta, Subhashree Roy, T Ramamurthy and Yosifumi Takeda) at the 4th Asian-African Research Forum on Emerging and Reemerging Infections held at Hokkaido University, Sapporo, Japan during December 15-16, 2008.

H. Koley: Presented an invited lecture on the occasion of UGC sponsored National Seminar, Current Trend in Human Physiology Research: It's Contemporary Relevance at the Presidency College, Kolkata on December 19 , 2008.

A. Palit: Presented a paper entitled "Transmission route of diarrheal pathogens: the problem of in-house contamination of potable water at community level (Authors)" at the International Symposium on Tribal Health held at Regional Medical Research Centre for Tribals (ICMR), Jabalpur, India during February 27-March 1, 2009.

Attended the 2nd India Probiotic symposium, "Role of probiotics in Intestinal Milieu and Disease management", held at Hotel International Eros, Nehru Place, New Delhi during November 7-8, 2008.

Participated at IMI workshop, sponsored by DSIR, Govt. of India, at New Delhi on May 3-5, 2008 and submitted report on "Managing Knowledge in R&D Organizations"

Participated in the workshop on Climate change and Health at NIMR, Delhi on July 1 , 2008.

Participated in the E.U. (European Union) information Seminar on "Research Funding Opportunities" in Kolkata, on July 8, 2008.

Participated in the Orientation meeting on WHO-GOI collaborative programme implementation: August 1, 2008 at New Delhi, India.

A. K. Mukhopadhyay: Visited two newly formed laboratories at Zanzibar as an invited expert to appraise quality control (QC) and quality assurance (QA) on current practices to isolate, identify and store Vibrio organisms during September 22-26, 2008.

Presented a talk entitled "The evolution of biotype hybrids of *Vibrio cholerae* O1 in Kolkata, India" in the Forum of Network of Research Center on Infectious Diseases at Hanoi, Vietnam on October 6, 2008.

Presented a talk entitled "The evolution of El Tor variant biotype of *Vibrio cholerae* O1 in Kolkata, India" at 43rd United Sates-Japan Conference on Cholera and Other Bacterial Enteric Infections at Fukuoka, Japan during November 17 - 19, 2008.

BIOCHEMISTRY

The focus of the Division of Biochemistry lies in elucidating the molecular mechanism of host-pathogen interactions in diarrheal diseases. With this objective, we attempt to identify and isolate surface-associated or soluble microbial proteins that are thought to play a critical role in pathogenesis of disease by mediating adhesion and colonization of host intestine by the organism, alteration of host cell physiology or cell death. In the next step, we characterize the proteins in terms of their solution structure, receptor-specificity and thermodynamics of association with host ligands and finally, elucidation of their biochemical functions. This involves extensive use of the techniques of molecular genetics and biophysical chemistry, like cloning and site-directed mutagenesis, amino acid and nucleotide sequencing, spectrofluorimetry, spectropolarimetry, microcalorimetry and analytical ultracentrifugation. This is a frontier area of biomedical research that is virtually the preserve of the developed countries and a few premier research Institutes in India. We have ventured into this area after the recent additions to our infrastructure. So far we have concentrated on the structure-function relationship and mode of action of *V. cholerae* cytolysin, the characterization of *V. cholerae* chitin-binding protein and its role in colonization in gut and the structure-function relationship of the colonization factor of enterotoxigenic *E. coli* (ETEC).

Scientist	:	Kalyan K. Banerjee, Scientist F
		Nabendu Sekhar Chatterjee, Scientist D
Staff	:	Keshab C. Paramanik, Technical Assistant
		Tapan Roy, Laboratory Technician
Senior Research Fellow	:	Abhisek Ghosal
Junior Research Fellows	:	Sreerupa Ganguly
		Avishek Ghosh
		Moumita Mondal
Research Assistant	:	Subrata Sabui

1. *Vibrio cholerae* cytolysin/hemolysin (VCC): A pore-forming toxin (PFT) with multiple biological functions

Investigator : Kalyan K. Banerjee

Co-investigator : Nabendu S. Chatterjee

Vibrio cholerae cytolysin/hemolysin (VCC) is a pore-forming toxin (PFT) causing lysis and death of a wide spectrum of eukaryotic cells at picomolar concentrations. VCC is expressed by most El Tor O1 and non-O1 strains and exhibits enterotoxic, cytotoxic and apoptogenic activity in animal models. In common with the mode of action of PFTs in general, the water-soluble, 65kDa VCC monomer interacts with the target plasma membrane, self-assembles by circular oligomerization to a heptameric β-barrel channel and inserts into the membrane lipid bilayer, forming a transmembrane diffusion channel leading eventually to destruction of the selective permeability of the plasma membrane and colloid osmotic lysis. The three-dimensional structure of the toxin has been resolved by X-ray crystallography. However, our understanding of the molecular details of the mechanism of transition of the toxin from a water-soluble protein to a multimeric integral membrane protein and of the relevance of the toxin in pathogenesis of human cholera is still inadequate.

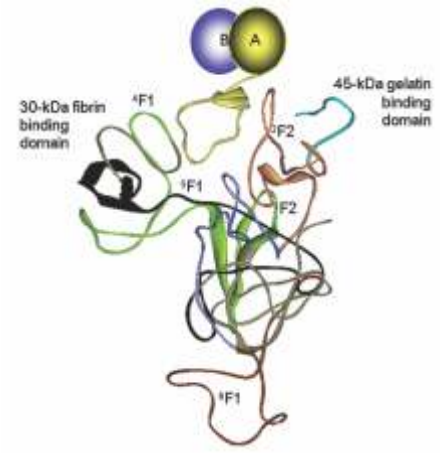
Study in this laboratory focuses broadly on receptor-specificity of VCC, mechanism of self-assembly and translocation of the β-barrel heptamer from water to the lipid bilayer. We reported that the toxin is unique among PFTs in possessing a lectin domain. Interestingly, we observed that although specific sugars strongly inhibit hemolysis, the cell surface sugars do not contribute to the initial interaction of the toxin with the target cell implying that they are unlikely to constitute functional receptors of the toxin. However, VCC is strongly amphipathic and partitions to the lipid-water interface in an essentially receptor-independent process. This is accompanied by movement of tryptophan residues from core of the protein to the surface triggering self-assembly of the monomer to the β-barrel heptamer.

Recently, we addressed the translocation of the toxin from the lipid-water interface to the nonpolar core of the membrane bilayer. Although there is consensus that the process is spontaneous, we showed that pore-formation got aborted at the oligomerization stage in absence of interaction of the C-terminus lectin domain of VCC with erythrocyte membrane glycophorin. This explains more than 1000-fold lower sensitivity of synthetic lipid vesicles than erythrocyte to VCC. The assignment of a critical role to the sugar-binding domain of the toxin in membrane translocation rather than membrane-binding is novel.

2. Molecular characterization of enterotoxigenic *Escherichia coli* colonization factors.

Investigator : Nabendu S. Chatterjee

Co-Investigator : T. Ramamurthy



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, and is a major cause of traveler's diarrhea. 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 is CFA/I, CS1, CS2,

CS3, CFA/III, CS4, CS5, CS6 etc. The overall goal is to develop a simple and specific method for detection of different CFAs for typing ETEC, identify the most prevalent CFAs and characterize them. This will also help us in tracking the movement ETECs round the globe.

ETEC colonize in the small bowel by means of different colonization factor antigens (CFAs). ETEC have been classified into several groups based on their distinct antigenicity. We have developed a multiplex PCR-based method to detect common CFAs for quicker analysis. Out of 50 strains tested, we found that 33 ETEC strains were expressing CS6, alone or in combination with other colonization factors, suggesting that CS6 is a common colonization factor in this region. However, based on serology, we could detect only 25 CS6-positive strains. Since CS6-expressing ETECs were common, we looked deeper into this CFA.

Our results also show that the structural subunits of CS6 (CssA and CssB) are in strong association, of which CssA has fatty acid modification. There are variations in the molecular weight of CssA, but not CssB in ETEC strains isolated in our strains. We have observed that 77% strains expressing CssA (18.5 kDa) with fatty acid modifications whereas 23% strains expressed differentially modified CssA subunit (16 kDa).

A loop-like structure in the C-terminal region of CssA subunit is responsible for CssA binding to the 70-kDa N-terminal domain of fibronectin for colonization. We propose a model of the interaction between the C-terminal loop of CssA and the 70-kDa domain of fibronectin and predict a mechanism for CS6-expressing ETEC adherence to the intestinal extracellular matrix.

3. Studies on *Vibrio cholerae* adherence and survival in gut and environment

Investigator : Nabendu S. Chatterjee

Co-Investigator : Kalyan K. Banerjee

Vibrio cholerae O1, a cause of epidemic diarrheal disease, normally resides as an indigenous component of riverine, estuarine and marine ecosystems. In these habitats, it associates with the chitinous exoskeletons of zooplankton. The principal objective of our study is to understand the mechanism as to how these bacteria adhere to the gut wall and survive in the environment by using different biochemical, biophysical and molecular biology approaches.

Chitin is an insoluble polymer of N-acetylglucosamine; *V. cholerae* can bind to chitinous exoskeletons of zooplankton and use chitin as a sole source of carbon and nitrogen in nutrient-poor aquatic habitats. The proteins that take part in this process are a chitin-binding protein (GbpA) and different chitinases.

V. cholerae chitinases have gathered immense interest due to their importance for survival of the bacteria in the environment as well as inside the host. We have recently shown that a chitin-binding protein GbpA plays a role in Intestinal adherence of *V. cholerae* by binding to intestinal mucin. GbpA is a key factor to initiate pathogenesis, although the association constant of this GbpA-mucin interaction is low (11.2 μM). GbpA, in turn increases mucin secretion in the intestine. GbpA and mucin upregulates each other, leading to increased levels of expression of both of these interactive factors for efficient colonization and pathogenesis by the organism.

We are also in a process of exploring the structure-function relationship of a secretory chitinase ChiA-2 in chitin utilization and survival of *V. cholerae in the environment*. We have generated a recombinant ChiA2, and have demonstrated that the enzymatic activity of this recombinant protein is very similar to the native enzyme when assayed with different substrates. Isogenic ΔChiA2 V. cholera mutant strain has been made. Further characterization of ChiA2 is in progress.

PRESENTATIONS & VISITS

N. S. Chatterjee: Presented a talk entitled "CS6- a unique colonization factor of enterotoxigenic Escherichia coli (Nabendu S. Chatterjee, Abhisek Ghosal, Subrata Sabui, T. Ramamurthy, T. Hamabata)" at the 43rd US-Japan Conference on Cholera and Other Bacterial Enteric Infections held in Kyushu University, Fukuoka, Japan during November 17-19, 2008.

Presented a talk entitled Characterization of colonization factor CS6 of enterotoxigenic Escherichia coli: molecular and genetic analysis (Nabendu S. Chatterjee, Abhisek Ghosal, Subrata Sabui, T. Ramamurthy, T. Hamabata) at the 4th Asian-African Research Forum on Emerging and Reemerging Infections" held in Hokkaido University, Sapporo City, Japan during December 15-16, 2008.

A. Ghosal: Presented a poster entitled "Purification and characterization of a colonization factor CS6 of enterotoxigenic Escherichia coli (Abhisek Ghosal, Subrata Sabui and Nabendu S. Chatterjee)" at 77th Annual Meeting of the Society of Biological Chemists (India) held at IIT-Chennai, during December 18-20, 2008.

A. Ghosh: Presented a poster entitled "Chitin-binding protein GbpA of *Vibrio cholerae* induces inflammation in the intestine (Avishek Ghosh, Rudra Bhowmick and Nabendu S. Chatterjee) at the 77th Annual Meeting of the Society of Biological Chemists (India)" held at IIT-Chennai, Chennai, India during December 18-20, 2008.

N. S. Chatterjee: Visited the National Institute of Infectious Diseases and International Medical Center of Japan, Tokyo, Japan during December 18-19, 2008.

CLINICAL MEDICINE

In this year, scientists of Division of Clinical Medicine are conducting two hospital based surveillance of diarrhoeal diseases. One surveillance project is conducted at Infectious Diseases Hospital where every 5th hospitalized patients of all age groups were surveyed in randomly selected two consecutive days in a week. Another surveillance project is in progress at Dr. BC Roy Memorial Hospital for Children, Kolkata where children up to the age of 12 years suffering from diarrhea or dysentery and attending Out Patient Department were included in the surveillance. Etiological agents associated in these diarrhoeal episodes were detected. Apart from applied research, scientists were also involved in basic research as intramural projects to explore the mechanism of immunomodulatory functions of Cholera Toxin and also to explore pro inflammatory functions of *V. cholerae* flagellins and their role in rectogenicity and immune response.

Scientists conducted 4 applied research projects funded by external funding agencies. In one study it was documented that attenuated measles vaccine given by the aerosol route was much safer as compared to sub-cutaneous route. Another study showed that two doses of rotavirus vaccine were immunogenic, showed good safety profile and were well tolerated when administered to healthy Indian infants.

Scientists are engaged to develop better formulation of Oral Rehydration Therapy with high amylase resistant maize starch in addition to reduced–osmolar ORS for treatment of dehydrating acute diarrhea in children. Scientists are also engaged to evaluate the role of probiotics for the better management of rotavirus associated diarrhea in children. A basic research project on the regulation of antimicrobial peptide expression in the intestinal epithelial cells was also funded by external funding agency.

Like that in previous years, scientists were involved for investigation of epidemics of diarrhoeal diseases and unknown fever. They are also involved for manpower development by providing training to the service providers like doctors and Para medical staff.

Scientist	:	P. Dutta, Scientist F [retired on 31.07.2008]
		U. Mitra, Scientist E
		M. K. Bhattacharya, Scientist E
		S. S. Das, Scientist C
Staff	:	A. Pal, Technical Assistant
		M. Dey, Senior Laboratory Assistant
		K.G. Saha, Laboratory Assistant
		S. Turi, Head
		S. Dey, Sweeper
Senior Research Fellows	:	Krishnendu Chakraborty
		Subhamoy Ghosh
		Nagaraja Theeya
Junior Research Fellows	:	Nirmalya Dasgupta
		Pujarini Dutta
		Atri Ta
		Bhupesh Kumar Thakur

1. Out patient based surveillance on diarrhoeal diseases at Dr. B. C. Roy Memorial Hospital for Children

Investigators : U. Mitra, P. Dutta, T. Rammurthy, T. Krishnan, S. Ganguly, K. Rajendran

A good number of children with diarrhoeal episodes attend OPD every day for specialized intervention on day care basis. Community Health Workers and clinicians practicing in the community fail to manage these episodes for which they are referred to the hospital. However, they are not severe enough for which they require hospitalization. These episodes can safely and efficiently be managed at OPD level by trained and experienced physicians. The objective of the present project is to establish a surveillance of diarrhoeal diseases and to identify the enteropathogens among children who attended outpatients department at Dr. B.C. Roy Memorial Hospital for Children, Kolkata.

Before starting the systematic surveillance, we conducted a trial survey to standardize the clinical set up with special reference to evaluation of Clinical Research Form (CRF), process of having written informed consent, sample collection and in-time transportation of sample to the laboratory.

A total of 120 children aged up to 5 years were enrolled in the trial survey during September, 2008 to April, 2009. Ninety children had the history of acute watery diarrhea and 30 children had dysentery. We did not face any difficulties to get informed written consent to enroll the children in the study and to get catheter specimen of stool samples or rectal swabs. Different enteropathogens detected from stool samples of sampled children were depicted in Table-1 and Table-2.

2. Safety of an aerosol attenuated Measles Vaccine in healthy Subjects with Trudell's nebulizer

Investigator: M.K. Bhattacharya

Measles vaccine is highly effective but does not achieve its full potential partly due to logistical constraints of administering a vaccine by injection to large populations in resource-poor settings. The burden of disease is also due to the under-utilization of the current measles vaccine. Failure to deliver at least one dose of measles vaccine to all infants remains one of the leading causes for the high measles morbidity and mortality that exists today. In addition, a number of safety concerns regarding the use and adequate disposal of syringes and sharps have been documented in a number of countries in recent years. For this, it was proposed to deliver the measles vaccine by the respiratory route by way of aerosolization. The objective of the project is primarily to study the safety of administration of live Edmonston Zagreb attenuated measles vaccine in healthy volunteers administered by the aerosol route and secondarily to measure the serum plaque reduction neutralization titers before and after aerosol administration of live attenuated measles vaccine given to healthy volunteers.

The clinical study used the same Serum Institute of India (SIIL) manufactured live, attenuated Measles Vaccine containing the Edmonston –Zagreb strain, which is used for routine immunization in India. The dose given was at least 1,000 PFU's (Particle Forming Units), and the vaccine was delivered by the Trudell's Nebulizer, which is a specialized aerosol delivery device. The study, being of an open, non-controlled and sequential by age and parallel group designs, proceeded in three phases, each involving one particular age group:

1) For Group 1 (18-35 yrs old): 20 healthy, measles immune subjects were vaccinated.

2) For Group 2 (05-17 yrs old): 19 healthy, measles immune subjects were vaccinated.

3) For Group 3 (01-04 yrs old): 17 healthy, measles immune subjects were vaccinated.

For each group, the subjects were followed up at 0, 1, 3, 7, 10, 14, 28, 90, 180, and 365 days post-vaccination, to assess whether they suffered any adverse events (AE's) or Serious Adverse Events (SAE's) related to the vaccination. Samples for PRNT and IgE (only for Groups 2 & 3) were drawn at Screening, Day-28, and Day-90 post vaccination to assess the immune response to the aerosol vaccine.

In view of the Eosinophilia seen in the study group 1, involving the age group 18-35 year, the DSMB recommended two additional studies. This special study was designed to observe the hematological response (Eosinophilia) between AEROSOL and SUBCUTANEOUS routes of vaccine administration.

Two additional studies were performed as follows:

1) One study, comparing the hematological response between adult subjects (18-35 years) using aerosol vaccine as compared to sub-cutaneous vaccine was conducted between November 2006 & January 2007. Ten subjects were vaccinated by subcutaneous route and ten subjects were vaccinated by aerosol route.

2) Another study, using only children in the age group 2 (05-17 years), vaccinated by the sub-cutaneous route was conducted concurrently with the main Group 2 study, between July & September, 2007. Twenty subjects were vaccinated by subcutaneous route.

Group 3 (01-04 years) subjects suffered the MOST AE's among all three study groups. Among the SPECIFIC AE's, Coryza /Running Nose/Nasal Congestion was observed as the MOST COMMON AE overall in all three study groups till date. No serious adverse effects were seen in any age group.

Regarding AE's seen in between AEROSOL & SUB-CUT Study groups, Sub-cut subjects had more AE's overall and more proportion of moderate & severe AE's as compared with subjects vaccinated via the aerosol route.

We may conclude, therefore, that the Measles vaccine given by aerosol route in all target age groups are much safer as compared to the sub-cutaneous route.

3. Exploring the Mechanism of the Immunomodulatory Functions of Cholera Toxin

Investigator: Santasabuj Das

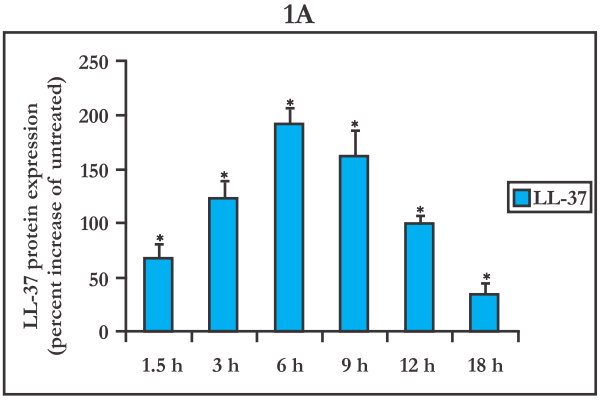
Cholera toxin (CT) is the most potent immunomodulatory agent known till date. Multiple mechanisms contribute to the immunomodulatory functions of CT. However, despite the critical role played by the mucosal epithelial cells (MECs) in the host innate immune responses, little is known regarding their contribution to CT-induced immunomodulation. CT has been reported to up and downregulate a large number of both pro- and anti-inflammatory genes in the intestinal mucosal cells. But its role in the regulation of small cationic antimicrobial peptides (CAMPs), which are pleotrophic immunomodulatory molecules abundantly released by different MECs, if any, is currently unknown. Our main objective was to study the

role of NF-κB and ERK signaling pathways in CT-induced immunomodulation. RESULTS AND DISCUSSION: We have observed that toxigenic V. cholerae and ETEC may significantly suppress human cathelicidin (hCAP18/LL-37) and β-defensin 1 (HBD-1) expression in the differentiated intestinal MECs, which line the luminal surface of the gut. This is achieved though their secreted toxins, namely CT and LT, both *in vitro* as well as *in vivo* in a cyclic AMP (cAMP)-dependent manner, thus evading the host immune system. Further studies revealed that cAMP signal transduction pathways stringently regulate cathelicidin expression at the transcriptional level in different MECs. A brief phase of induction is followed by rapid restoration of the original levels (Fig 1A). Promoter analysis revealed consensus CRE (cAMP response element) and ARE (AP-1 response element) sequences (Fig 1B) that are occupied by CRE-binding protein (CREB) and activator protein 1 (AP-1), respectively upon activation of the cAMP pathways as shown EMSA (Fig 1C). The above binding drives transcription of a reporter gene by the promoter (Fig 1D) and CHIP assays while over-expression of CREB and AP-1 (c-jun and c-fos) significantly augments cathelicidin expression in the MECs. siRNA-mediated silencing of the genes encoding the above transcription factors revealed their ctitical role in cAMP-induced cathelicidin expression (Fig 1E). On the other hand, the counter-regulatory mechanisms operate through inducible cAMP early repressor (ICER). This is manisfested by the abrogation of cathelicidin induction by CREB and AP-1 when ICER is co-expressed. In contrast, cathelicidin levels remain persistently elevated in the MECs where the ICER gene is silenced (Fig 1F). ICER was also found to replace CREB and AP-1 from the transcriptional complexes over the cathelicidin promoter.

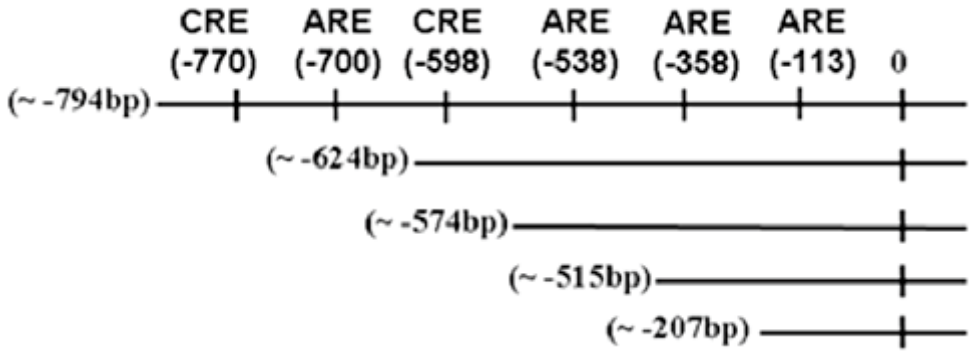
4. Study of the pro-inflammatory functions of V. cholerae flagellins and their role in reactogenicity and immune response.

Investigator: Santasabuj Das

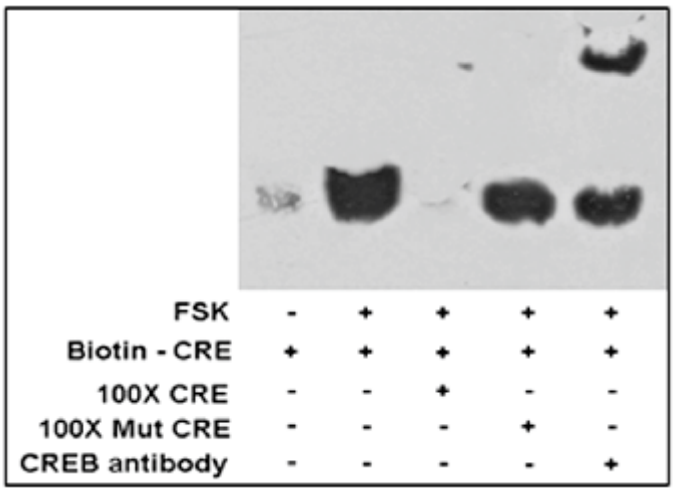
Bacterial motility protein flagellin binds, as monomers, to the cell surface TLR5 receptor through their N- and C-terminal evolutionary conserved domains. This binding results in the activation of pro-inflammatory signal transduction pathways, most notably the NF-κB and MAPKinase pathways, inside the cells. Vibrio cholerae possess a single polar flagellum, consisting of five subunit proteins (Fla A to E). Although, vibrio flagellins were shown to induce pro-inflammatory genes in cell culture studies and this has been implicated in the reactogenicity of the vaccine strains, the inflammatory potential of the individual flagellin subunits is not clearly known. Moreover, although the conserved domain is also responsible for motility of the bacteria, only FlaA is required for vibrio motility. Our principal objective is



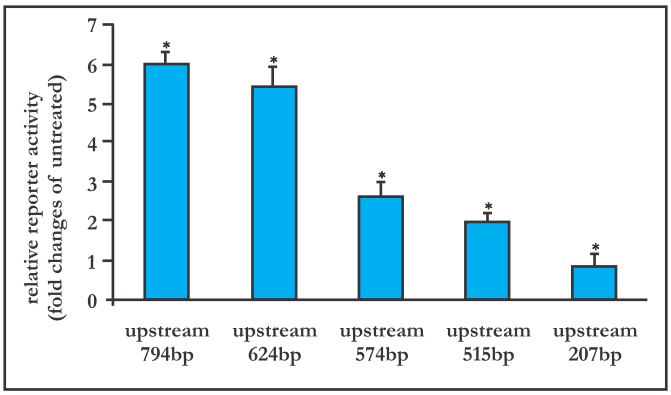
1B



1C



1D



to study the pro-inflammatory functions of the individual flagellin subunits of Vibrio cholerae and to identify the critical motifs/residues of the proteins that are required for these functions, but not for motility. We have future plans to study the nature of immune responses generated by V. cholerae strains expressing flagellins mutated over the above motifs/residues. We have found that Vibrio flagellin subunits have differential ability to induce pro-inflammatory genes like IL-8, with FlaE having the highest and FlaB the least pro-inflammatory potential (Fig 2A). Studies with various signaling pathway inhibitors revealed that both the above subunits primarily depend on ERK MAPK for their activities, while FlaB also equally depends on NF-κB activation (Fig 2B). EMSAs revealed equal potential for both the proteins to form in vitro DNA/protein complexes with the consensus NF-κB-binding sequences over the IL-8 promoter, but a significantly larger complex in case of FlaE with the AP-1 binding sequences (Fig 2C). Role of AP-1 in conferring higher pro-inflammatory potential to FlaE was proved by silencing the gene in the intestinal epithelial cells that resulted in almost complete abolition of IL-8 induction (Fig 2D). The amino acid residues critical for inflammation were searched for by homology

1E

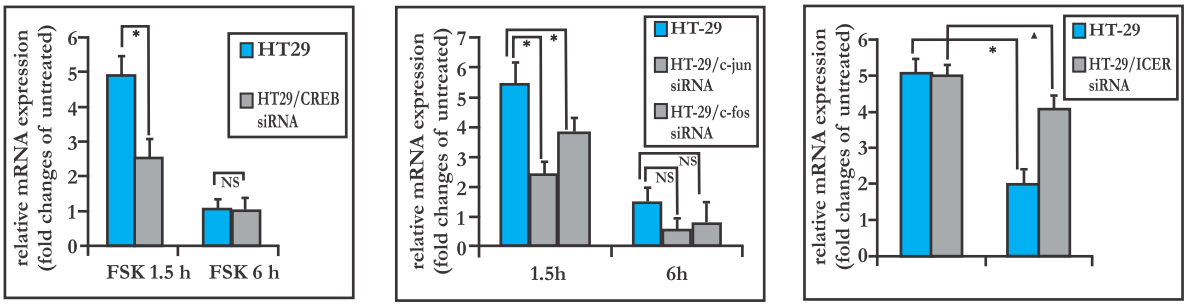
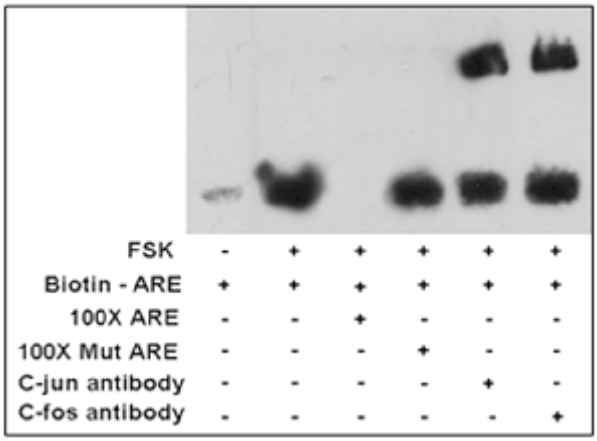


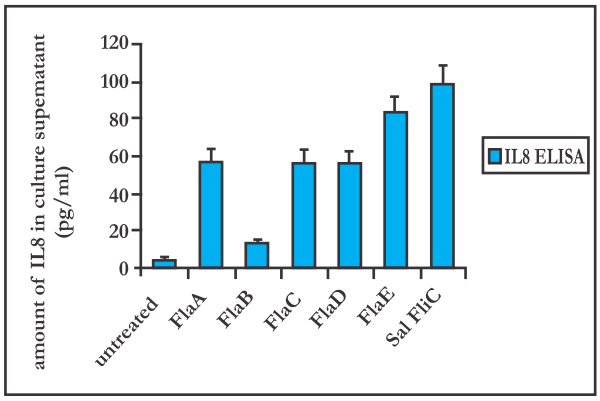
Fig. 1

modeling of the flagellins followed by TLR/flagellin docking (Fig 2E). We have identified several such residues, which have been mutated and the mutants are currently under study.

1F



2A

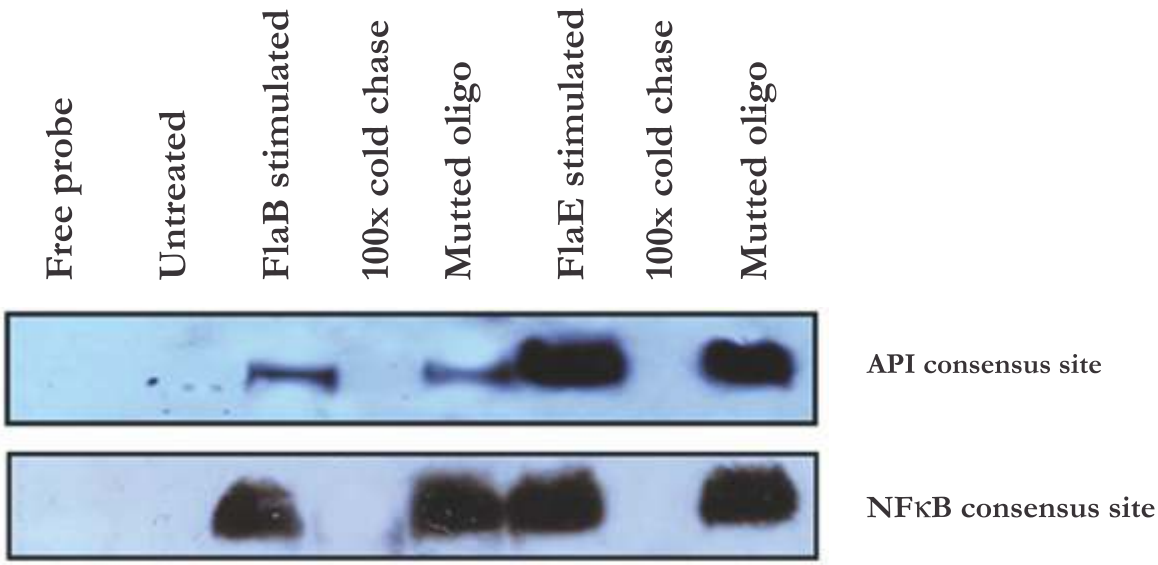


2B

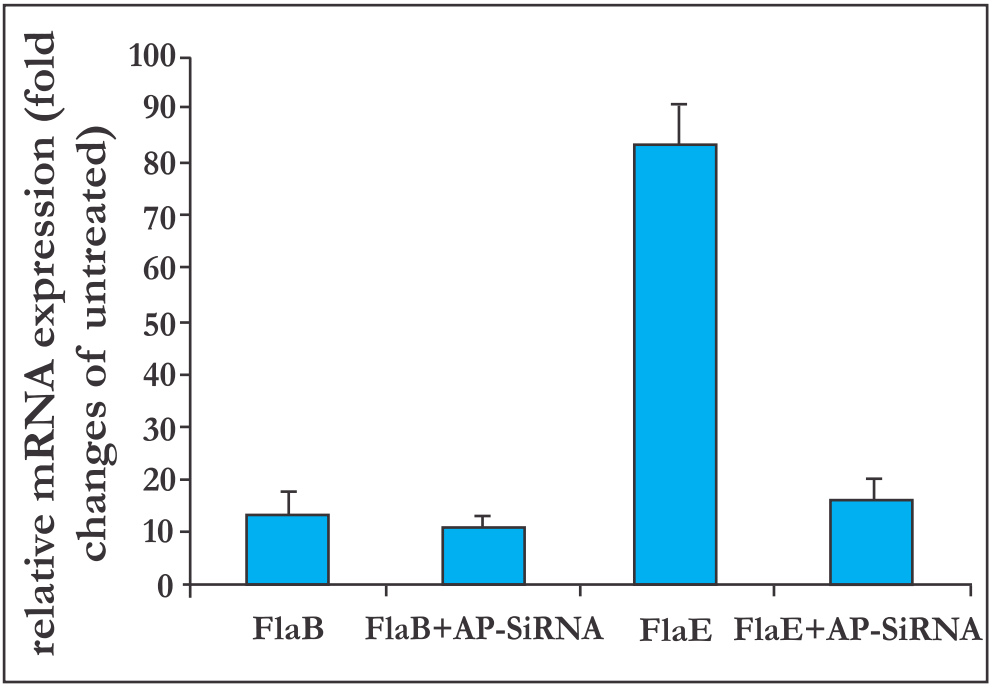
TLR5 signaling pathways activated by different Flagellins

Flagellin	NF-κB	ERK	P38	JNK
FlaA	+	++	+	+
FlaB	+	+	±	±
FlaC	+	++	+	+
FlaD	+	++	+	+
FlaE	+	+++	+	+
FliC	+++	+	+++	±

2C



2D


relative mRNA expression (fold changes of untreated)
100
90
80
70
60
50
40
30
20
10
0
FlaB
FlaB+AP-SiRNA
FlaE
FlaE+AP-SiRNA

2E

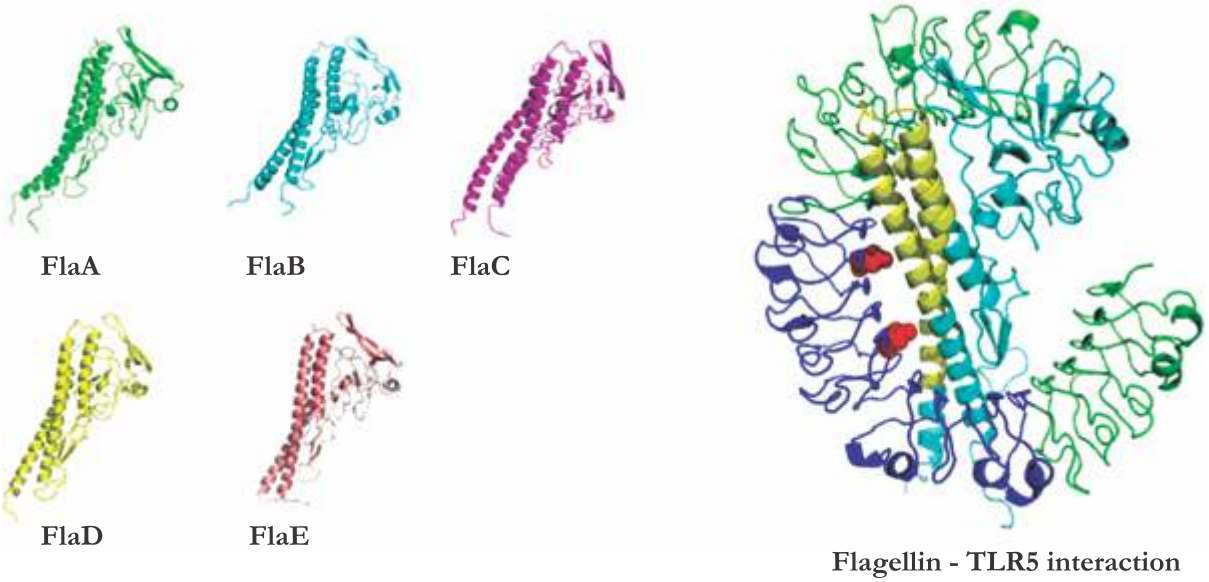

FlaA
FlaB
FlaC
FlaD
FlaE
Flagellin - TLR5 interaction

Fig. 2

PRESENTATIONS & VISITS

U. Mitra: Attended 52nd State Conference of Indian Association of Public Health association held at Rabindraa Bhaban, Barasat, North 24 Parganas on October 26, 2008.

Attended 2nd Probiotic Symposium held at Hotel International Eros, Nehru Place, New Delhi during November 7-8, 2008.

M. K. Bhattacharya: Attended Training Mission in cholera case Management and Research, sponsored by NIH during September 7-14, 2008, Kolkata, India and delivered lecture on Hot Topics in clinical intervention.

Presented a paper entitled "Clinical Profile and Etiology of Acute Diarrheal Cases Admitted at the Infectious Diseases Hospital, Kolkata, India" at the Forum of the Network of Research Center on Infectious Diseases held at the National Institute of Hygiene and Epidemiology, (NIHE) Hanoi, Vietnam on October 6, 2008.

Attended 2nd India probiotics Symposium on Evidence bases Health Benefits of Probiotics held at Hotel International Eros, Nehru Place, New Delhi during November 7-8, 2008.

S. S. Das: Presented a talk entitled "TLR5 Signaling: Differential Regulation by Different Flagellin (Authors)" at the 43nd United States-Japan conference on Cholera and Other Bacterial Enteric Infections held at Fukuoka, Japan during November 17-19, 2008

Presented a talk entitled "Regulation of Cationic Antimicrobial Peptide Expression by Enteric Pathogen-Derived Virulence Factors (Authors)" at the 4th Asian-African Research Forum on Emerging and Reemerging Infections held at Sapporo, Japan during December 15-17, 2008.

Visited the National Institute of Infectious Diseases and International Medical Center of Japan, Tokyo, Japan during December 18-19, 2008.

DATA MANAGEMENT

The division of Data management is primarily focusing on good data management practices to produce reliable, complete and accurate data from the various health research projects of this Institute as well as involvement in Hospital based diarrhoeal diseases surveillance study at Infectious Disease Hospital (IDH), Kolkata to identify the pattern of diarroeagenic enteric pathogens. The information on causative organism and antimicrobial resistance pattern is being communicated on weekly basis to IDH and different departments of State Government to help physicians for proper treatment and management of diarrhoeal diseases.

Because the division has direct access to the data from all divisions, it is in a position to provide data management support including data entry/verification to various studies undertaken by this institute in collaboration with the project on HIV sentinel surveillance of National AIDS Control Organization (NACO) of Ministry of Health and Family Welfare, Government of India and Integrated Diseases Surveillance Project (IDSP) and International Collaborators like International Vaccine Institute, Korea, and Centre for Vaccine Development, University of Maryland, Baltimore. This division is capable of advanced electronic data transfer from country to country and also GIS implementation. This division rendered statistical help for epidemiological, clinical and microbiological research. It has also future plans to conduct local and country level training on research methodology, basic Bio-Statistics, Epi-info and SPSS for health researchers. This would eventually provide us with a comprehensible vision of basic and operational research in diarrheal diseases.

Scientist : B. Manna, Scientist E

K. Rajendran, Scientist B

1. Time series model study for prediction of cholera and diarrhoea using atmospheric temperature, relative humidity and rainfall in Kolkata, India.

Investigator: K. Rajendran

The objectives of the study is to compare the climatic characteristics with observed infection of diarrhoea and cholera in the Infectious Diseases Hospital, Kolkata and to assess long term changes to develop Time series model as well as statistical models. They are:

a) To compare the climatic factors such as Temperature, Relative Humidity and Rainfall, SST Bengal, SST El Niño, Sunspot number with observed infection of diarrhoea and Cholera in Kolkata;

b) To assess long term changes to develop Time series model as well as statistical models for early prediction; and

c) To generate disease clustering in time & place. From the comparative statistical analyses, the characteristics of the climatic factors will also be elucidated for the more effective prediction and diseases management.

Emerging and re-emerging cholera infection in the global is major public health disaster and endangering <5 years children and progressive mortality rate in developing countries even though the modesty of scientific development around the world. In late 1970's, transmission of cholera was believed to involve person-to- person contact during epidemic outbreak. However, it is now understood that the epidemic strains of *V.cholerae* occur in the aquatic environment. The climatologist is more certain of the likelihood of human induced global climate change resultant the possible consequences of climate changes alter local weather pattern and by disturbing life supporting natural systems and process would affect the health of human.

Maximum Entropy Method – Power Spectral Analysis for Frequency Range

MEM power spectral density (MEM-PSD) $P(f)$ (f: frequency) for the time series with equal sampling interval Δt, can be expressed by

$$P(f) = \frac{P_m \Delta t}{\left| 1 + \sum_{k=-m}^{m} g_{m,k} \exp[-i 2\pi f k \Delta t] \right|^2}$$

where the values of P_m are the output power of a prediction-error m and $_{m,k}$ the corresponding filter order. The details are described in ref.[8].The value of MEM-estimated period of the n-th peak component T_n (=$1/f_n$:f_n is the frequency of the n-th peak component) can be determined by the positions of peaks in the MEM-PSD.

El Niño-southern Oscillation (ENSO) is closely related to Indian Ocean Dipole (IOD) and has strong correlation with the Indian monsoon. In the past, many coastal regions of India have experienced severe rainfall during 1983, 1994 and 1997 due to positive dipole. We correlated the changing weather patterns and prevalence of cholera, using the data collected from the hospital based surveillance on diarrheal diseases in Kolkata. In this study, the monthly *V. cholerae* prevalence from 1996 to 2007 in Kolkata, satellite data of sunspot numbers, sea surface temperature (SST) of Bay of Bengal and rainfall in relation to El Niño occurrence were considered for Time series analysis that was based on Maximum Entropy and least square

methods. The box plot explored the difference of variation among the climatical factors and *V. cholerae* (Fig-1-1a). The similar years mode of V. *cholerae* infection with aforesaid climatic factors were 3, 2, 1.5, 1, .9, .5, .52 and .54 years (Table-1). The dominant periodic modes of power spectral density were used to identify long and short term influence on *V. cholerae* infection. Prevalence of cholera was high when the sea surface temperature reached about 28°C and positive correlation was detected between rainfall and *V. cholerae* infection (Fig-2). When the sun spot numbers were more, prevalence of cholera cases was low during the study period. During 1996, 1997 and 1998, single cholera peak was recorded with more number of cases in the monsoon season due to highest rain fall while SST was below 28°C. However, the above said event was exceptional in 1998 when the El Niño had occurred (1997-1998). After 1998, oscillation in the monsoon seasons led predominant changes in the rainfall, which was not recorded in the history of cholera in Kolkata. The climatic research with relational impact of cholera infection at Kolkata is being monitored and resultant of experience above research explored that strong relation of *V. cholerae* infection with local climate changes.

PRESENTATIONS & VISITS

B. Manna: Participated in the meeting on Impact Evaluation of Cholera Vaccine Introduction held at Dhaka, Bangladesh during April 30- May 1, 2008.

Participated in "The Ninth Advanced Vaccinology Course (ADVAC 2008)" Course at Annecy, France during May 19-30, 2008.

Participated in the investigator's meeting for the Global Enterics Multi-center Study (GEMS) at Seattle, Washington, USA during September 10-12, 2008.

Participated in the Annual Meeting of American Society of Tropical Medicine & Hygiene (ASTMH) and symposium organized by Global Enterics Multi-center Study (GEMS) at New Orleance, Louisiana, USA during December 8-10, 2008.

K. Rajendran: Attended Workshop on "Data Mining & Data Warehousing" held during September 15-20, 2008 at center for soft computing research, Indian Statistical Institute, India.

Visited Sapporo Medical University, Sapporo, Japan for collaborative research and training on "Time series analysis of cholera outbreaks and climate changes" during October 16 –November 15, 2008.

EPIDEMIOLOGY

The Division of Epidemiology is involved in various community based research projects such as observational, operational and intervention studies with the objective of finding out ways to reduce diarrhea disease morbidity and mortality. The Division is engaged in collaborative projects with international research institutes like International Vaccine Institute and universities like Johns' Hopkins, University of Maryland, USA. The division has successfully completed large scale vaccine trials.

Epidemiologists of the division are not only engaged in community based research, but are also frequently called for outbreak investigation for diarrhea, unknown fever, H5N1 surveillance and helped local government in micro planning during flood affected situations. Other than diarrhea, HIV related observational and operational research work is also being carried out by scientists of the division. Other activities include training in the fields of Diarrhoea and HIV surveillance.

Scientist	:	S. Ghosh, Scientist F
		D. Sur, Scientist E
		S. Panda, Scientist E
		K. Sarkar, Scientist E
		A. K. Deb, Scientist D
		S. Kanungo, Scientist B
Staff	:	D. C. Das, Technical Officer
		S. Shil, Data Processing Assistant, Grade A
		S. Manna (nee Sur), Senior Technical Assistant
		R. L. Saha, Senior Technical Assistant
		C. Mondal, Field Worker
		A. Chakraborty, Assistant Social Worker
Senior Research Fellow	:	Baishali Bal
		Joydeep Majumder

1. Randomized Controlled Field Trial of a Probiotic to assess its role in the Prevention of Acute Diarrhoeal Diseases in Children—in urban slums of Kolkata (2007- 2008)

Investigator : D. Sur

Co-Investigators : B. Manna, A. Palit, S. K. Neogi, T. Ramamurthy

The objectives of the study were to assess the impact of probiotic in the prevention of acute diarrhoeal diseases, on nutrition and growth of children and identification of pathogens causing diarrhoea. A double-blind randomized controlled field trial involving 3,758 children was conducted in an urban slum community in Kolkata, India. The participants were given either a probiotic drink containing *Lactobacillus casei* strain Shirota or a nutrient drink daily for 12 weeks and were followed up for another 12 weeks. The incidence of diarrhoea was lower in the probiotic group (1.08/child/year) compared to nutrient group (1.27/child/year) and the difference was statistically significant ($p<0.05$). The proportion of children suffering from diarrhea was significantly lower ($p<0.05$) in the probiotic group (33.7%) compared to that in the nutrient group (37.8%). The probiotic efficacy was 15% (95% CI 8%-23%, $p <0.05$). The survival distribution curves were compared through Kaplan-Meier technique on periodical time interval from the point of intake of the drinks to the end of follow-up. It was observed that after 12 weeks of the intake of the drink the survival curve in probiotic group was significantly higher (Log Rank Test: Chi-Square = 7.6: $p=0.01$) compared to the nutrient group. The detection of different enteric pathogens was similar between the two groups.

The study establishes that daily intake of probiotic is efficacious in decreasing the incidence of acute diarrhoea in young children in a community setting of a developing country.

Group	# Children received Probiotic/Nutrient	# Children* with diarrhoea	Total diarrhoea 1Episodes	Incidence/child/yr*
Probiotc	1802	608 (33.7%)	900	1.08
Nutrient	1783	674 (37.8%)	1048	1.27
TOTAL	**3585**	**1282(35.8%)**	**1948**	**1.18**

Occurrence of diarrhoea in children in the probiotic and nutrient groups during 12 weeks intake and 12 weeks follow-up.

incidence = no. of episodes / person-weeks X 52 ; * $p <0.05$

2. Randomised controlled evaluation of protection by Vi polysaccharide vaccine against typhoid fever in Eastern Kolkata (2004-2008)

Investigator : S. K. Bhattacharya

Co-Investigators : D. Sur, B. Manna, S. Dutta, S. Kanungo

The objective of the study was to determine the protective effectiveness of the Vi polysaccharide vaccine following routine administration in a 1-dose schedule. To address the programmatic impact of Vi vaccine in a typhoid-endemic setting, an effectiveness trial was conducted among slum-dwelling residents of Kolkata, India aged two years and above. They were randomized by geographic cluster (40 clusters per arm) to receive a single

dose of either Vi vaccine or a control vaccine (hepatitis A vaccine) in a mass immunization campaign. All residents were followed up for two years with treatment center-based surveillance for typhoid fever. Of the 54,674 eligible subjects, 69% i.e., 37,673 subjects were vaccinated (18,869 in the Vi vaccine group and 18,804 in the hepatitis A vaccine group). Protective effectiveness (PE) at the end of 2 years post-vaccination in all age groups was 61% (95% CI: 41%, 75%, P<.0001). For children under five years of age, PE was 80% (95%CI: 53%, 91%, P<.001) and for older persons (PE was 53% (95%CI: 24%, 71% P< .01). An overall herd protection was 44% i.e., non-vaccinated members of the Vi vaccine clusters had a rate of typhoid fever that was 44% (95%CI: 1%, 69%; P<.05) lower than that for non-vaccinated members of the hepatitis A vaccine clusters. It may be concluded that Vi vaccine protected both young children and older persons and conferred protection to non-vaccinated neighbors of Vi vaccinees. The potential for combined direct and herd protection by Vi vaccine should be considered in future deliberations about introducing this vaccine in typhoid-endemic settings.

Table showing Total Protection against Typhoid Fever by age at vaccination

Age Group	Total Protection (95%CI)
2-4 years	82% (P<.001; 95% CI: 58%, 92%)
5-14 years	59% (P<.05; 95% CI: 18%, 79%)
≥15 years	48% (P>.05; 95% CI: -44%, 81%)
All age groups	65% (P<.0001; 95%CI: 42%,79%)

3. A randomized controlled trial of the bivalent killed whole cell oral cholera vaccine in Eastern Kolkata, West Bengal, India (2006-2009)

Investigator : G.B Nair

Co-Investigators : D. Sur, B. Manna, S. K. Neogi, B. L. Sarkar, S. Kanungo

This study is being conducted to estimate the efficacy of a two-dose regimen of the oral whole cell killed bivalent cholera vaccine in preventing culture-proven *V. cholerae* O1 diarrhoea episodes severe enough to require treatment in a health care facility in persons over one year of age.

A cluster randomized trial was conducted in Kolkata, among non-pregnant residents aged 1 year and older. Participants were randomized by residential premise to receive two doses of either reformulated oral WC cholera vaccine or *E. coli* K12 placebo. Cholera was detected during two years of post vaccination follow-up using a network of all outpatient and inpatient diarrheal treatment facilities serving the study population. The protective efficacy (PE) of all age group was 67%; (99%CI, lower bound=35%) at the end of two years post vaccination. The PE in different age group showed 49 % in1-4 years of age, 87% in children aged 5-14 years, and 63% in older persons. No serious adverse events related to the vaccine were detected. Presently the post vaccination surveillance for the third year is ongoing.

Thirty years have elapsed since the parenteral cholera vaccine was withdrawn from India at the behest of the World Health Organization because of its short duration of protection and painful side effects. The burden of cholera in India however, has been steadily increasing. Based on results of this study, we have established the protective efficacy of this vaccine which has been chiefly instrumental for the new cholera vaccine being licensed in India in March 2009. As a public health tool, this vaccine will make a dramatic change in reducing the burden of cholera in India.

PROTECTIVE EFFICACY OF CHOLERA VACCINE

Age Group (yrs)	Protective Efficacy
1- <5	53% (p <0.05)
5- < 15	88% (p<0.01)
>15	66% (p<0.005)
All age groups	68% (p<0.005)

4. Immune Responses Following One Dose versus Two Doses of Killed Oral Cholera Vaccine in Eastern Kolkata, West Bengal, India (2008)

Investigator : D. Sur

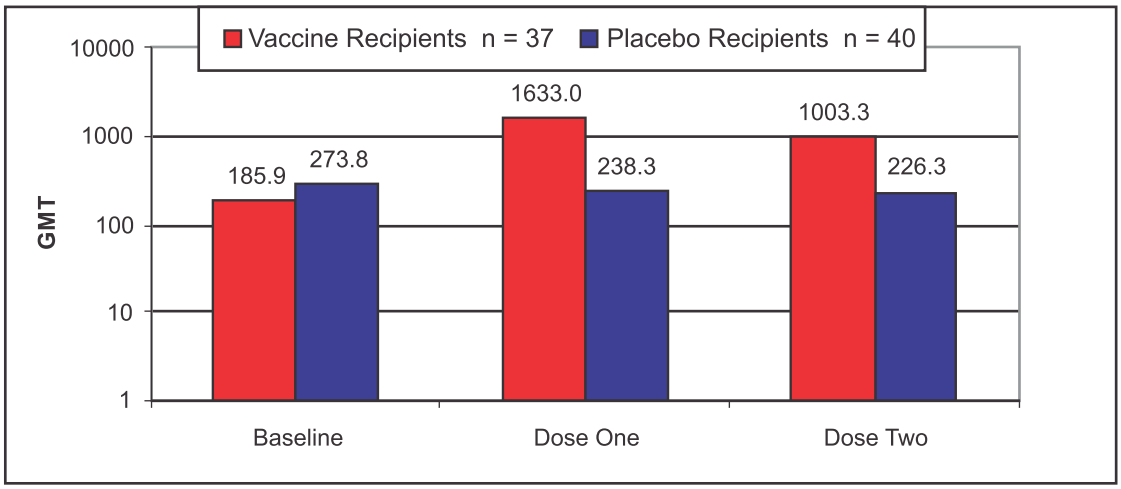
Co-Principal Investigator : S.K. Bhattacharya

Co-investigators : M.K. Bhattacharya, B. Manna, S.Kanungo

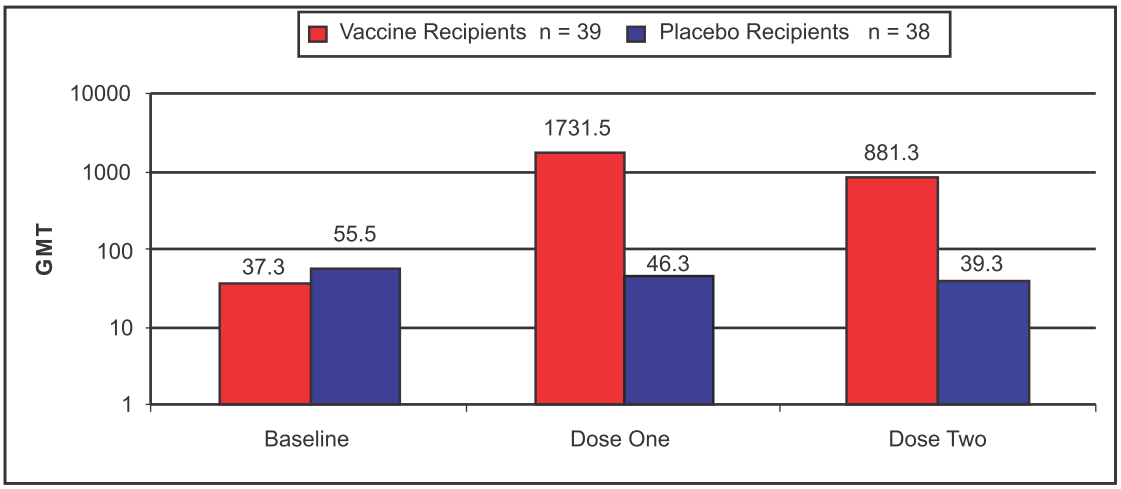
The objective was to compare immune responses following one dose and two doses of killed oral cholera vaccine among adults and children.The participants were 85 healthy adults (males and non-pregnant females) aged 18 – 40 years and 82 healthy children (males and non-pregnant females) aged 1 – 17 years. They were randomized to receive either the bivalent killed whole-cell oral cholera vaccine or placebo (killed oral *Escherichia coli* K12). For immunogenicity, it was identified as proportion of subjects exhibiting 4-fold or greater rises in titers of serum vibriocidal antibodies, relative to baseline, 14 days after dose 1 and 14 days after dose 2 of vaccine or placebo. Safety was assessed by proportion of subjects with adverse events during the duration of study participation.

Following immunization with dose 1 of the vaccine, 65% of adults and 87% of children exhibited a $\geqslant$4 fold rise in serum *V. cholerae* O1 vibriocidal antibody titers. After dose 2, 46% of adults and 82% of children exhibited a $\geqslant$ 4 fold rise in serum V. cholerae O1 vibriocidal antibody titers. Adverse reactions were observed with a similar frequency among vaccine and placebo recipients in adults and children. The vaccine elicited similar vibriocidal responses to dose 1 compared to dose 2. An efficacy trial of a single dose of the vaccine is required to determine if these results translate to protection against cholera.

Immunogenicity by Geometric Mean Titre



Adult



Paediatric

5. Diarrheal Disease in Infants and Young Children in Developing Countries (2007-2011)

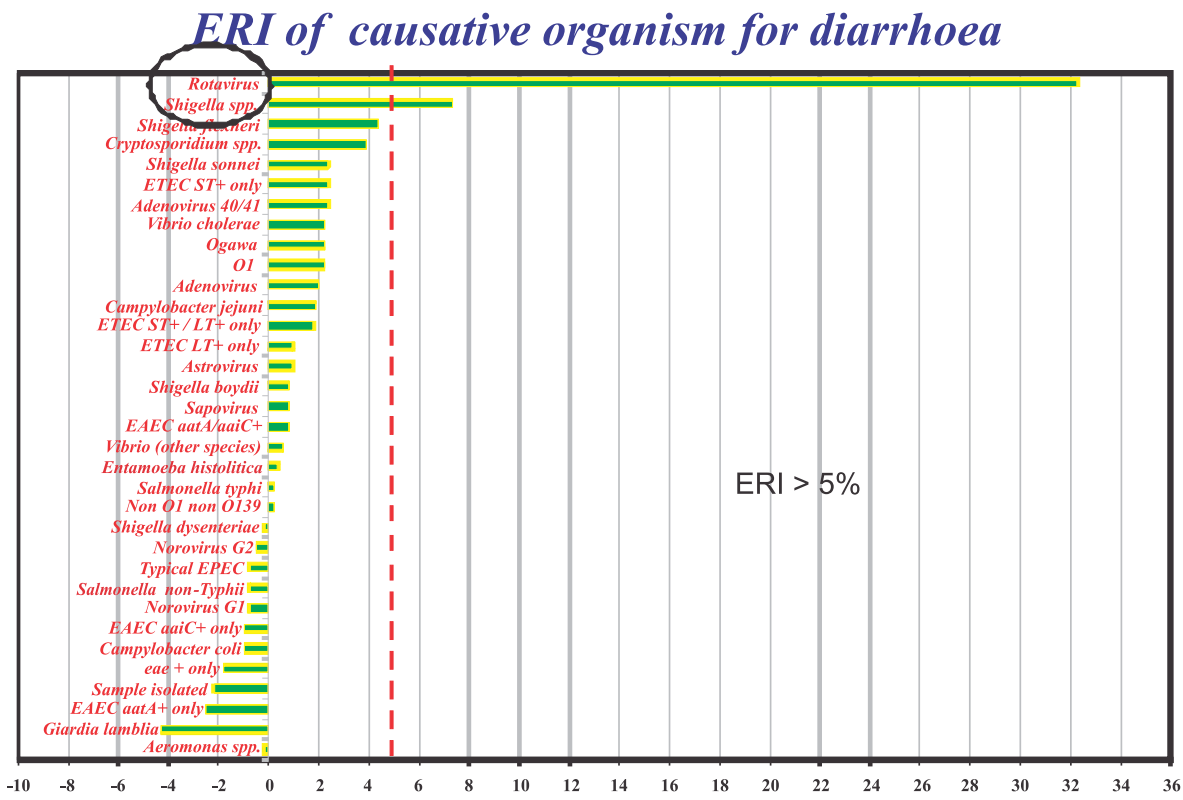
Investigator : Dipika Sur

Co-Investigators : T. Ramamurthy, B. Manna & S. Kanungo

The objective of the study is to estimate the population-based burden, etiology and adverse clinical consequences of severe diarrhea among children 0-59 months of age in multicentric sites including India to guide the development and implementation of vaccines and other interventions. The study site is being conducted in the slum areas within Wards 14, 31, 34, 58 and 59 of Kolkata Municipal Corporation (KMC) in eastern Kolkata. Currently, a total of 2,05,516 populations including approximately 13,000 children are under Demographic Surveillance System (DSS) at 4 months interval for collection of demographic events. A Case-Control study is being conducted with cases (from the study sites only) being enrolled from Two Sentinel Health Centres (Infectious Diseases Hospital and B.C. Roy Children Hospital) and controls matched for age and sex from the neighborhood of the case. The target is to enroll 8 cases fortnightly with 1:1

case–control ratio in each stratum of 0-11 month; 12-23 month and 24-59 months of children from the dynamic cohort.

Since the beginning of the study, (Dec'07 – Mar'09), a total of 2,923 births were detected and 112 deaths were reported from Demographic Surveillance System. A total of 646 cases and 646 controls of under five children were enrolled with 298 cases in 0-11 month, 221 cases from 12-23 month and 127 from 24-59 months of age. The excess risk of infection (ERI) as depicted in the graph, shows the risk of infection the cases have over the controls by different organisms. The value 5% has been taken as an arbitrary cut-off value.



6. Surveillance for dengue fever in eastern Kolkata, West Bengal, India (2008-2010)

Principal Investigator : Sekhar Chakrabarti

Co-Principal Investigator : Dipika Sur

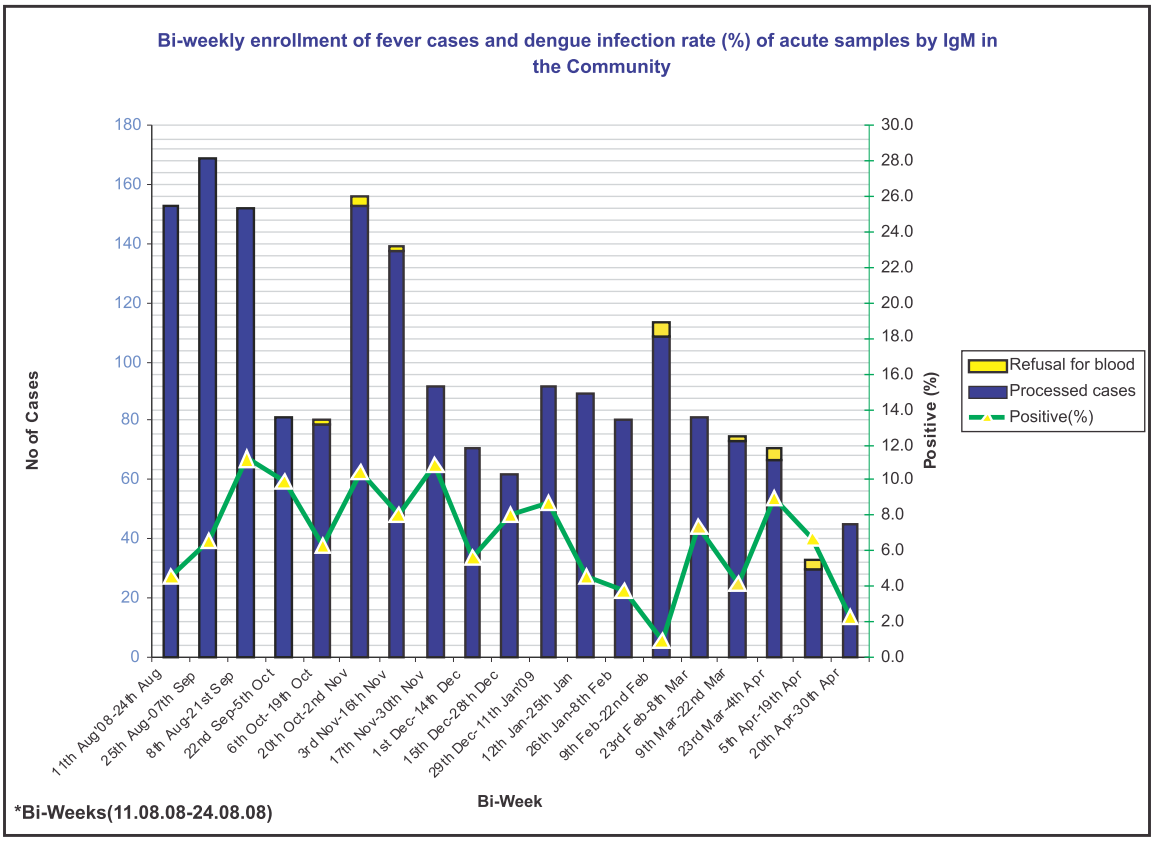
Co-Investigators : Suman Kanungo, Byomkesh Manna, Shyamalendu Chatterjee, Provash Sadhukhan, Shanta Dutta

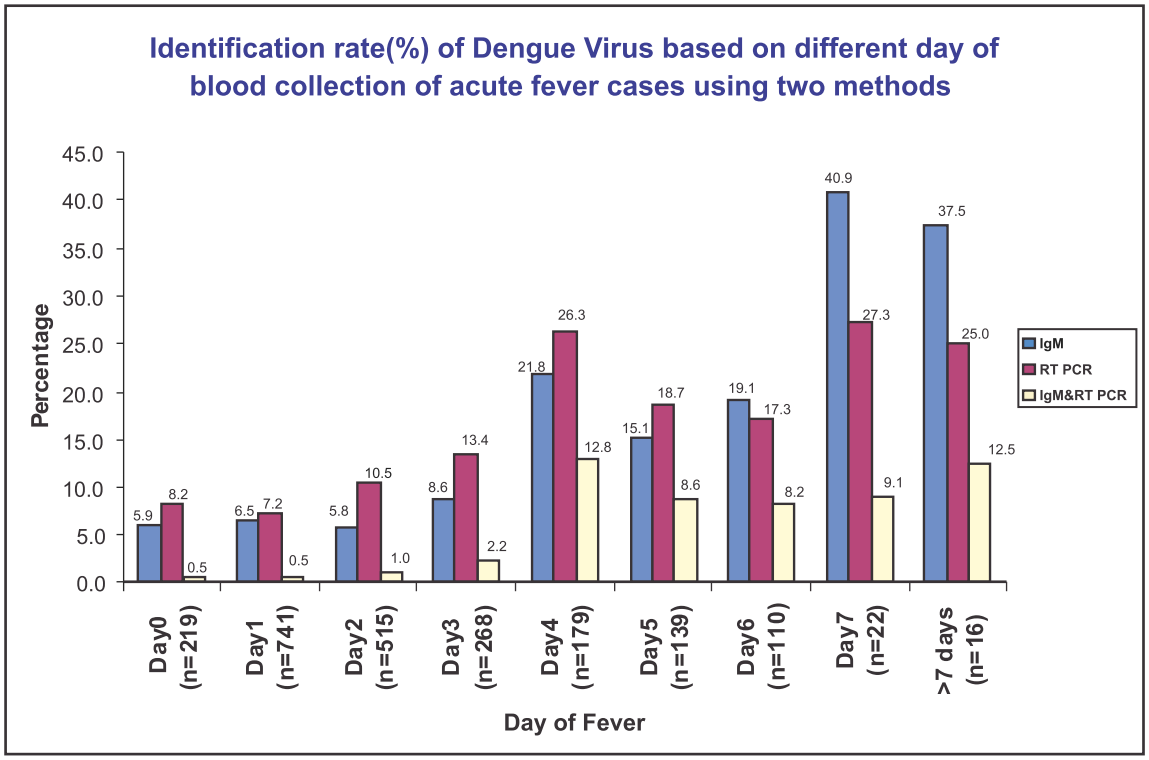
The study is being conducted to determine the incidence and burden attributable to dengue along with the epidemiologic, clinical and virologic details and to assess the characteristics of severe dengue through health care facility based enhanced sentinel surveillance for febrile illness. Health clinics have been established among urban slum population of Kolkata. Two referral hospitals (Infectious Diseases Hospital and B.C. Roy Children Hospital) were identified to study severe dengue cases.

IgM anti-DENV was determined by microplate ELISA (Dengue IgM capture ELISA, PANBIO Diagnostics) and DENV detection and

molecular typing were done by RT-PCR. During 9 months of surveillance, a total of 1848 patients presented at the field outposts with 0-7 days of febrile illness and among them 1825 (99%) samples were collected. Out of these, 207 (12%) samples were from 0-5 year, 442 (24%) from 5-15 years and 1176 (64%) from >15 years age group respectively. Overall, dengue detection rate by IgM ELISA among fever cases was 6.8%. It was similar (8.1%) in 0-5 years and 5-15 years age group but slightly lower (6%) in >15 years of age group. Based on 9 month IgM ELISA data, the crude yearly dengue incidence was estimated on current population of 22,010. The overall crude dengue incidence was 8/1000/year but the highest incidence (20/1000/year) was observed in 0-5 year age group of children. So far, DEN-1 is the most prevailing strain. These data suggest that dengue is a major public health problem in Kolkata. High incidence in younger age group makes it important for decision making for future trials of dengue vaccines for targeting this particular age group.

The primary objectives of this study were (a) to determine overall incidence of diarrheal illnesses in the study population, (b) to determine drug sensitivity pattern of the major isolated pathogens, (c) to determine risk factors for development of diarrhea and dehydration in the study population, and (d) to determine cause-specific mortality among the children and proportionate mortality due to diarrhea. Children aged 0-4 years of either gender residing within the Langalberia PHC area in Sonarpur block of South 24-Parganas district, West Bengal, will be eligible for participation in the study if their parents give consent to participate. Within the framework of the surveillance program, there will be several





components of the study – each one having specific design meant for evaluation of specific aspects of diarrheal illnesses, such as - (a) Prospective surveillance of diarrhea among under-5 children within a defined population. The surveillance will have two complimentary components – (i) Community-based surveillance of diarrheal illnesses, and (ii) Hospital-based surveillance of diarrheal illnesses; (b) Case-control study to determine risk factors for diarrhea and dehydration; (c) Verbal autopsy to assess mortality among under-5 children. Written informed consent will be obtained in local languages from the parents / guardians before enrollment of children, obtaining any information and collecting any specimen. So far we have developed necessary study instruments, including the informed consent forms. We have also pre-tested the study instruments, results from which will be utilized to guide us for finalization of the questionnaires and other processes, determination of the health care seeking patterns, and to assess various study needs, including logistic requirements. The actual study procedures are expected to start in July 2009.

7. To study the epidemiology of Human Papilloma Virus infection, a co-morbidity to HIV infection, in HIV infected female population of West Bengal – a pilot study

Investigator : Kamalesh Sarkar

Co-investigators : Sekhar Chakrabarti, Bibhuti Saha[1], Sudeep Saha[2]

[1]School of Tropical Medicine, Kolkata-700 073 and [2]Calcutta Medical College, Kolkata-700 073

The objectives of the study are to understand the prevalence of oncogenic HPV and associated pre-cancerous lesions in HIV infected female population of West Bengal along with the epidemiology of HPV infection to find out strategies for prevention /control of carcinoma cervix in them.Field work has been initiated and samples are being collected from

HIV infected female patients attending STM. A total of 94 HIV infected subjects were interviewed followed by collection of cervical specimens for HPV testing. PAP smear was collected from all of them to study cervical cytological abnormality. Three subjects showed pre-cancerous lesions. HPV genotype 16 is the commonest pathogen among them. The study is in progress.

8. A comprehensive population-based diarrheal disease surveillance program among under-five children in rural West Bengal, India

Investigator : Alok K. Deb

The primary objectives of this study were (a) to determine overall incidence of diarrheal illnesses in the study population, (b) to determine drug sensitivity pattern of the major isolated pathogens, (c) to determine risk factors for development of diarrhea and dehydration in the study population, and (d) to determine cause-specific mortality among the children and proportionate mortality due to diarrhea. Children aged 0-4 years of either gender residing within the Langalberia PHC area in Sonarpur block of South 24-Parganas district, West Bengal, will be eligible for participation in the study if their parents give consent to participate. Within the framework of the surveillance program, there will be several components of the study – each one having specific design meant for evaluation of specific aspects of diarrheal illnesses, such as - (a) *Prospective surveillance of diarrhea among under-5 children within a defined population. The surveillance will have two complimentary components – (i)* Community-based surveillance of diarrheal illnesses, and (ii) Hospital-based surveillance of diarrheal illnesses; (b) *Case-control study to determine risk factors for diarrhea and dehydration;* (c) *Verbal autopsy to assess mortality among under-5 children.* Written informed consent will be obtained in local languages from the parents / guardians before enrollment of children, obtaining any information and collecting any specimen. So far we have developed necessary study instruments, including the informed consent forms. We have also pre-tested the study instruments, results from which will be utilized to guide us for finalization of the questionnaires and other processes, determination of the health care seeking patterns, and to assess various study needs, including logistic requirements. The actual study procedures are expected to start in July 2009.

9. Study on biological markers of HIV-1 resistance conferring polymorphism and their distribution in injecting drug users' population of north-eastern India

Investigator : Kamalesh Sarkar

Co-Investigators : Sekhar Chakrabarti, Santasabuj Das

This project was completed officially on September 2008. This is a community based cross-sectional study. It was conducted among injecting drug users' population of north-eastern states of India to understand their genetic susceptibility to HIV infection. The objective was to assess the existence and magnitude of genetic mutations of chemokine receptors of CCR2-64I, CCR-5 D-32 and SDF-1-3`A in them that are known to confer resistance to HIV infection in some set up. A total of 711 injecting drug users from Manipur, Mizoram, Nagaland and Meghalaya were subjected for this study. The selected participants were interviewed to study their socio-demography, risk behaviors and risk perceptions after obtaining their verbal informed consent. The interview was followed by collection of about 5ml of blood samples by unlinked anonymous method for studying genetic

mutation and HIV infection. All samples were transported and processed at the virology laboratory of National Institute of Cholera and Enteric Diseases, Kolkata, India. The genetic mutations were detected by polymerase chain reaction (PCR), RFLP (restriction fragment length polymorphism) assay techniques. The study revealed that most IDUs belonged to the age group of 20 – 29 years (46.1%; n=328) followed by 30 – 39 years (42.9%; n=305) and the lowest number of IDUs belonged to the age group of above 49 years (0.3%; n=2). The HIV sero-positivity rate varied widely among IDUs living in different north-eastern states that ranged in between 4.5% to 61%. There was no single injecting drug users' with CCR5 mutation. Mutated genes of CCR2-64I and SDF-1-3`A were detected in the frequencies of 37% and 25% respectively in them. The HIV sero-positivity rate in IDUs having CCR2 mutant gene was (30%, n=79) and without mutation was (32%, n=142). Similarly HIV sero-positivity in IDUs with and without SDF1 mutation was 32% (n=57) and 31% (n=164) respectively. Both the differences were not statistically significant. The absence of CCR 5 mutant gene in this studied population appear to make them genetically susceptible to HIV infection as CCR 5 mutation is known to be the most prominent marker that confer resistance against HIV infection. Analysis also revealed that although mutation to CCR2 and SDF1 was present to some extent in this studied population, but that didn't confer any additional resistance against HIV. This indicates that north-eastern IDUs are susceptible to HIV infection genetically apart from vulnerabilities caused by behavioral and other relevant factors.

10. Art and testimonial: A Unique Community Based Approach to Reduce HIV/AIDS Stigma in villages of West Bengal

Investigator : Samiran Panda

Starting in July 2008, the research project based on community based randomized controlled trial design, has recently completed its one year of existence in two districts of West Bengal. The baseline assessment and first repeat survey show significant difference in change in negative attitudes towards HIV between intervention and control clusters. Final evaluation of the effectiveness of the conducted trial will take place during the end of 2009. Interventions accomplished under this extra-mural project comprise first round of community based event that combines one-on-one as well as one-to group interaction with personal testimony and folk songs on HIV/AIDS. Moreover small group meetings were organized with community stakeholders wherein also questions on HIV/AIDS were answered and concerns were addressed. Community engagement has remained central throughout implementation of this research project and 'Stigma Reduction Committees' (SRCs) have been formed in the respective villages that house the intervention clusters. Currently school based component of the intervention is on-going.

Individuals and families impacted negatively by HIV related stigma and discrimination have been assisted through the project to link up with service providing non-government/community based organizations so that they could cope better with the situation.Apart from the community health workers (some of whom live with HIV) working in the project being trained on ‘how to define cluster’, ‘preparation of master list’, ‘conducting interviews’ and ‘carrying out focus group discussions’, local performing artists were trained on issues around HIV/AIDS and how to weave HIV/AIDS themes in their art form.

PRESENTATIONS & VISITS

B. Manna: Participated in the meeting on Impact Evaluation of Cholera Vaccine Introduction held at Dhaka, Bangladesh during April 30- May 1, 2008.

Participated in "The Ninth Advanced Vaccinology Course (ADVAC 2008)" Course at Annecy, France during May 19-30, 2008.

Participated in the investigator's meeting for the Global Enterics Multi-center Study (GEMS) at Seattle, Washington, USA during September 10-12, 2008.

Presented paper entitled "Role of probiotics in prevention of diarrhoea in children (Authors)" at the 2nd India Probiotic Symposium and held at Hotel International Eros, Nehru Place, New Delhi during November 7-8, 2008.

Participated in the Annual Meeting of American Society of Tropical Medicine & Hygiene (ASTMH) and symposium organized by Global Enterics Multi-center Study (GEMS) at New Orleance, Louisiana, USA during December 8-10, 2008.

K. Rajendran: Attended Workshop on "Data Mining & Data Warehousing" held during September 15-20, 2008 at center for soft computing research, Indian Statistical Institute, India.

Visited Sapporo Medical University, Sapporo, Japan for collaborative research and training on "Time series analysis of cholera outbreaks and climate changes" during October 16 –November 15, 2008.

D. Sur: Presented on "IVI-NICED Collaboration in Kolkata at the Board of Trustees meeting and Symposium held at International Vaccine Institute, Seoul, Korea during April 2–4, 2008.

Presented lecture on "Impact of climate change on diarrhoeal diseases" at the programme on Climate Change and Human Health-Risks and Responses- organized by WBPCB, West Bengal supported by WHO on April 7, 2008.

Presented lecture on "Water quality and disease burden due to diarrhoea and enteric diseases" at National Conference on Safe Drinking Water for rural areas-Community Based Approaches organized by WaterAid India at New Delhi on April 8, 2008.

Attended meeting on Impact evaluation of Cholera Vaccine Introduction" held at ICDDR, B, Bangladesh during April 30 – May 1, 2008.

Attended Planning meeting on "Disease burden due to inadequate water and sanitation facilities and poor level of hygiene practices in India" organized by Sulabh International academy of Environmental Sanitation in Kolkata on August 2, 2008.

Presented updated data of the Indian study at the "Investigators meeting on the study of diarrhoeal disease burden" held at Seattle, Washington, USA during September 10-12, 2008.

Participated in "PDVI Field Consortium Meeting" in Jeju, Korea during September 25-27, 2008.

Presented paper entitled "Role of probiotics in prevention of diarrhoea in children" at the 2nd India Probiotic Symposium and held at Hotel International Eros, Nehru Place, New Delhi during November 7-8, 2008.

Presented research work done at NICED on pediatric diarrheal diseases at the Annual Meeting of American Society of Tropical Medicine and Hygiene held at New Orleans, Louisiana, USA during December 8-10, 2008.

Presented a paper entitled "Role of Probiotics in prevention of diarrhea in children" at the 12th Annual Scientific Conference (ASCON) held at ICDDRB, Dhaka, Bangladesh during February 9-12, 2009.

Presented "Results from a cluster randomized controlled effectiveness trial with typhoid Vi vaccine in Kolkata" at the Meeting on Typhoid Fever Vaccination in the Asia-Pacific Regions held at Bangkok, Thailand during March 10-11, 2009.

A. K. Deb: Attended a meeting of Strategic Advisory Board, Diarrheal Disease Program of Institute for OneWorld Health held in London, UK. during April 28-29, 2008

Attended dissemination workshop on CTRI (Clinical Trial Registry – India) organized by NIMS, New Delhi and NICED (funded by WHO) at NICED, Kolkata on August 18, 2008.

Attended "Pre-Surveillance Orientation and Planning Workshop for Regional Institutes and Focal Persons for Surveillance at SACS" organized by NACO and NIHFW, New Delhi and held in Chennai during September 20-21, 2008.

Attended and supervised training workshops for HIV Sentinel Surveillance 2008 for ANC and STD clinic attendees (as member, NACO Regional Institute, Eastern Region) in the states of West Bengal, Chhattisgarh, Sikkim and Andaman & Nicobar Islands during October 2008.

Dr. S. Kanungo

1. Participation in the Meeting titled "Impact evaluation of cholera vaccine introduction. to Bangladesh" from April 30 to May 1, 2008 in Bangladesh.
2. Participation in United Nations/India/ESA Regional Workshop on Using Space Technology for Tele-Health/Tele-Epidemiology to Benefit Asia and the Pacific Region held on October 20-23, 2008 at the School of Telemedicine & Biomedical Informatics, Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow.
3. Attended 2nd India Probiotic Symposium on November 7 -8, 2008 at New Delhi.
4. Attended course on Observational Epidemiological Studies Workshop in Lucknow, UP, India, December 10-12, 2008, sponsored by the National Institute of Health, USA and the International Clinical Sciences Support Centre.
5. Presented an oral presentation on the topic titled "Treatment cost of typhoid fever at two hospitals in Kolkata," at the 12th Annual Scientific Conference held by International Centre for diarrhoeal Disease Research, Bangladesh at Dhaka, Bangladesh from February 9 to 12, 2009.
6. Presented an oral presentation titled "Vaccine desirability during an effectiveness trial of the Typhoid Polysaccharide Vi Vaccine, Kolkata, India" at "International Symposium on tribal health" held at Regional Medical Research for the tribal, Jabbalpur from March 27 to April 1, 2009.

K. Sarkar: Presented findings on collaborative study on injecting drug use titled 'Baseline epidemiological study on injecting drug use' at the conference of International Society for Addiction Medicine held in Cape Town, South Africa during November 16-20, 2009

ELECTRON MICROSCOPY

The division of Electron Microscopy is engaged in research and diagnosis in the field of diarrheal diseases. There are several projects going on in the laboratory that can be categorized as follows.

Cryoelectron microscopy and 3-D image reconstruction

Three-dimensional structure of protein molecules are being worked out by employing cryoelectron microscopy and single-particle analysis methods. The 3-D structure of hemolysin oligomer, a pore-forming toxin of *Vibrio cholerae,* has already been worked out. Also the 3-D structure of several vibriophages and packaging pattern of DNA inside the phage head have been determined using cryoelectron microscopy. Three-dimensional structure of pili that play a vital role in the attachment of bacteria to the intestinal cell wall are being worked out using cryoelectron microscopy.

Vibriophage research

Morphology of different vibriophages isolated from different sources as well as those used in different phage typing schemes has been determined. Conformation of the genomes of these phages, genetic relatedness amongst them and studies on the biological processes like replication of these vibriophages, packaging of the genome inside the phage head have been carried out. This laboratory, for the first time, showed the filamentous nature of RS1-KmΦ phage of *V. cholerae*.

Nanobiotechnology

The bacterial flagellum consists of a flagellar motor, a hook and a long filament. The flagellar motor, not more than 40 nm wide, can rotate at a tremendous speed of about 1,00,000 rpm which propels the cell. How torque is generated for such high speed and also how the cell changes its direction of swimming are very important factors in the swimming process of the cell. Knowledge of these factors is essential for the design of an artificial nanomachine like a propeller-driven one that can dispense drug. Elastic properties of the flagella of several *Vibrio* spp. have been studied.

Histopathological studies

Histopathological changes caused by different enteric pathogens have been studied by light microscopy. Surface structural changes and in-depth ulltrastructural changes are being studied using scanning and transmission electron microscopes. Few of the important enteropathogens studied so far are: *Vibrio cholerae, Helicobacter pylori, Shigella* and *Aeromonas hydrophila.*

Scientist	:	A. N. Ghosh, Scientist F
		D. R. Saha, Scientist E
Staff	:	A. Sarbajna, Technical Assistant
		S. Kumar, Sr. Laboratory Assistant
		B. R. Mallick, Lab Attendant
Senior Research Fellow	:	Somnath Dutta

1. **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 gastro duodenal diseases. About 60-90% people in developing world acquire infection in early childhood and develop persistent infection, which lasts for decades unless treated with antibiotics. Severe gastritis is believed to be the denominator of peptic ulcer diseases and atrophic gastritis may lead to gastric cancer. However it is not clear why few strains are associated with ulcer formation with relevant clinical symptoms, while others are not associated with any disease manifestation. *H. pylori* is genetically more diverse than most bacterial species. Strain specific genetic changes are thought to be involved in the organism's ability to produce different diseases. The aim of our study is to look for the tissue changes in the stomach in *H. pylori* infected diseased and asymptomatic individuals and to correlate the *H. pylori* genotypes with the different histological changes. Active gastritis was present in 94% of asymptomatic healthy volunteers (Hv) and 84% of duodenal ulcer (Du) patients. Atrophic changes were observed in 27.7% Hvs and 60% Du cases. Metaplastic changes which are can change to cancerous conditions were less in both the groups. Genotyping of *H. pylori* strains between Du and Hvs were not much different. Metaplasia was detected in 14% of Du and 8.3% cases of Hv subjects. The present study suggests that inspite if the evidence of active histologic gastritis, Hvs form Tribal group were free from any clinical symptoms and they had much lower atrophic and metaplastic changes in stomach tissue. Individual immune response, bacterial genotype and environmental factors may have important influence in the disease outcome of *H. pylori* infected people.

PRESENTATIONS AND VISITS

1. **A. N. Ghosh:** Presented a paper entitled "Cryoelectron Microscopy and Single Particle analysis of *Vibrio cholerae* hemolysin" at the EMSI-2009 held at Bundelkhand University, Jhansi, during January 17-20, 2009.
2. **A. N. Ghosh:** Delivered invited talk entitled "Specimen Preparation Techniques and Applications of TEM in Biological Sciences" in UGC-Refresher Course on 'Analytical Instruments and their Applications' held in Jadavpur University, Kolkata, during February 2-21, 2009.

IMMUNOLOGY

The Division of Immunology is exploring the regulation of mucosal immune cells by two proteins: porin, the major outer membrane protein with pore-forming activity of *Shigella dysenteriae*, and hemolysin, a pore-forming toxin of *Vibrio cholerae*. The major focus of the immunology group centers on understanding how the two proteins are recognized by the cells that steer the signaling machinery either towards activation or apoptosis. The study of porin is aimed at establishing it as a potential adjuvant in vaccine strategies, while the work with hemolysin reveals the putative mechanism of how the two forms of the exotoxin differentially interact with the cells of the mucosal immune system.

Scientist	:	T. Biswas, Scientist E
Staff	:	S. K. Shaw, Lab. Attendant
Senior Research Fellows	:	Pallavi Banerjee
Junior Research Fellows	:	Deep Chandan Chakraborty
		Tanmoy Paul
		Krittika Sasmal

1. Porin-induced costimulation through Toll-like receptors for cytokine regulation of naïve $CD4^+$ T cells

Investigator: Tapas Biswas

Apart from triggering adaptive immunity through stimulation of innate immune system, TLRs are now known to be present on T cells, thus directly influencing the function of adaptive immunity. This information led us to investigate whether porin can influence TLRs on T cells, thus directly evoking T cell activity.

Among the TLRs involved in recognition of bacterial products, porin induced the expression of TLR2 on T cells (Fig. 1). The direct involvement of TLR2 was further confirmed through up-regulation of the TLR by varying concentrations of porin. Porin-induced up-regulation of TLR2 on activated $CD4^+$ T cells led us to track the involvement of TLR2 in downstream signaling. Detection of MyD88-TLR2 complex showed that the immunogen elicits downstream signaling through MyD88-TLR2 association (Fig. 2A). This eventually leads to activation of MAPK and NF-B, as evident from phosphorylation of the stress kinases ERK, p38 and JNK, and degradation of IB (Fig. 2B) along with TLR2-dependent nuclear translocation of NF-B.

Dilution of CFSE fluorescence in the porin-treated cells indicated their proliferative response (Fig. 3), as the level of the CFSE dye, which gets divided equally among the daughter cells decreased upon cell division. Next, effect of porin on viability of $CD4^+$ T cells was assessed, as proliferative response could be a reflection of cell survival. Our study demonstrated that porin promotes the survival of CD3-activated T cells in a TLR2-dependent manner. Moreover, the induction of Bcl-X_L by porin indicated the involvement of Bcl-X_L in porin-mediated cell survival.

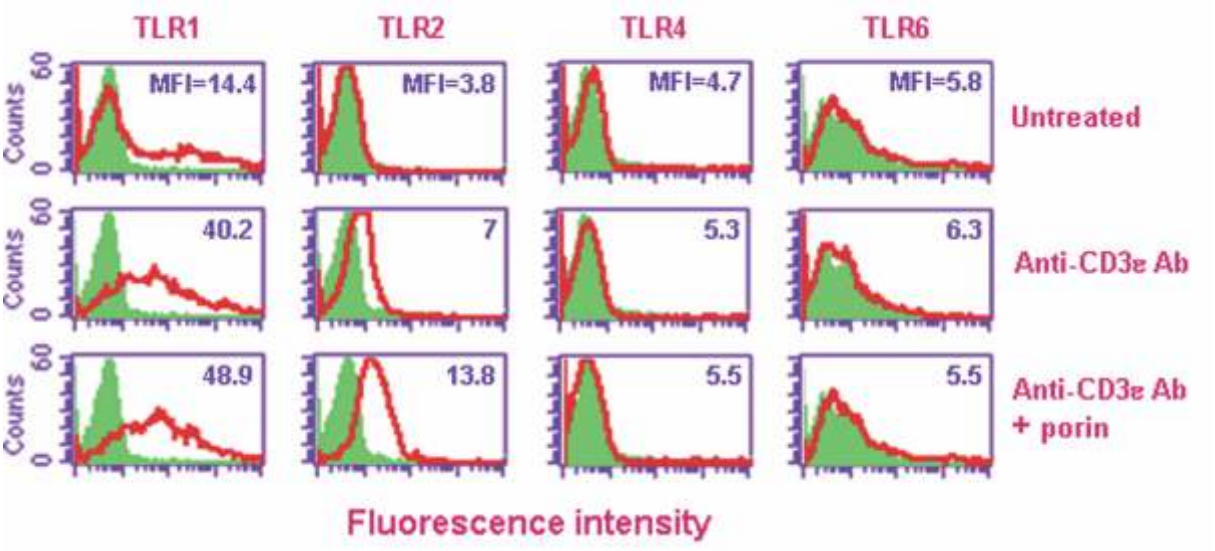


Figure 1

The data suggest that porin of *S. dysenteriae* type 1 proliferates T cells both by inducing cell division and protecting the cells from apoptosis through direct costimulation by TLR2. We are now investigating if TLR2 costimulation could only induce survival and proliferation of the T cells, or could work beyond that by influencing T cell effector functions.

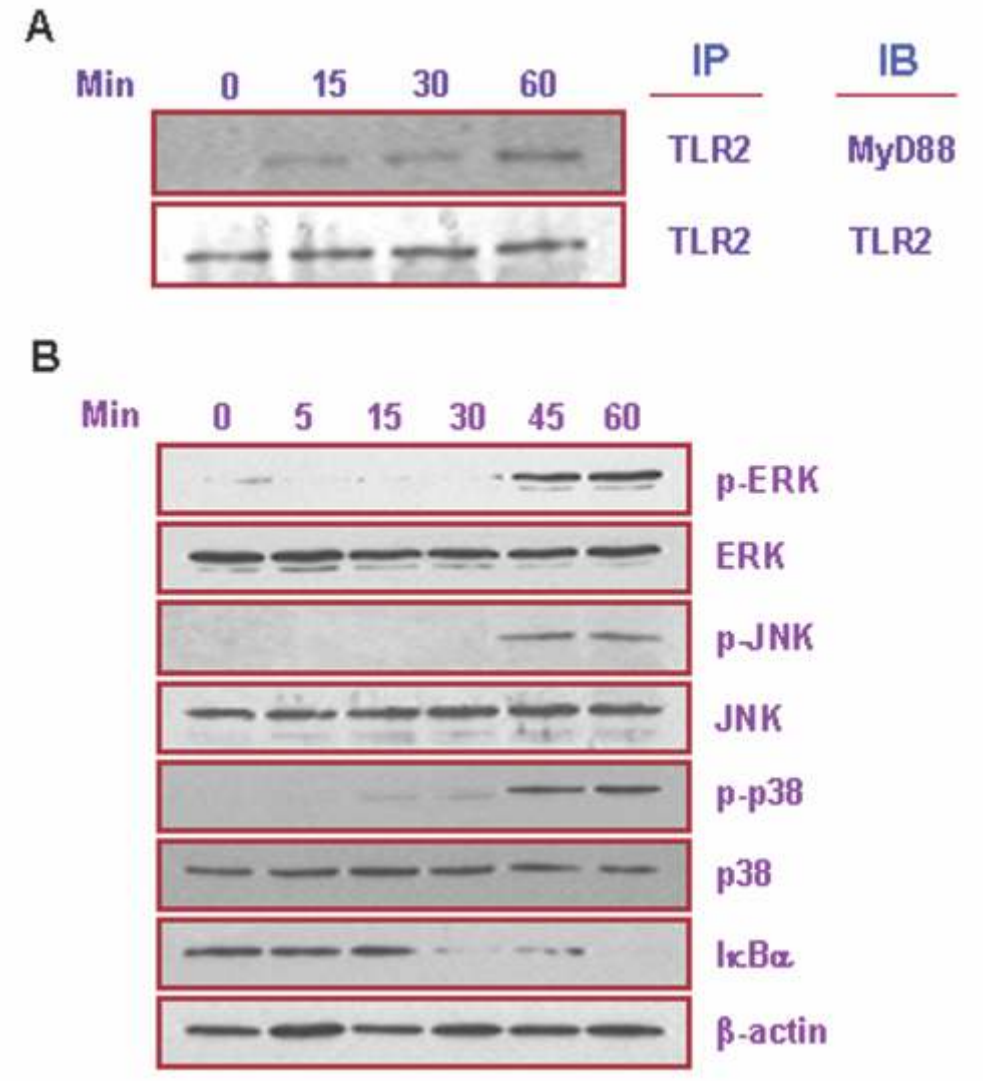


Figure 2

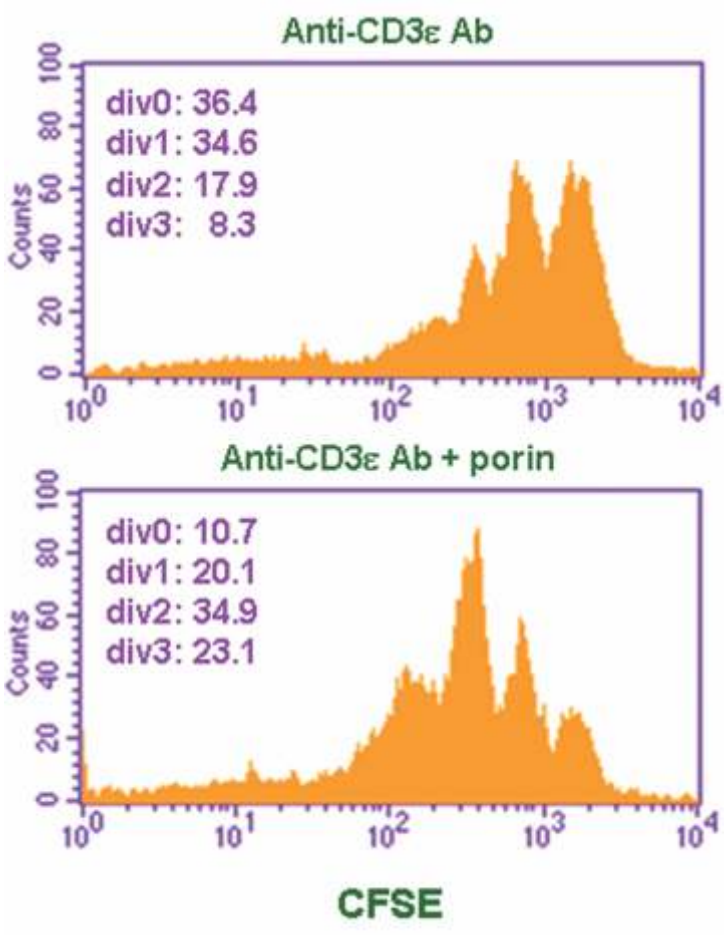


Figure 3

PRESENTATIONS AND VISITS

T. Biswas: Presented a poster entitled "Regulation of mucosal immune system by porin of *Shigella dysenteriae* and hemolysin of *Vibrio cholerae*" at the Training Mission in cholera case Management and Research, sponsored by NIH held at NICED, Kolkata, during September 8-10, 2008.

PhD DEGREE AWARDED

Dr. Amlan Biswas was awarded by Jadavpur University for his work entitled "Activation and Response of Peritoneal Macrophage for T Cell Polarization by Porin of *Shigella dysenteriae* Type 1" under the supervision of Dr. Tapas Biswas.

Dr. Gayatri Mukherjee was awarded by Jadavpur University for her work entitled "Differential Regulation of B-la Cell by *Vibrio cholerae* Hemolysin and Its Oligomer" under the supervision of Dr. Tapas Biswas and Dr. Kalyan K. Banerjee.

PARASITOLOGY

The Division of Parasitology at NICED actively integrates research into the mechanisms of parasitic diarrheal diseases at the molecular and cellular levels with epidemiological investigation of parasitic diagnosis from hospital and community patients. While ensuring an increasing understanding of human parasitic diseases, like amoebiasis, giardiasis, cryptosporoidis etc., this provides the foundation for further developments in diagnosis and future therapeutics.

Research efforts are built upon understanding the mechanism of ribosome biogenesis in Giardia, macromolecular interactions, mechanism of macromolecular complex formation and its use as a drug target in Giardiasis. Genomic DNA microarray chip of Giardia has been constructed in this division and is utilized for studying the effects of oxidative stress regulation in microaerophilic Giardia at its transcriptomic and proteomic level. A surveillance of enteric parasites from stool samples collected from different hospitals are regularly done in this lab to get the current scenario of parasitic diarrhoea in Kolkata as well as to establish the prime aetiology with parasitic co-infections.

This division is the eastern node as well as the central unit of a parasitic network under Indo-US joint collaboration for training and manpower generation and quality control of parasitic diagnosis across India.

This division has strong collaborations with Okayama University, Japan, NIID, Japan, CDC, USA, City University of New York, USA, Childrens International, USA, ICDDR, Bangladesh, Amsterdam Medical University, Netherlands etc.

This division offers PhD and Post Doctoral training program in different aspects of enteric parasitology. Beside its PhD and post doctoral program, this department organizes workshops and training for scientists, students and technicians.

Different prestigious grants and awards from National and International level have enriched this department time to time.

Scientist	:	Sandipan Ganguly, Scientist C
Staff	:	Trailokya Nath Boral, Senior Technical Assistant
		Shiv Laxman Prasad Singh, Laboratory Assistant
Senior Research Fellows	:	Arjun Ghosh
		Esha Ghosh
Junior Research Fellow	:	Punam Chowdhury
Research Assistant	:	Avik Kumar Mukherjee

1. Study of the effects of different Reactive Oxygen Species (ROS) generating factors in *Giardia lamblia* at molecular level

Investigator: Sandipan Ganguly

Giardia lamblia, a binucleate flagellated intestinal protozoan parasite, is a frequent cause of both epidemic and endemic diarrhoeal illness in developed and developing countries. *G. lamblia* is regarded as one of the most conserved eukaryotes evolved from the prokaryotes. Living aerobic organisms, from prokaryotes to complex eukaryotes, have developed elaborate sequences of adaptive mechanisms to maintain oxygen homeostasis and equilibrium. Any deviation from homeostasis, or physiological change in oxygen pressure, is recognized as an exposure to oxidative stress. Generation of reactive oxygen species (ROS) is a characteristic for such oxidative stress. *G. lamblia* is a micro-aerophilic organism, which does not usually tolerate elevated oxygen pressure. In the upper intestinal lining, where this organism generally resides, the O_2 concentration has been measured at 60μM. This amitochondriate lacks cytochrome and other conventional respiratory oxidases (catalases, superoxide dismutasases, glutathione reductase etc.) responsible for the scavenging of reactive oxygen species. The detailed mechanism is unknown by which the parasite could aid in the detoxification of hydroperoxides produced during an oxidative stress and reactive oxygen species generation. In the present project attempt has been made to know how does *G. lamblia* survive and perform vital activities in the gastro-intestinal tract where high reactive oxygen species generation takes place.

Full genomic DNA library of *G. lamblia* has been prepared and spotted on aminosilane coated glass slides to construct the full genomic DNA microarray. cDNA was extracted from both control and oxidative stressed cells and coupled with Cy3 and Cy5 respectively. After hybridisation the slides were scanned in microarray scanner and analyzed. The upregulated and downregulated spots were identified and had been sequenced to identify the genes responsible for oxidative stress regulation.

Differentially Expressed Genes	(Hypothetical) Function
Hsp70B2, cytosolic form	Cell defense/rescue
Hsp90	,,
Giardin	Cell attachment
NADH Oxidase	Oxygen metabolism
NADH Ferrredoxin Oxidoreductase	,,
Pyruvate Ferredoxin Oxidoreductase	,,
Alcohol dehydrogenase (lateral transfer candidate)	,,
Disulfide Reductase	Thiol redox management
Nitroreductase	Nitro radical detoxification
Arginine dihydrolase	Alternative arginine metabolism
Protein kinase	Signal transduction
Cathepsin B precursor	Cell death proteins
Variant Surface Proteins (VSP)	Surface antigen

In vitro oxidative stress generation in *Giardia* led us to some exciting results. The genes that got upregulated due to oxidative stress signify a bacteria-like oxygen metabolism in *Giardia lamblia*. Cell cycle analyses revealed that increased oxygen tension affects the cell cycle progression and during death we found a sub G_1 peak in flow cytometer. Further studies confirmed that the parasite cells are not dying through necrosis but via a programmed cell death mechanism. In Giardia, cellular membrane distension and exposure of annexin V-associated phosphatidyl serine residues on the outer leaflet of the plasma membrane were interpreted as common denominators with canonical programmed cell death (PCD). Apart from this, DNA is known to undergo a recursive sharing process that in turn generate fragments of defined length, as seen from the typical electrophoretic ladder profile during PCD. When the same analysis was performed on *Giardia lamblia* dead cells, a low molecular weight persistent smear devoid of any banding pattern was observed recurrently. This feature unambiguously differentiates *Giardia lamblia* PCD from other described cell death forms and suggests the existence of a different underlying DNA fragmentation mechanism in organisms devoid of mitochondria. Similar type of report has been obtained in case of *Trichomonas vaginalis*. Propidium Iodide has shown typical DNA degradation in the dead cells. But caspase or other proteases have been found not to be responsible for this type of death. Cells after incubating with protease inhibitors when underwent stress, still they committed death in programmed pattern which is confirmed by Annexin V test and DNA fragmentation, same as before. Two cases in Leishmania and Blastocystis have been reported where also the PCD is independent of caspase pathway or other proteases. From all these above facts it may be hypothesized that these early branching eukaryotes (EBE) including our model organism *Giardia lamblia* undergo PCD using a novel pathway other than caspase or other known proteases dependent pathways.

2. Studies on rRNA maturation and processing in *Giardia lamblia*

Investigator: Sandipan Ganguly

The gut protozoan parasite, *Giardia lamblia*, is the best-characterized example of the most ancient extant amitrochondrial eukaryote. Apart from its obvious medical importance, Giardia is fascinating in its own right.

In higher eukaryotes, ribosomal RNA (rRNA) biogenesis and processing was found to involve small nucleolar RNAs (snoRNAs) which interact with nuclear proteins to form the ribonucleo protein particles (RNPP). These RNPPs play a key role in ribosome biogenesis. Previously Giardia was considered as an anucleolate organism but having small nuclear RNAs (snRNAs) which are homologues to snoRNAs of higher eukaryotes and involved in rRNA processing. But after the identification of nucleolar like structure in this organism the scenario has been changed suddenly. Hence Giardial r-RNA processing is no doubt an attention grabbing incident.

In the present study we have cloned three snRNAs of *Giardia lamblia* and in vitro transcribed them under bacterial promoter and RNA polymerase followed by crosslinking with nuclear extract of the parasite to identify the protein(s) involved in RNPP formation and their characterization with respect to their role in rRNA processing and biogenesis. We have identified several proteins which specifically binds to these snRNAs after crosslinking followed by RNase protection assay using cold transcript competition to understand the specificity of binding. one of these proteins have been identified as fibrillarin which is also a very well reported protein involved in RNPP formation in other higher eukaryotes. Recombinant fibrillarin was cloned, sequenced, overexpressed and purified to homogeneity and antibody was raised against this purified recombinant *Giardia* fibrillarin.

In vivo identification of cross linking to find out binding domains of fibrillarin as well as protein binding motifs of snRNA are also under progress. Mutants are being raised and electroporated to cells for this study.

PRESENTATIONS AND VISITS

Dr. S. Ganguly

1. Invited participation in DMID International Research in Infectious Diseases Meeting (ICTDR Annual Meeting) in Bethesda, Maryland, USA from May 28 -30, 2008.
2. Invited participation as resource person in workshop on Spectral Confocal Laser Scanning Microscopy organized by Institute of Cytology and Preventive Oncology, Indian Council for Medical Research, Noida, India from August 4-6, 2008.
3. Participation and paper presentation in NIH Indo-US symposium, Kolkata, September, 2008.
4. Invited participation and paper presentation in 2nd National Conference of Tropical Parasitology (TROPACON 2) and pre conference CME on Parasitic Diseases in AIDS" at AIIMS, New Delhi, India from October 30 to November 2, 2008.
5. Invited participation and paper presentation in 20th National Congress of Parasitology at NEHU, Shillong, India in November, 2008.
6. Participation and paper presentation in Asian-African Research Forum on Emerging and Reemerging Infections, Sapporo, Japan, December, 2008.
7. Scientific visit to International Medical Center of Japan and National Institute of Infectious Diseases at Tokyo, December, 2008.
8. Invited participation as resource person in workshop on Confocal Laser Scanning Microscopy organized by NCBS, Bangalore, March, 2009.

PATHOPHYSIOLOGY

The research interest of the Division of Pathophysiology is related to the understanding of pathogenesis and signal transduction mechanism of different diarrhoeagenic bacteria, development of candidate vaccine, Super ORS and use of proper antibiotics against diarrhoea.

This Division is involved in the purification and characterization of different toxins and virulence factors secreted by diarrheal pathogens and interested to study the in depth signaling mechanisms of action performed by them.

The Division is well conversant in identification, purification and characterization of receptors, bacterial adhesins, toxins and proteases.

The involvement of different intracellular signal molecules in the induction of intestinal secretion by *E.coli* heat-stable toxin (STa), non-O1 V.cholerae (NAG–ST), Yersinia enterocolitica heat-stable toxin (Y-STa) have been evaluated. Moreover, calcium sensing receptor mediated downregulation of colonic carcinoma cell proliferation by thermostable direct hemolysin (TDH) has also been studied. It has been reported that COLO-205 cell line might be used as a model cell line to study the mechanism of action of E.coli STa.

The pathogenic mechanism of nonO1, nonO139 *V. cholerae* is not yet known clearly. In a study at this Division two forms of Hemagglutinin Protease (HAP), one is mature 45-kDa and processed 35-kDa forms have been purified from a nonO1, nonO139 strains and subsequent studies suggest that HAP may be an important virulence factor of these strains.

A study on vaccine development revealed that oral administration of heat-killed *Shigella flexneri* 2a could give 100% protection against homologues challenge which may lead to develop a simple, practical and effective vaccine against shigellosis.

These studies undertaken by the division are important for the development of vaccines and other therapeutic agents which can stop the signaling mechanisms of diarrheagenic pathogens at a particular stage which ultimately may prevent diarrhoeal diseases.

Scientist	:	Manoj Kumar Chakrabarti, Scientist F
		Amit Pal, Scientist D
Staff	:	Sankar Sen, Senior Technical Assistant.
		Jaglal Ram, Laboratory Technician.
Senior Research Fellows :		Pinki Chowdhury
		Debasis Pore
		Nibedita Mahata
		Aurelia Syngkon
		Elluri Sridhar

1. **Title of the Project: Characterization of the 34kDa outer membrane protein of *Shigella flexneri* 2a and study of its immune response.**

Investigator: M. K. Chakrabarti

It has been reported earlier by us that oral immunization with heat-killed whole cell *Shigella flexneri* 2a gives protection against challenge with homologous strain in rabbits. Amongst different outer membrane proteins, the gel cut 34 kDa protein is capable of providing significant protection in rabbits against the challenge with virulent *Shigella flexneri* 2a. Moreover, it has been found that electro eluted gel cut band of 34 kDa OMP induces the release of IL-12, tumor necrosis factor alpha (TNF-α) and production of nitric oxide (NO) by murine peritoneal macrophages in a dose dependent manner which established itself as a protective antigen and a potent candidate of subunit vaccine against shigellosis. However, it was thought that LPS and denaturation might influence immunogenecity of the gel cut electroeluted protein. Therefore, in the present study an effort has been made to purify and characterize the 34 kDa OMP of *S. flexneri* 2a. and study of its immune response.

For purification of 34 kDa OMP of *S. flexneri* 2a, the N-lauroylsarcosyl-insoluble outer membrane protein of *S. flexneri* 2a was isolated and the major outer membrane protein [MOMP] was prepared by selective extraction of OMP with the extraction buffer. The soluble fraction was then concentrated and applied to a Sephacryl S-200 HR (Pharmacia) column equilibrated with the extraction buffer (Fig. 1). The eluted fraction containing the 34 kDa MOMP was identified by SDS-PAGE and pooled and dialysed. The dialysed fraction was then concentrated and applied to a DE-52 (Whatman) column and the MOMP was eluted in 1.5 ml fraction with a linear gradient of 0 to 0.5 M NaCl. (Fig. 2). The SDS-PAGE analysis of this fraction revealed that it was essentially pure 34-kDa protein (Fig. 3), which was then dialysed and finally stored at -20°C. Further studies are going on to characterize the purified 34 kDa OMP.

2. **Studies on enterotoxigenicity of a cholera toxin gene negative *V. cholerae* non-O1, non-O139 strain in an in-vitro rat intestinal model.**

Investigator : Amit Pal

In an earlier study we had reported that hemagglutinin protease (HAP) may play a role in pathogenesis of ctx gene negative *V. cholerae* non-O1, non-O139 strain. In the present study we constructed hapA deletion mutants in

WO-6 a ctx gene negative *V. cholerae* O1 strain, WHA 6.8. Earlier reports suggested increased virulence in hapA deleted *V. cholerae* O1 strains. The strains were tested in *C. elegans* model and the WHA6.8 strain showed it was toxic to the worms and caused them to run away from the bacterial lawn (Fig1) WHA6.8 strain showed presence of 97 and 98 kDa bands in SDS-PAGE. The N terminal sequence of the 97 kDa band showed homology with prtV a metalloprotease earlier reported to play a role in predator grazing in C. elegans model. The 98 kDa protein band showed homology with aminopeptidase N. A high molecular weight protein was also detected in WO-6 strain and its N terminal sequence showed homology with El Tor hemolysin. Mutants were constructed on WO-6: WO-6 ΔhapA, WO-6ΔprtV, WO-6ΔhlyA and WO-6Δapn. Mutants were also constructed on WHA 6.8 : WHA6.8ΔhlyA, WHA6.8ΔprtV and WHA 6.8 Δapn. Except for WHA 6.8 strain all other double knock out mutants WHA6.8ΔhlyA, WHA6.8 ΔprtV and WHA6.8Δapn failed to prevent the toxic effect on C. elegans. The *C. elegans* running away factor in WHA 6.8 is still unknown. Though HAP is the major protease in C6709 a *V. cholerae* O1 Peruvian strain. hapA deleted CHA6.8 strain showed protease activity in azocaesin assay. This protease was partially purified after ammonium sulphate precipitation, dialysis and run in DEAE ion exchange column chromatography. The protease activity was recovered in the non binding fraction of DEAE column. Protease activity was partially inhibited by EDTA (50%). Further work is in progress to purify and characterize this protease.

PRESENTATIONS AND VISITS

M.K. Chakrabarti

1. Mahata N, Chowdhury P, Pore D, Pal A and M.K. Chakrabarti. "Reorganization of cytoskeletal proteins by *Escherichia coli* heat-stable enterotoxin (STa)" at the International Conference on "Perspective on Cell Signaling & Molecular Medicine" at Bose Institute, Kolkata on November 27-29, 2008.
2. Pore D, Chowdhury P, Mahata N, Mahalanabis D and M.K. Chakrabarti. "An approach towards the development of a candidate Shigella vaccine" at the International Conference on "Perspective on Cell Signaling & Molecular Medicine" at Bose Institute, Kolkata on November 27-29, 2008.
3. Chowdhury P, Pore D, Mahata N, Pal A and M.K. Chakrabarti. "Thermostable Direct Hemolysin: A possible therapeutic target to colon cancer" at the International Conference on "Perspective on Cell Signaling & Molecular Medicine" at Bose Institute, Kolkata on November 27-29, 2008.
4. Mahata N and M.K. Chakrabarti. "Rearrangement of cytoskeletal proteins by *E. coli* Heat-stable Enterotoxin" at International Congress on Advanced Medicare, December 9-11, 2008, Kolkata.
5. Pore D and M.K. Chakrabarti. "A study towards the development of candidate vaccine against Shigellosis" at International Congress on Advanced Medicare, December 9-11, 2008, Kolkata.
6. Chowdhury P and M.K.Chakrabarti. "Amplification and Cloning of *E. coli* Heat-stable Enterotoxin gene from a Human Colonic Carcinoma Cell line HT-29" at International Congress on Advanced Medicare, December 9-11, 2008, Kolkata.
7. M.K. Chakrabarti. Invited lecture delivered on "Cellular Signaling" at NIPER, Patna on December, 2008.
8. M.K. Chakrabarti. "A study towards the development of a candidate Shigellavaccine" Invited lecture delivered at the Section of Medical Science including Physiology. 96th Session of Indian Science Congress, January 3-7, 2009, NEHU, Shillong.
9. M.K. Chakrabarti. "Chronological Development of Technology and Research on Diarrhea" Invited lecture delivered at J.N.T.University, Kankinada, A.P. on February 18, 2009.

A. Pal

Presented a lecture entitled "Studies on pathogenesis in ctx gene negative *Vibrio cholerae* non-O1, non-O139 strains" at the Research workshop with research groups of Mizunoe-Wai - Uhlin at Umea University, Sweden held at the Department of Molecular Biology, Umea University during June 18-19, 2008.

PhD DEGREE AWARDED

Amit Ghose was awarded Ph.D. from Jadavpur University for his thesis entitled "Studies on enterotoxigenicity of non-toxigenic *Vibrio cholerae* non-O1, non-O139 strains" under the supervision of Dr. Amit Pal.

VIROLOGY

The Division of Virology focuses on Enteric viruses and Human Immunodeficiency Virus (HIV) with three basic components namely, service, training and research.

For service, the division plays a key role in the surveillance studies undertaken by the institute to understand the etiological role of different diarrhoeagenic viruses in and around Kolkata to gather information with relation to the disease burden. The division provides laboratory diagnostics for viral pathogens like rotavirus, norovirus, astroviruses, adeno viruses and picobiRNA viruses during the diarrhoeal outbreaks in the state or country. In addition, epidemiological and molecular characterization of HIV strains in high risk groups in West Bengal and Manipur is done in collaboration with epidemiology division.

The Division also serves to impart training to graduate and doctoral students and staff so as to improve the human resources capable of studying viral diarrhoeal diseases across the country.

The research programs in the division include intramural projects and extramural projects with national and international funding and collaborating scientists. The current programs are associated with DBT, ICMR, CDC Atlanta, Sapporo Medical University, Okayama University etc. The division is involved in basic research involving studies on genetic diversity, vaccine development, host-virus interactions related to enteric viruses and Human immunodeficiency virus (HIV).

The Division has extended it's activites to include studies on influenza viruses and has organized a routine surveillance program in collaboration with World Health Organization and Centers for Disease Control and Prevention, Atlanta, USA for close monitoring of genetic diversity among circulating strains. The Division also maintains Biosafety level 3 laboratory to carry out investigations during an outbreak of suspected highly pathogenic viral diseases such as SARS or avian influenza.

Objectives of Division:

1. Molecular characterization of crucial HIV encoded genes with focus on understanding immunogenicity for developing vaccine candidates.
2. Surveillance and disease burden of diarrhoea induced by Enteric Viruses.
3. Molecular phylogenetic analysis of the circulating enteric viruses in and around Kolkata with focus on Rotaviruses, Caliciviruses (Norovirus and Sapovirus), Astroviruses, Picobirnaviruses and Adenoviruses.
4. Analysis of the signaling mechanisms during Rotavirus-host cell interactions: Study of host cellular proteins required for viral replication and pathogenesis.

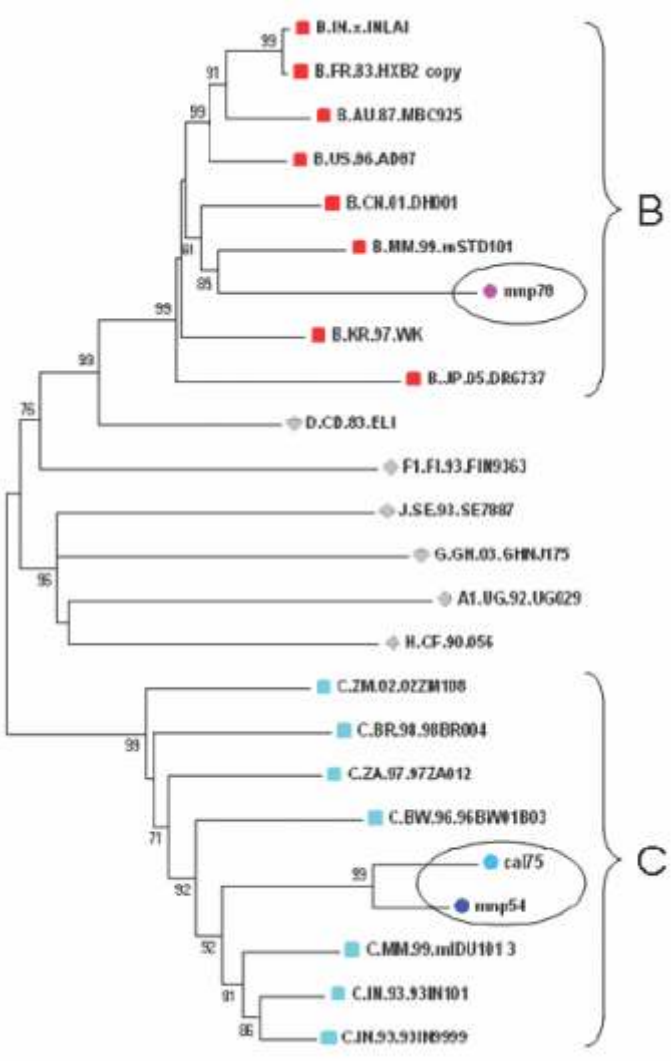
Scientist	:	Sekhar Chakrabarti, Scientist F
		Triveni Krishnan, Scientist D
		Mamta Chawla-Sarkar, Scientist C
		B. Ganesh, Scientist B
Staff	:	Sudhir Omesh, Sr. Technical Assistant
		Mousam Mallick, Technical Assistant
		Khokon Sen, Sr. Laboratory Assistant
		Papiya De, Sr. Laboratory Assistant
		Bimal K Bera, Laboratory Assistant
		Md. Mussaraf Hossain, Laboratory Assistant
Senior Research Fellows	:	Dipanjan Dutta
		Ranajay Mullick
		Roni Sarkar
		Satarupa Sengupta
Junior Research Fellows	:	Anurodh S Agrawal
		Parikshit Bagchi
		Nilanjana Biswas
		Anupam Mukherjee
		SM Nataraj
		Mehuli Sarkar
Research Assistant	:	Madhusadan Pativada

1. Genetic Characterization of Full-length *gag* gene of HIV-1 from India

Investigator: S. Chakrabarti

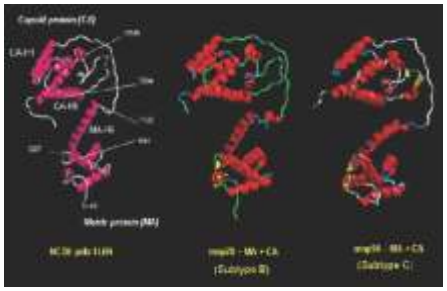
The HIV-1 *gag* gene, like all other retroviruses, encodes the polyprotein Gag which contains all the necessary as well as sufficient elements required for virus assembly. Roles played by HIV-1 Gag proteins during the life cycle are numerous and complex, involving not only assembly but also virion maturation after particle release and early post-entry steps in virus replication. Therefore, detailed molecular characterization of full length gag gene of HIV-1 has always been important to study, especially in India, where since the first case of HIV infection as well as AIDS case report in 1986, the epidemic has spread throughout the country.

In order to study the full length gag gene (1.5 kb) of HIV-1 among the high risk groups from eastern and north-eastern India, proviral DNA was isolated from PBMC and was used to amplify the gag gene (1.5 kb) of HIV-1. The primers were designed in such a way that the Kozak sequence along with the start and stop codons became incorporated in the amplicon so that the gene could be expressed by recombinant vector under suitable promoter. One Calcutta strain (cal75) and two Manipur strains (mnp54 and mnp78) were amplified for 1.5 kb gag gene with specific primer pairs, cloned in pGEM-T, TA-TOPO and pUC18 vectors respectively and sequenced. The full-length gag (1.5 kb) sequences were then put into the software program for restriction map analysis and were found to be in agreement with previous restriction digestion pattern of the clones. BLAST search revealed the homology of mnp54gag and cal75gag to subtype C while mnp78gag to subtype B strains in HIV database. Phylogenetic analysis (Fig.1) and simplot analysis of these strains again detected the homology of mnp54 and cal75 to subtype C and mnp78 to B. Genetic distance computed by the pairwise distance calculation method were- 0.03% between mnp54 and cal75 (both subtype C); 18.7% between mnp54 (C) and mnp78 (B) while 19.1% between cal75 (C) and mnp78 (B).



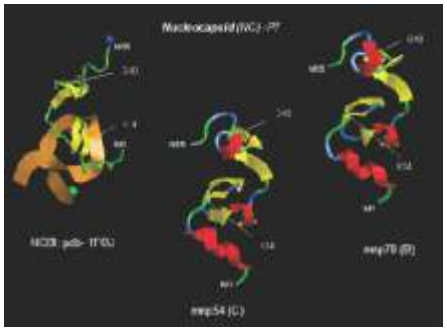
The predicted amino acid sequences from the gag gene nucleotide sequences of the Calcutta C (cal75), Manipur C (mnp54) and Manipur Thai-B (mnp78) strains were aligned with subtype C strain IN301999 and subtype B HXB2 references. It clearly showed amino acid similarities of cal75 and mnp54 to C.IN301999 while mnp78 to HXB2. Subtype specific divergences were there between the C and B strains. Genetic distances computed from pairwise amino-alignment based revealed that the subtype C strains cal75 and mnp54 were less divergent to Indian C strain IN301999 (11.2% and 11.7% respectively) and more divergent to reference B HXB2 strain with 21.1% and

20.8% respectively. While on the other hand, subtype B strain mnp78 was also more close to the reference B.HXB2 strain with only 12.2% divergence to it and it was also distantly related to C.IN301999 strain with a greater divergence of 22.4%. However, the two representative C strains, cal75 and mnp54 of India showed the least variation between them and had only a minimum divergence of 6.3%.

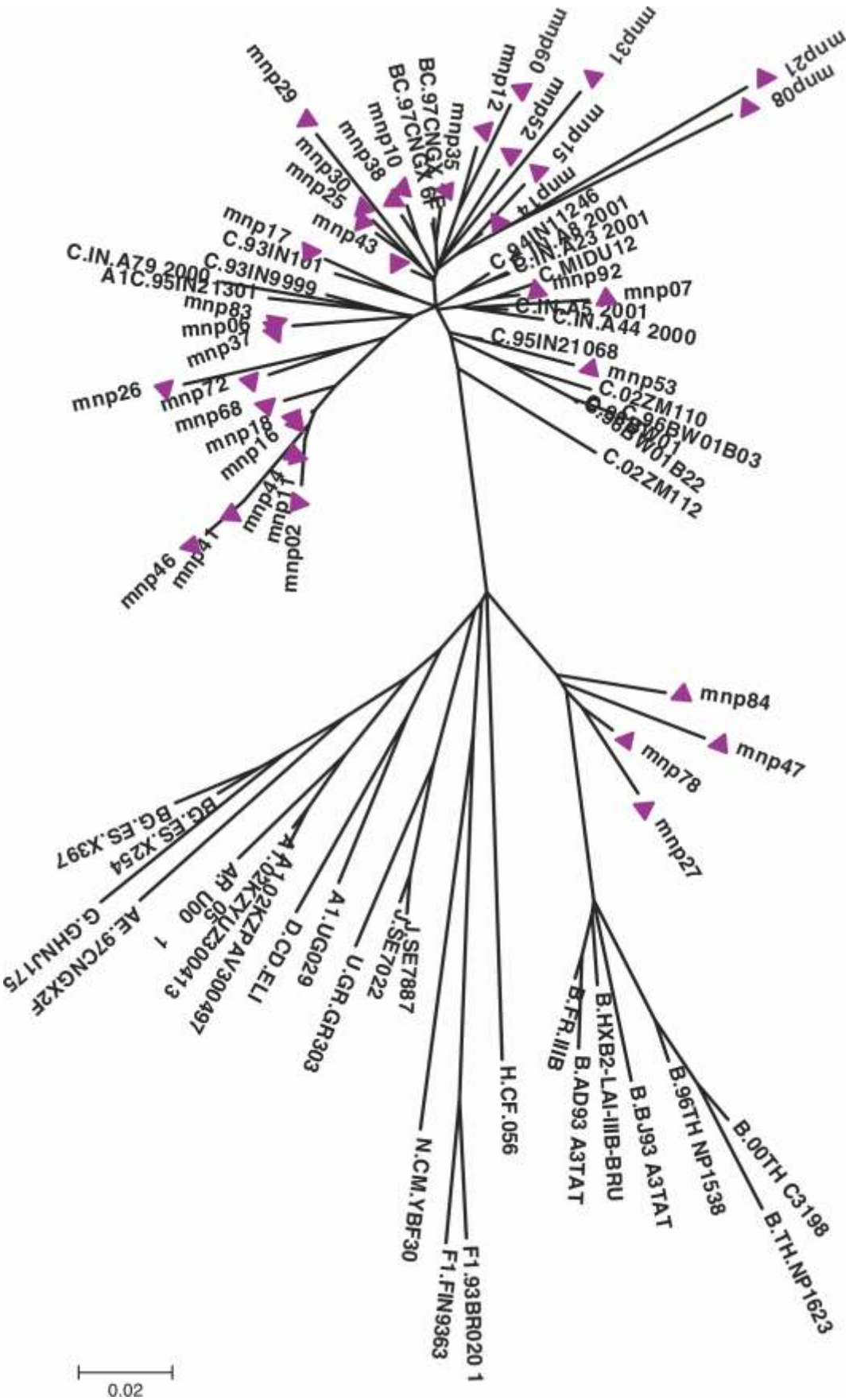


Further, the three major domains of HIV-1 Gag, namely the matrix (MA), capsid (CA) and nucleocapsid (NC), which upon protease mediated processing, constitute the architecture of the infectious mature viral particle have been studied. Molecular modeling was done to view and analyze the HIV-1 Gag structure based on the matrix, capsid and nucleocapsid region from the predicted precursor Gag (Pr55) sequences of samples – mnp54 and mnp78. These two samples were found to belong to subtype C and subtype B respectively in the previous analyses and therefore it was interesting to know whether these subtype variations at the nucleotide level infer any significant comparative difference in the structural analysis. The HXB2 strain of HIV-1 was taken into consideration during the study because its protein motifs and structural components were well documented. Template selected for the matrix and capsid modeling was the NMR structure of N-terminal 283-residue of immature HIV-1 Gag polyprotein [PDB: 1L6N; MMDB: 19925]. Fig.2a clearly showed maximum structural similarities of matrix-capsid domains of the reference NMR structure 1L6N with the predicted structures of mnp54 and mnp78 irrespective of any major subtype discrepancy. The MA-H5 region (91R…122T in HXB2) related to viral entry indicates a maximal imperfect DNA mirror repeats with ⩾50% symmetry and the corresponding region is highly conserved especially in the subtype B strains mnp78 with HXB2. The helical structure (MA-H5) has been indicated in Fig.2a.

HIV-1 NC plays key roles in virus structure and replication via its nucleic acid binding and chaperoning properties and controls proviral DNA synthesis. The 55 amino acids (M1…N55) of nucleocapsid region were taken for 3D construction. Fig.2b depicted the 3D structures of mnp54 and mnp78 along with a reference NMR model [PDB: 1F6U; MMDB: 14914]. HIV-1 NC protein is formed of two CCHC zinc finger motifs (ZF1 and ZF2) which were highly conserved in all the cases. K14 to G40 of NC indicated the corresponding K391 to G417 of full gag sequence (HXB2) respectively, spanning the region of zinc knuckles and spacer between them. In this study, structural similarities were found in HIV-1 subtype C and subtype B strains from India. However, the amino acid variations might occur in similar groups causing the viral epitopes to look different to host immune system to escape it, in spite of keeping protein structure stable. Interaction between DNA and protein structure and function is interwoven over entire length of Gag while the gene sequence exhibits a high degree of regularity and consists of sequence segments associated with functional attributes of the protein segments that they translate.



The 1.5 kb full length gag gene of subtype C strain which is the major prevalent strain in India was further cloned in vaccinia expression vector and recombinant vaccinia virus containing the full gag gene was constructed. The infection/transfection method allowed the formation of recombinants by homologous recombinantion between the TK flanking regions and TK$^-$ selection was done. The expressed 55 kD precursor Gag protein was found to be immunoreactive in western blot analysis. This recombinant vaccinia construct of HIV-1 gag gene could prove to be useful for the studies of antigenic properties of HIV-1 Gag proteins.


mnp29
mnp30
mnp25
mnp38
mnp10
BC.97CNGX 6F
mnp35
mnp12
mnp60
mnp31
mnp52
mnp15
mnp14
mnp21
mnp08
mnp17
C.93IN101
C.IN.A79 2000
A1C.95IN21301
C.93IN9999
mnp83
mnp06
mnp37
mnp43
C.84IN11246
C.IN.A8 2001
C.IN.A23 2001
C.MIDU12
mnp92
mnp07
C.IN.A5 2001
C.IN.A44 2000
C.95IN21068
mnp53
C.02ZM110
C.96BW01B03
C.98BW01
C.02ZM112
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mnp72
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mnp84
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AE.97CNGX2F
G.GHNJ175
AR U00 1
05
A1.02KZPAV30041 3
D.CD.ELI
A1.UG029
U.GR.GR303
J.SE7887
J.SE7022
N.CM.YBF30
F1.FIN9363
F1.93BR020 1
H.CF.056
B.FR.IIIB
B.AD93 A3TAT
B.HXB2-LAI-IIIB-BRU
B.BJ93 A3TAT
B.96TH NP1538
B.00TH C3198
B.TH.NP1623
0.02

2. Genotyping of HIV-1 strains based on env, gag and tat genes among Injecting Drug Users from West Bengal

Investigator: S. Chakrabarti

One of the most important routes of HIV-1 transmission worldwide is through sharing of needles and syringes among injecting drug users (IDUs) and ten percent of the HIV/AIDS cases globally, have been attributed to IDUs. A recent occurrence of HIV-1 seropositivity among a group of Injecting Drug Users (IDUs) in Darjeeling, a hilly district in northern West Bengal revealed over all 11.8% HIV seroprevalence. Siliguri, the only subdivision located on plain, serves as the gateway for Nepal, Bhutan, and Bangladesh as well as all north-eastern states and Sikkim. Therefore it is the gateway for drug trafficking in India through the various international boundaries. The IDU samples from Darjeeling were screened for HIV-1 seropositivity by an unlinked anonymous method. HIV was tested by ELISA (Immunogenetics, Belgium) followed by tridot assay as per policy of country's National AIDS Control Programme. Blood samples were collected in Na-citrate solution and peripheral blood mononuclear cells (PBMCs) were separated from whole blood by Ficoll-Hypaque gradient centrifugation and DNA was extracted by using the QIAamp DNA Blood Mini Kit 250 (QIAGEN, Germany). Nested PCR were performed to amplify the env C2-V3 and gag p24-p7 regions. Heteroduplex Mobility Assay (HMA), sequencing and phylogenetic analysis showed that Darjeeling IDU sequences belonged to subtype C both env & gag genes. Interestingly, the IDU sequences from Darjeeling were again found to be more close to the C-strains from Manipur, a northeastern state in India, which is linked to the Golden Triangle via Manipur-Myanmar border, rather than the IDU C-sequences from Nepal, a neighboring country of India. After assigning of the subtypes with respect to env and gag genes and their analysis, polymorphic variation of the tat regulatory gene was further studied among these Darjeeling IDU samples. The tat exon-1 (~216bp) was amplified and subjected to sequencing and phylogenetic analysis. Darjeeling IDU tat sequences formed characteristic cluster among them and were close to the Myanmar and China IDU sequences along with the prototypic Indian and African C sequences (Fig.3). The outgroup reference strains from different sites of IDU-driven epidemics in the world like Russia, Vietnam, Thailand, Spain etc belonged to non-subtype C group and formed separate clusters from the subtype C cluster in our analysis. As reported earlier, drug traffickers actively use porous Indo-Nepal border through Siliguri, which comes to Nepal from "Golden Triangle" and some of the drugs filter to the hilly part of the Darjeeling district for abuse by the young ones. But our study suggests possible new route of transmission of HIV-1 from the Manipur-Myanmar border towards West Bengal and subsequent spread of HIV infection to the eastern parts of India. The IDU sequences of Darjeeling also tended to form a strong monophyletic cluster among themselves in cases of env, gag and tat genes indicating an isolated spread of HIV-1 strains within this geographic region. Such kind of star-shaped phylogeny, suggests horizontal mode of transfer of the virus in a short time period. Until this report, other than the epidemiological survey, no genotyping and subtype determination studies based on phylogenetic analysis at the molecular level had been done among the HIV-1 infected IDU population from West Bengal. This is also a first time reporting of studies based on HIV-1 tat gene among seropositive IDUs from India. Thus, it gives an alarming signal of the increasing population of injecting drug users in West Bengal those who are potent carriers of HIV infection and of transmission of HIV-1 through the northeastern borders of India to West

Bengal which may lead to emergence of new recombinant strains. It would be interesting to continue further studies on other genomic regions of HIV-1 in this population in order to monitor possible emerging routes of HIV-1 transmission in India and to see whether any new strain is entering in this part of the country.

3. Prevalence and genetic diversity of diarrhoeagenic viruses viz. Rotaviruses, Norovirus, Sapovirus, Astrovirus and Adenovirus detected in Kolkata

Investigator: Triveni Krishnan

Group A rotaviruses are major pathogens causing life threatening dehydrating gastroenteritis in children and other viruses viz Norovirus, Sapovirus, Astrovirus and Adenovirus are found to be associated sporadically with acute watery diarrhea in Kolkata among children and adults.

The main objective was to study the prevalence and genetic diversity of diarrhoeagenic viruses circulating among acute watery diarrhea cases in Kolkata.

Group A Rotaviruses are largely associated with diarrhea among children aged below 5 years. Molecular characterization of various gene segments encoding (a) VP4 [P-type specific] (b) VP6 [group specific] (c) VP7 [G-type specific] and (d) NSP4 [viral enterotoxin/genogroup specific] provides better understanding of genetic diversity among rotavirus strains in Kolkata. We had reported the new evidence that RdRp and capsid gene of Norovirus genogroup I and Norovirus genogroup II can undergo recombination leading to a novel recombinant strain. Moreover, intergenotype recombinants of Norovirus have also been detected among Norovirus genogroup II strains. The analysis of a complete genome sequence of the astrovirus strain detected in Kolkata, India had shown interesting features that ORF1a had two unique nucleotide sequence stretches; the phylogenetic relationship varied considerably within the three different ORFs for the Kolkata strain unlike hitherto reported astroviruses.

The surveillance data shows that rotavirus, adenovirus and Sapovirus are more closely associated with acute watery diarrhea among children below five years and were seen to infect few adults whereas Norovirus and Astrovirus affected children above five years and adults.

4. To analyze Host-Rotavirus Interactions: Pivotal role of host proteins during virus replication and pathogenesis

Investigator: Mamta Chawla-Sarkar

Rotaviruses are the single most important cause of severe dehydrating diarrhea in children worldwide with estimated 100,000 deaths and 400,000 hospitalizations in India. Since viruses in general depend on its host cell for replication and pathogenesis, we hypothesize that, identifying the host cellular proteins and virus encoded proteins, which facilitate virus replication and propagation by evading immune responses will lead to better understanding of the mechanisms of pathogenesis. Rotavirus primarily infects intestinal epithelial cells and leads to transcriptional regulation of a number of cellular genes, including IFN inducible anti-viral genes, anti-apoptotic signaling and Hsp90 chaperone protein. Hsp90 has been shown to regulate replication of many viruses. During this study too we observed dose and time dependent decrease (1.6-2.0 log) in rotavirus growth in presence of Hsp90 inhibitor, 17-allylamono-demethoxygeldanamycin (17-

AAG). Inhibition of HSP90 function has been shown to cause degradation of client proteins via the ubiquitin-proteasome pathway, which results in the simultaneous depletion of other related proteins and the combinatorial downregulation of signals propagated through numerous signaling pathways. 17-AAG resulted inhibition of virus induced p-Akt and p-NFκB activation (Fig 1). Direct Hsp90-Akt interaction in virus infected cells was also reduced in presence of 17AAG.

To understand functional significance and mechanism of rotavirus induced activation of PI3K/Akt signaling, rotavirus encoded proteins were studied. Nonstructural protein-1 (NSP1) was found to activate pro-survival pathways like PI3K/Akt and NFκB during initial stages of infection, to delay virus induced apoptosis. The NSP1 mutant strain could only weakly PI3K/Akt and NFκB pathways compared to the isogenic NSP1 wild type strain A5-13 and resulted in early induction of apoptosis (Fig 2). In virus infected cells NSP1 coimmunoprecipitated with p85-subunit of PI3K (phosphoinositide 3-kinase) confirming direct interaction between cellular and virus protein. Furthermore PI3K/Akt inhibitors attenuated rotavirus growth significantly confirming importance of cellular proteins during rotavirus infection and possibility of targeting cellular chaperones for developing new anti-rotaviral strategies.

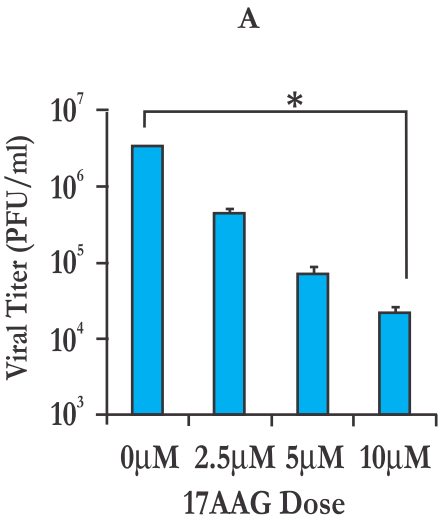
5. Surveillance and molecular characterization of Influenza Virus strains circulating in Eastern India.

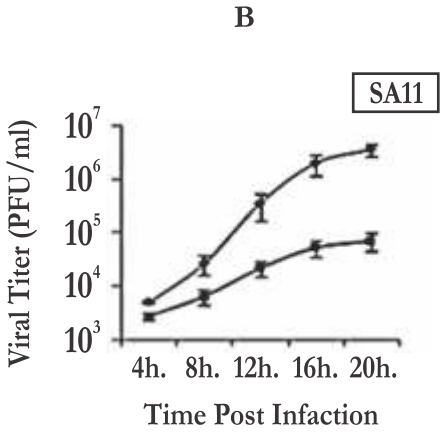
Investigator: Mamta Chawla-Sarkar

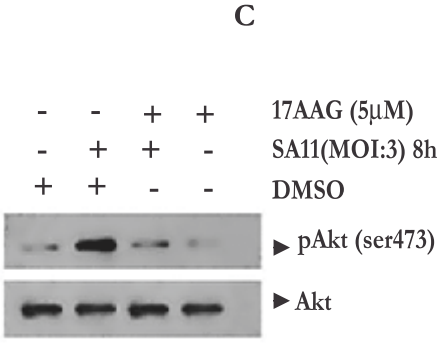
Influenza virus is a major human pathogen that causes epidemics and pandemics with increased morbidity and, especially in the elderly and those with pre-existing medical conditions. In recent years, outbreak of highly pathogenic avian influenza H5N1 and the swine flu H1N1 with pandemic potential has been reported worldwide with high mortality rate. However there was no systemic surveillance or report on Influenza disease burden in West Bengal in the past decade. During the last three years, the Influenza surveillance project at NICED has been crucial in providing information regarding Influenza strains in Kolkata and also the laboratory setup provided quick diagnosis of suspected human samples to the State government and ICMR HQ during the Avian Influenza outbreaks in West Bengal.

A total of 822 patients were enrolled during the study period. The sample collection was maximum during July-Oct due to higher load of various viral infections in Kolkata during monsoon season. The clinical samples were classified based on sex and duration of fever though no direct correlation was observed with Influenza positivity. Maximum number of symptomatic patients were in age group of 1-5 years. Out of 822 samples, obtained 53 isolates were obtained which were subtyped as H1N1 (1), H3N2 (29) and Inf B (23). The isolation rate was thus around 6.4%. The maximum number of isolates were obtained during June-July co-relating with monsoon season. All 822 samples were screened in parallel with Real time PCR to validate the sensitivity of tests and compare it with virus isolation. We observed 11.9 % positivity by Real time PCR (92/822) which is 2 fold higher than virus isolation.

In addition, during the H5N1 outbreak in poultry in West Bengal, clinical samples from suspected human cases were tested by RT-PCR and reports were sent to the state health department for patient management.







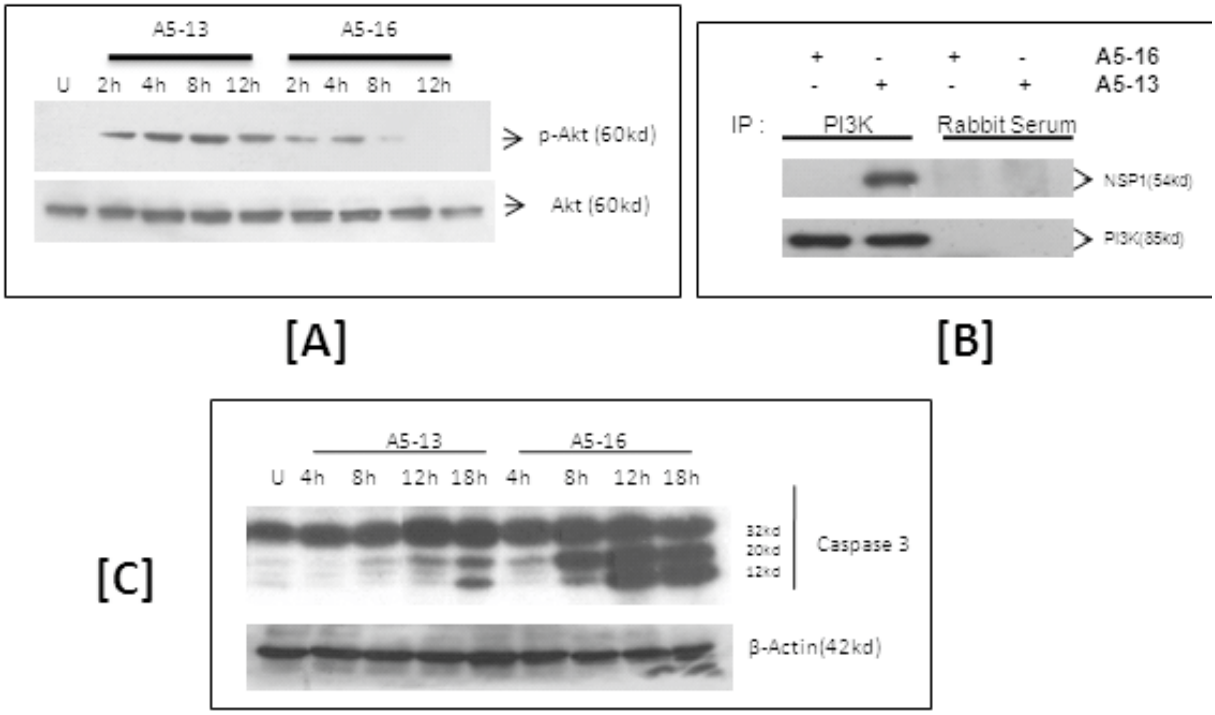
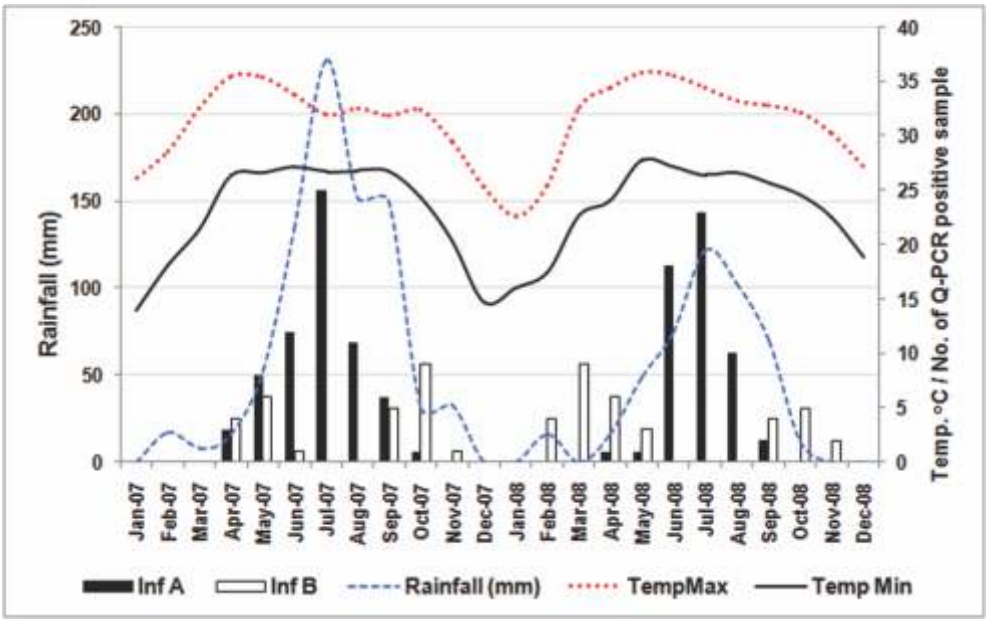


Figure 2



6. Detection and molecular characterization of complete nucleotide sequence of human picobirnavirus causing acute watery diarrhoea among children in Kolkata.

Investigator: B. Ganesh

The results of screening for picobirnaviruses in faecal specimens collected during a community based study showed that thirty four cases (3%; n=1112) were picobirnavirus positive. Based on their migration in PAGE, there were 26 (76.5%) large genome profile PBV and 8 (23.5%) small genome profile PBV. Picobirnavirus in 20 (59%) samples could be characterized further by RT-PCR. Molecular characterization using the reported primers PicoB25-PicoB43 for genogroup I and PicoB23-PicoB24 for genogroup II in RT-

PCR showed the presence of Genogroup I PBVs (47%; n=16) and genogroup II PBVs (12%; n=4); but the above primer sets could not successfully amplify 14 (41%) of the 34 PBV positive samples.

In the hospital based study between November 2007 and September 2008, five Picobirnavirus positive samples (0.6%; n=807) were collected. PBV showed 4 (80%) large genome profile and 1 (20%) small genome profile. As some picobirnavirus positive samples could not be successfully amplified, it is possible that the above primer sets may have a limited efficiency in detecting all the picobirnaviruses circulating in Kolkata.

The detection and partial molecular characterization of human isolates of picobirnaviruses were Genogroup I porcine picobirnavirus strains reported from Hungary. The phylogenetic analysis showed varying degree of genetic diversity amongst PBV strains detected within Kolkata and other countries.

To the best our knowledge, this is the first report of detection of several genogroup I porcine picobirnaviruses from children suffering from acute watery diarrhoea in Kolkata, India. Partial molecular characterization and sequence analyses of the Kolkata strains show that there exists distinct sequence heterogeneity among human PBVs warrants stringent surveillance of newly emerging variants of PBVs. Comparison of sequence data of emerging genotypes in the RdRp region as well as from other variable regions of the genome will be valuable to monitor the genetic diversity among these novel diarrhoeagenic strains of human PBVs. Thus, to understand the evolutionary relationships among different PBV isolates and to establish their taxonomy, further complete nucleotide sequence studies are in progress to analyze both RNA dependent RNA polymerase (RdRp) region as well as capsid protein encoding genomic segments respectively.

PRESENTATIONS AND VISITS

S. Chakrabarti

Delivered a talk on "HIV Vaccine-Any light at the end of the tunnel?" at the Calcutta School of Tropical Medicine, Kolkata in May, 2008.

Delivered a talk on "HIV/AIDS in India" at the conference sponsored by National Academy of Sciences, Kolkata Chapter during June, 2008.

Delivered a talk entitled "Highlight of the HIV/AIDS Research by ICMR" as an invited speaker at the Bigyan Mancha of Kolkata in a scientific awareness program.

Attended the investigators' meeting of PDVI, Seoul, Korea during September 2008.

Delivered a talk on "HIV Vaccine in India" at the 93rd Indian Science Congress Association meeting held at Shillong during January 3-7, 2009.

Presented a talk entitled "Molecular Characterization of HIV and development of the appropriate vaccine" as an invited speaker in the Indo-German workshop on Emerging Infectious Diseases held at the University of Hyderabad during February 2009.

Presented a poster entitled "Molecular Chracterization of HIV-1 circulating in north-eastern and eastern regions of India: Development of an appropriate vaccine" at the Keystone conference on Prevention of HIV/AIDS held at Keystone, Colorado, USA during March 23-26, 2009.

T. Krishnan

Presented a poster entitled "Genetic Diversity of Rotavirus Strains From Diarrhoea Cases in Eastern India (Triveni Krishnan*, Ganesh Chandra Sahoo, Debarati Chatterjee, Ganesh Balasubramanian, S.M. Nataraju, Anupam Mukherjee, Muktikant Nayak, Rittwika Bhattacharya, Trailokya Nath Naik, Mamta Chawla Sarkar, Ng Brajachand Singh and Nobumichi Kobayashi) at the 8th International Rotavirus Symposium held at Istanbul during June 3-4, 2008.

M. Chawla-Sarkar

Presented paper entitled "Natural plant products: an effective source for developing new anti-influenza virus drugs (Chawla-Sarkar M, Sarkar M, Agrawal A.S, Dey R)" at The Third European Influenza Conference held at Vilamoura, Portugal during September 14-17, 2008.

Presented paper entitled "Host Cellular Proteins - Key Determinants of rotavirus infection and pathogenesis (M. Chawla-Sarkar, D. Dutta, P. Bagchi and N. Kobayashi)" at the Asian-African Research forum on Emerging and Reemerging Infections held at Sapporo, Japan during December 15-16, 2008.

D. Dutta

Presented a paper entitled "Study of host cellular proteins required for viral replication and pathogenesis during Rotavirus-host cell interactions (D. Dutta, P. Bagchi and M. Chawla-Sarkar)" at the Keystone symposium on Pathogenesis and Control of Emerging Infections and Drug Resistant Organisms held at Bangkok during October 22-27, 2008.

M. Sarkar

Presented a poster entitled "Screening of Influenza Virus Strains by Real time RT-PCR: Rapid and Highly Sensitive Method compared to Virus Isolation (M.Sarkar, A. S. Agrawal, R. Dey, H. Kaur, M.S.Chadha, A.C.Mishra and M. Chawla-Sarkar)" at the 96th Annual Meeting on Indian Science Congress held at Shillong, India during January 3-7, 2009.

A. S. Agarwal

Presented a poster entitled "Respiratory Syncytial Virus Group B BA-IV Genotype Strains with Six Nucleotide Deletion are Circulating among Children with Acute Respiratory Tract Infection in Kolkata, Eastern India (A. S. Agrawal, M. Sarkar, S. Ghosh and M. Chawla-Sarkar)" at the 96th Annual Meeting on Indian Science Congress, Shillong, India during Jaunary 3-7, 2009.

SERVICE

SERVICES

1. Antisera Supply

Antisera prepared by the Division of Bacteriology for serodiagnosis of *V. cholerae* O1 (Ogawa and Inaba) and O139 are supplied in 2 ml vials free of cost to different non-profit Public Health Laboratories in the country.

2. Culture confirmation and serotyping

Culture confirmation and serotyping of *Vibrio cholerae*, *Shigella*, *Salmonella* and Diarrhoeagenic *Escherichia coli* received from different states was done in the Division of Bacteriology.

3. Phage typing of *V. cholerae* O1 and O139 strains

As a WHO Collaborating Center for Diarrhoeal Diseases Research and Training, NICED is working as a Vibriophage Reference Laboratory since 1968. We receive strains of *V. cholerae* from all parts of India and abroad for biotyping, serotyping and phage typing. This year we received a total of 555 strains from different institutions located in 7 states across the country. Of these, 493 (88.83%) representative strains confirmed as *V. cholerae* O1 biotype ElTor were included in phage typing study and reports have been sent to the appropriate authority. No *V. cholerae* O139 strain was received this year for phage typing.

4. Bioinformatics Centre

The Biomedical Informatics Center of the institute has stepped into the fourth year. Initiated with an extramural fund from ICMR, it has developed as a valuable adjunct to the current research activities of the institute and provides computational supports to research efforts in NICED and other Institutes and Medical Colleges in the area.

The center is located at the top floor of the new NICED building within the I.D. & B.G. Hospital campus. It is manned by two dedicated scientists (temporary positions, funded by ICMR) and its activities are supervised by Dr. S. S. Das, Scientist C of the institute. The center is equipped with 9 PCs connected by structured LAN and one GBPS internet connectivity, one LINUX Server (XEON 3.2 GHz dual processor with 2GB RAM, 72X5 GB HDD), one workstation (HP) with intel core, two printers (HP Color LaserJet 3800 and HP LaserJet 3052 Series PCL 6), one digital flatbed scanner with auto-feeder (HP Scanjet 8390) and several commercial software including GCG (for sequence analysis), Discovery Studio 2.0 (for molecular modeling and simulation), GOLD (for protein-protein interaction) and SPSS (for statistical analysis). A two day workshop on genome sequence analysis and protein modeling was organized on May 22-23, 2008 which was attended by young scientists, PhD and postdoctoral students as well as medical students from within and outside the institute.

5. Clinical Laboratory

The Public Health Laboratory is involved in providing service to the medical community by diagnostic testing, disease surveillance, applied/operational research, laboratory training and other services related to problems affecting human health.

The diagnostic facilities in the Research Support Laboratory employing state of art automated hematology, biochemistry and dry chemistry analyzer for testing clinical samples compliant with the Good Clinical Laboratory Practice (GCLP) as per the requirement of World Health Organization. The NACO Reference Laboratory (NACO-NRL) provides referral service for

confirmation of HIV detection. Immuno-assay and Molecular diagnostics techniques are employed for detection of blood borne and sexually transmitted agents like HIV, HBV, HCV, CT/NG and syphilis. The newly established Integrated Counseling and Testing Centre (ICTC) offers service to the community for HIV testing.The activities include (i) training of resource persons, officials, doctors, counselors and technicians (ii) monitoring of sentinel sites activities and testing laboratories and (iii) evaluation through data management.

Applied/Operational Research areas include clinical trial for development / application of new vaccine or drug, population health study with aim of identifying health risk factors, application of newer methods such as Dried Blood Spot (DBS) for immune / molecular diagnosis for reduction of operational complications.

Laboratory training and workshops are conducted for resource persons and trainers, laboratory specialists, doctors, technicians and students. Special emphasis is given for Good Clinical Practice (GCP) for scientists and Good Clinical Laboratory Practice (GCLP) and bio-safety for laboratory technicians. Expertise for the improvement of laboratories / program is provided to other governmental and non-governmental organizations including regional, national and international agencies and reference laboratories. Other activities include development of NACO Consortium for Quality Testing, evaluation of diagnostic kits for blood safety for national and international agencies, participating in external quality assurance scheme (EQAS) for clinical biochemistry and external quality assurance programme (EQAP) for hematology. The NICED has taken initiative to establish the NACO-Regional Institute-East for HIV Sentinel Surveillance for the states of West Bengal, Sikkim, Chhattisgarh and Andaman. The UNOPS (United Nation Office for Project Services) recognized NACO-National Reference Laboratory at NICED for Evaluation of diagnostic kits for HIV, HBV and HCV. The NACO recognized the National Reference Laboratory at NICED as a member of Consortium for Quality Testing where other members are NARI Pune, NICD New Delhi and NIMHANS Bangalore.

Activities of NACO National Reference Laboratory

National AIDS Control Organization (NACO) of Ministry of Health and Family Welfare, Government of India funds the HIV National Reference Laboratory of the Institute since 1992. The activities of the Reference Laboratory comprise of:

1) Confirmation of serum / plasma samples received from different surveillance and zonal blood testing centers located in different states of Eastern India.

2) Sentinel Surveillance for HIV infection.

3) Training man-power (Doctors and Medical Laboratory Technologists etc.) for HIV surveillance and laboratory diagnosis of HIV infection as and when requested by Institute of Serology, Govt. of India, State Health authorities, Hospitals etc.

4) EQAS and Panel Sera preparation for SRL of different states of Eastern India.

5) HIV, HCV, HBV & RPR kit evaluation for National AIDS Control Organization, West Bengal State AIDS Prevention and Control Society and other National and State agencies.

Between April 2008 and March 2009 a total of 2962 serum samples were screened by highly sensitive ELISA and positive samples were confirmed by either highly specific ELISA or Western Blot tests.

A) Sample screened for HIV Antibody by ELISA, Rapid and/or Confirmatory Test: (from April 1, 2008 to March 31, 2009).

Source of Samples	No. of Tested	No. of Positive
A. WEST BENGAL		
1. Drug Users	Nil	Nil
2. Command Hospital	77	74
3. People with High Risk Behavior	Nil	Nil
4. Patients from Hospitals	90	47
5. Miscellaneous	02	Nil
SUB TOTAL	**169**	**121**
B. OTHER STATES		
1. Jharkhand	04	04
2. Meghalaya	03	03
SUB TOTAL	**07**	**07**
GRAND TOTAL	**176**	**128**

B) HIV Sentinel Surveillance 2008

Sentinel surveillance was organized by West Bengal State AIDS Prevention and Control Society among the Antenatal Mother (ANC), STD & Gynae patients of West Bengal. Six sites allotted us, five ANC sites and one was STD & Gynae site. We screened 2250 samples for HIV, VDRL from November, 2008 to January, 2009 and the results (Positive individuals were 0.36% & 1.02% for HIV & VDRL respectively) of the same were communicated to the Project Director West Bengal AIDS Prevention & Control Society, Kolkata.

C) Kit Evaluation

We have evaluated diagnostic kits for West Bengal AIDS Prevention & Control Society, NACO, United Nations Office for Project Services (UNOPS) and manufacturers for detection of HIV, HBsAg, HCV antibody in serum / plasma by ELISA and Rapid tests. We evaluated 65 nos. of HIV kits, 86 nos. of HBsAg kits, 46 nos. of HCV kits and 11 nos. of RPR kits for detection of antibody by ELISA and Rapid tests from time to time for West Bengal State AIDS Prevention and Control Society, UNOPS and different kit manufacturing companies.

D) EQAS Programme of NACO

The serum samples for HIV testing under EQAS Programme were received from different states viz. Assam, Jharkhand, Meghalaya, Orissa and Andaman & Nicobar Islands and the results of the same were communicated to NACO and respective State AIDS Control Society. We also sent the serum panel set (10 Nos. each) to different State Reference Laboratories of Assam, Jharkhand, Meghalaya, Orissa & Andaman & Nicobar Islands. They sent the report in time.

Different State Reference Laboratories of Assam, Jharkhand, Meghalaya, Orissa and Andaman & Nicobar Islands sent the serum samples of Sentinel Surveillance '2008 for EQAS. We received total 1480 serum samples. Break up of samples from other states given below:

Serial No.	Name of SRL/ Testing Centres	Samples Sent		Sample rejected		Confirmed Result		No of Discordant
		HIV - ve	HIV + ve	HIV - ve	HIV + ve	HIV - ve	HIV + ve	
1	G. B. Pant Hospital, Andaman	112	10			112	10	Nil
2	Rajendra Institute of Medical Science, Ranchi, Jharkhand	215	11			215	11	Nil
3	MGM Medical College, Jamshedpur, Jharkhand	83	10			83	10	Nil
4	Patuliputra Medical College, Dhanbad, Jharkhand	193	24			193	24	Nil
5	Pasteur Institute, Shillong, Meghalaya	81	02			81	02	Nil
6	SCB Medical College, Cuttack, Orissa	290	19		10	Not sent any transport sheet		
7	VSS Medical College, Burla, Orissa	213	21	07		206	18	03
8	MKCG Medical College, Berhampur, Orissa	70	10			70	10	Nil
9	Guwahati Medical College, Guwahati, Assam	86	10			86	10	Nil
10	Silchar Medical College, Silchar, Assam	63	06	56		Not sent any transport sheet		
11	Assam Medical College, Dibrugarh, Assam	83	04	52		Not sent any transport sheet		
12	B. P. Civil Hospital, Assam	60	37		23			Nil
13	Dhubri Civil Hospital, Assam	73		03		Not sent any transport sheet		
14	Kanaklata Civil Hosp. Nagaon, Assam	73		11		62		Nil

Integrated Counseling and Testing Centre (ICTC)

Since the beginning of HIV/AIDS programme in the country NICED has been providing the testing support to the community and detected the second case of HIV positive in the country from Kolkata as early as in 1986-87. After NACO has taken charge of HIV/AIDS activities in India the counseling and testing service was provided with partial support from NACO/ WBSAP&CS.

From 2008 a fully NACO supported ICTC has started functioning at NICED.

The centre started functioning at NICED new building, ID&BG hospital campus, and providing service to local community, patients from ID hospital and different community service organizations. Service is available six days in the week (Mon-Sat) from 10AM to 5PM. Test results are available in the same day and in case of emergency within one hour.

This centre also providing Post Exposure Prophylaxis (PEP) to the ID hospital staff as and when required.

PRESENTATIONS AND VISITS

S. Panda, A. Deb and Dr. M. K. Saha

Participated in the Pre-Surveillance Orientation and Planning Workshop for Regional Institutes and Focal Persons for Surveillance at SACS organized by NACO and National Institute of Health & Family Welfare (NIH&FW) held at Chennai, India during September 20-21, 2008.

M. K. Saha

Participated in the Review meeting for HIV Sentinel Surveillance 2008 held at NACO, New Delhi on February 26, 2009.

6. Epidemic Investigations

A) Outbreak Investigations of Bihar Flood

S. Ghose and H. Koley

Four districts in North Bihar, Supaul, Araria, Madhepura and Purnea were devastated by the Kosi Flood in August, 2008 caused by breaching of embankment in the upstream of the river at the India-Nepal border and also by the periodic change in the river course. More than 2.5 million people were affected by the flood and the reported casualty was 1000. Two of our scientists, Dr. S. Ghosh and Dr. H. Koley of the Divisions of Epidemiology and Bacteriology, respectively were sent to the affected area to join the Central Team that included Dr. R. Katiyal, an entomologist of the National Institute of Communicable Diseases. The task of the Team was to suggest appropriate measures to control spread of infectious diseases and, in the case of a possible outbreak, suggest remedial measures.

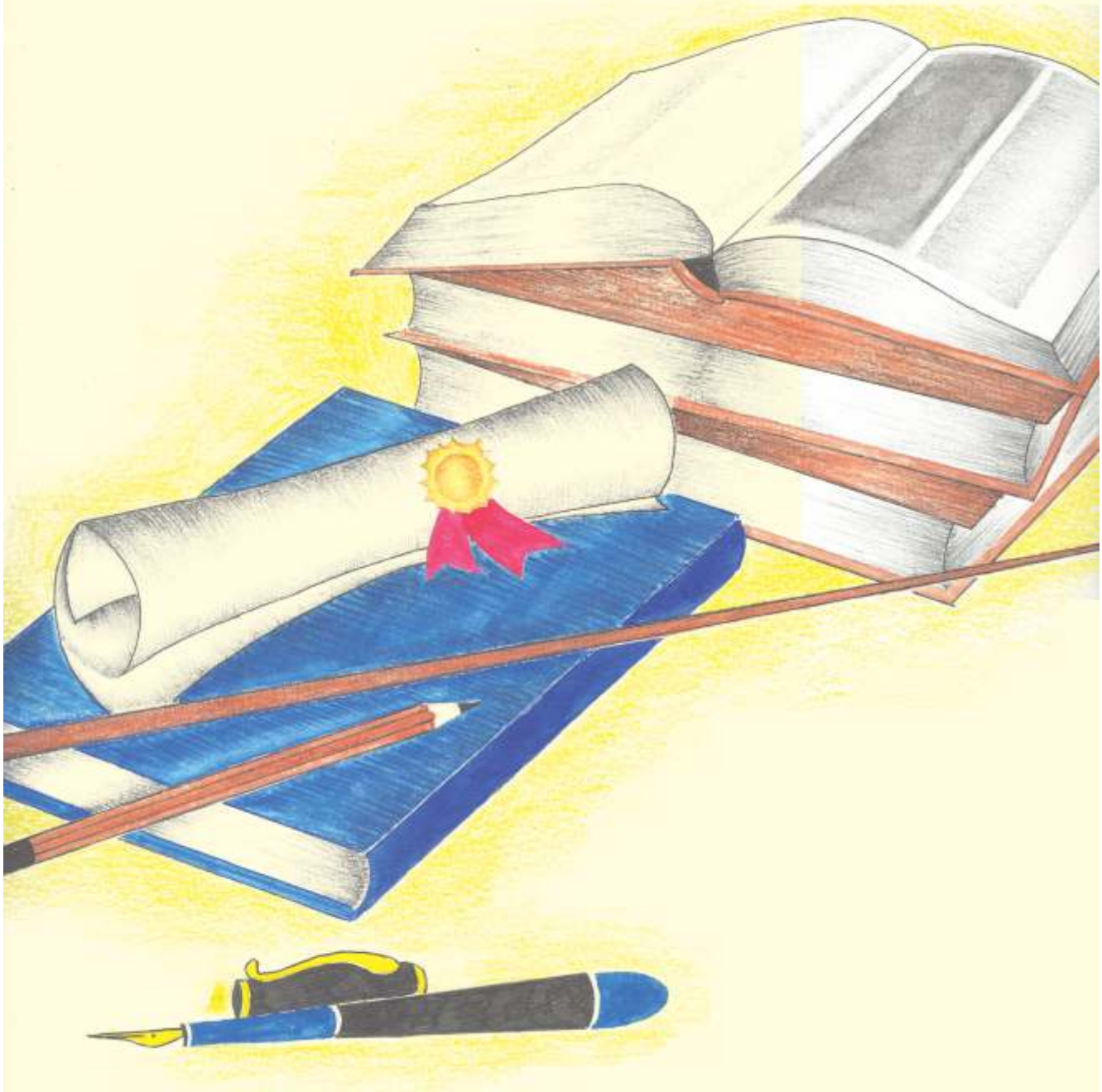
In the week beginning 15th September, 0.66 per cent of an estimated population of 1.5 million was affected with diarrhea. *V. cholerae* was isolated in 65.6% of the 32 stool samples collected from hospitalized patients with severe diarrhea. No death was reported. The strains were *V. cholerae* 01 Ogawa and were sensitive to ampicillin, ciprofloxacin, norfloxacin and tetracycline.

B) Outbreak of Avian Influenza in Assam

B. L. Sarkar

A suspected outbreak of avian influenza was reported from the district of Nalbari, Assam in December, 2008. Dr B. L. Sarkar, Division of Bacteriology, was deputed to assist the Central Public Health Team to assess the public health situation and to provide necessary assistance to the state health authorities. The Central Team visited several block Public Health Centres in and around Nalbari district for surveillance. No poultry birds were observed in the community. Nor was there any report of Avian Influenza from this area.

TRAINING



TRAINING

Bioinformatics workshop

Biomedical Informatics Centre of the National Institute of Cholera & Enteric Diseases (NICED) organized a two-day workshop during May 22-23, 2008. The aim of the workshop was to share know-how of the techniques and tools of Bioinformatics in present day research. The participants were from the NICED and other academic institutes. Two noted bioinformatics scientists, Prof. Subhasish Mukherjee of University of Calcutta and Prof. Dhananjay Bhattacharyya of Saha Institute of Nuclear Physics, Kolkata delivered lectures in the workshop.

Training Mission in Cholera Case Management and Research September 7-15, 2008

The training mission in Cholera Case Management and Research, jointly hosted by NICED, Bose Institute and Indian Institute of Chemical Biology (IICB), Kolkata, was held during September 7-15, 2008. The National Institute for Allergy and Infectious Diseases (NIAID) and National Institutes of Health (NIH), USA sponsored this training mission for the scientists working in the field of diarrhoeal diseases research. The aim was to reach out to the researchers who are working in the area of diarrhoeal diseases in particular, and other infectious diseases, in general. The objectives of the training included exchange of knowledge and experience, and develop collaborations that will hopefully blossom into a new era of collaborative science in the fields of diarrhoeal diseases and other infectious diseases research with the understanding of each others' perspectives. Twenty nine participants from Bangladesh, Canada, Japan, Kenya, Malaysia, USA, and Vietnam joined the programme.

Dissemination Workshop of Clinical Trial Registry in India (CTRI)

The workshop on Dissemination of Clinical Trial Registry in India was held on August 18, 2008 in the B. C. Deb auditorium of the NICED. This workshop was organized by the National Institute of Medical Statistics (NIMS), New Delhi with funding from World Health Organization. Participants from different parts of India attended the workshop. The mission of the workshop was to encourage all prospective clinical trials conducted in India to be registered before the enrollment of the first subject and to disclose details of the WHO's International Clinical Trials Registry Platform (ICTRP) as well as the additional items of CTRI data set. Dr. G. B. Nair, Director, NICED welcomed all the participants. The speakers on the occasion were Prof. Arvind Pandey, Director, NIMS and administrator of CTRI,

Dr. Surinder Singh, Drug Controller General of India, Dr. Atul Juneja, Research Officer, NIMS, ICMR and Dr. Abha Agarwal, Deputy Director, NIMS and coordinator of CTRI, NIMS, New Delhi. Prof. S. D. Seth Advisor to CTRI, NIMS, ICMR, New Delhi chaired the inaugural session.

Good Clinical Laboratory Practice Course organized by WHO / TDR Clinical Coordination & Training Centre in Collaboration with International Vaccine Institute and National Institute of Cholera and Enteric Diseases held from February 24 – 2 , 2009 at NICED, Kolkata, India.

Training of Laboratory Technician

We trained 12 Laboratory Technicians from different hospitals from different states for laboratory detection of HIV antibody along with a lecture on principles and operation of above tests. The training programme (one in each month) was organized at the request of Institute of Serology, Govt. of India.

Laboratory Staff Training

a) NACO and National Serology Reference Laboratory Australia workshop for harmonization of NRLs for kit evaluation and batch testing, July 21-24, 2009, NARI, Pune, India.

b) NACO workshop for External assessment of HIV testing quality of the National Reference Laboratories (NRLs), Sept 23-25, 2009. NIB, Noida, India.

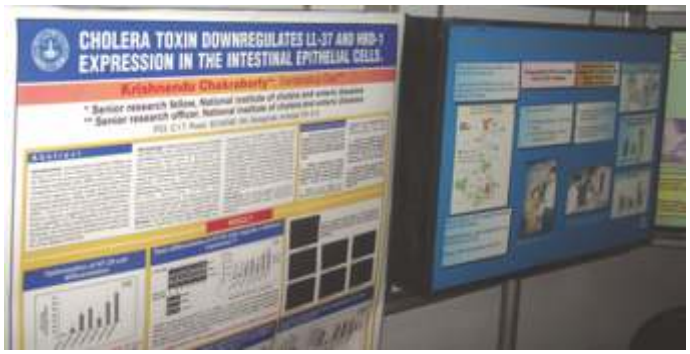
c) NACO Training workshop on testing of dried blood spot under HIV Sentinel Surveillance held on March 19-20, 2009 at National AIDS Research Institute, Pune.

EVENTS

EVENTS

Closing ceremony of JICA–NICED collaborative project on "Prevention of diarrhoeal diseases": The closing ceremony of the JICA-NICED project on "Prevention of Diarrhoeal Diseases" was held at ICMR Building at I.D. & B.G. Hospital Campus on June10, 2008. The guests of honour were Dr. S. K Bhattacharya, Additional Director General, ICMR, Prof. N. K Ganguly, former Director General, ICMR, Mr. Sanjiv Datta, Financial Advisor, ICMR, Mr. Mitsuo Takamatsu, Senior Consul, Consulate General of Japan, Kolkata, Mr. Tomoyuki Fuji, resident representative of JICA India office, New Delhi and Prof. Yoshifumi Takeda, former Chief Adviser, JICA-NICED collaborative project.

Felicitation of the employees on completion of 25 years of service: ICMR felicitates its employees who complete their 25 years of service in recognition of their service in the Council. Dr. G. B. Nair, Director of the Institute and Dr. Sekhar Chakrabarti, Scientist F, felicitated the employees of the Institute with flower bouquet, memento, a watch and a certificate on August 28, 2008.



Scientific Advisory Committee (SAC) Meeting: The Scientific Advisory Committee Meeting of the Institute was held during September 1-2, 2008. The meeting was chaired by Prof. N. K. Ganguly, former Director General, ICMR and Advisor, Translational Health Science & Technology Institute, National Institute of Immunology. In the meeting discussions were held on the ongoing and future projects proposals of the scientists of the Institute. The SAC members provided their inputs for modification of the project proposals and their proper execution.

XII National Expo 2008: NICED participated at the XII National Expo 2008 held during September 5-10, 2008 at Central Park, Salk Lake, Kolkata, organised by the Central Calcutta Science & Culture Organisation for Youth. NICED received one of the awards of excellence from the organisers of the Expo.

Hindi Divas: Hindi divas was celebrated on 14th September 2008, in the auditorium of this institute. All administrative and technical employees of the institute attended the program organized by the Institutional Rajbhasha Committee. Two eminent guests from the Rajbhasha Teaching Scheme, Kolkata Rajbhasha Department, Home Ministry, Govt. of India were invited to grace the occasion. Program started with inaugural speech by Director, Dr. G. B. Nair, where he encouraged the staff to use Hindi in their day to day activities. To encourage the employees, two competitions namely Dictation and Speech in Hindi were held. More that 30 employees participated

in each competition and 1st, 2nd and 3rd prizes were announced. Program ended with prize distribution by the Director, NICED and Vote of Thanks by the Chairperson, Hindi Committee.

Celebration of the Foundation Day of the Institute: The National Institute of Cholera & Enteric Diseases (NICED) began its journey in the year 1962. The Foundation Day of NICED was celebrated for the first time on February 18, 2008 at the initiative of the present Director, Dr. G. B. Nair. This year the day was celebrated on February 18. The Director, Dr. G. B. Nair felicitated all the alumni on this day for their contribution towards the growth of this Institute. The whole day ceremony concluded with a cultural programme.

Science Day: The National Science Day was observed on March 2, 2009. Prof T.K. Bose, Head, Department Of Forensic and State Medicine, Medical College, Kolkata delivered a lecture entitled "OVERVIEW OF FORENSIC MEDICINE." In his speech, he explained lucidly to the faculties, staff and research fellows of this institute how the science of Forensic Medicine can serve as a powerful legal tool in deliverance of justice.

Handing over ceremony of ELISPOT analyzer (CTL) to NICED: US Company, Cellular Technology Ltd. (CTL) has donated an advanced immune monitoring instrument (ImmunoSpot Analyser) to NICED, Kolkata, to assist its vaccine efforts. This effort is supported and funded by IVI and Bill & Melinda Gates Foundation. On March 3, 2009 a ceremony was held at the NICED, in which Prof. Paul V. Lehmann, founder and CEO of CTL, officially handed over the instrument to the scientist of the Institute. He also delivered a lecture on the recent advances in immune monitoring. Labindia, as the representation for CTL in India, also joined the event.

Young Scientist Fest: Young scientist's Fest was held at the NICED on March 16, 2009 to encourage research fellows to present their work. Fifteen research

scholars from several divisions of the Institute were nominated by the faculty members of the Institute. Prof. Arun K. Singh, Prof. Sujay Dasgupta, Prof. Indranil Chakraborty and Dr. Syamal Roy acted as the external judges. From amongst the participants, Mr. Abhisek Ghosal came first. His topic was"Molecular characterization of a prevalent colonization factor of enterotoxigenic Escherichia coli." Mr. Dipanjan Dutta came second. His topic was "Host Cellular Proteins: key determinants of rotavirus infection and pathogenesis." Mr. Krishnendu Chakraborty came third. His topic was "Stringent regulation of human cathelicidin (hCAP18/LL-37) expression in the mucosal epithelium by cAMP signal transduction pathway." The three research scholars were awarded certificates and cash prizes on April 6, 2009 at the Dr. S. C. Pal Oration lecture ceremony. Dr. S. C. Pal, a renouned microbiologist was one of the Directors of the NICED. Prof. Rita Colwel who delivered this year's Dr. S. C. Pal Oration lecture presented the awards to the research scholars.

Inauguration of Immunomonitoring laboratory: This facility was started at the NICED on March 19, 2009 to handle various types of assays and to assure quality data on various immunological parameters of clinical specimens from the vaccine trails. The laboratory was inaugurated by Dr. Tom Brewer, Senior Program Officer, Global Health Program, Bill & Melinda Gates Foundation in the presence of Dr. John Clemens, Director General, Dr. Cecil Czerkinsky, Deputy Director General, both from International Vaccine Institute, Seoul, Korea and Dr. G. Balakrish Nair, Director of the NICED.

Dr. S. C. Pal Memorial Oration 2009: Dr. S. C. Pal Memorial Oration was held on April 6, 2009. Late Dr. Pal, former Director of NICED and scientist par excellence had the rare insight in both fundamental and applied research activities. Prof. Dr. Rita R. Colwell, University of Maryland College Park and Johns Hopkins University Bloomberg School of Public Health and Senior Advisor and Chairman Emeritus, Canon U. S. Life Sciences; President and CEO, Cosmos ID, Inc delivered Dr. S. C. Pal Memorial Oration 2009. The title of her oration was "Climate, oceans and infectious diseases: the saga of cholera".

NICED Award 2009: This award has been instituted from the year 2008 onwards to recognize an Indian or a foreign scientist who has contributed substantially to the growth of the NICED. The award consists of a

silver plaque given to the Awardees. The 2009 NICED award has been given to Dr. John D. Clemens, Director-General of the International Vaccine Institute (IVI). Dr. John D. Clemens is an international expert on vaccine evaluation in developing countries. He is the chief architect to bring the IVI project at NICED that has enormously contributed to the growth of the Institute. The Director of NICED, Dr. G. B. Nair presented the NICED Award to Dr. John D. Clemens on April 6, 2009.

Dissemination meeting following completion of baseline study of the project titled 'Art and testimonial: a unique community based approach to reduce HIV/AIDS stigma in villages of West Bengal'.

A dissemination meeting was held following completion of baseline study and preliminary analysis of the Project Art & testimonial: a unique community based approach to reduce HIV/AIDS stigma in villages of West Bengal.' This is one of the 26 award winning projects of 1000 proposals submitted to the World Bank from South Asia. NICED provided space for this meeting and is evaluating the effectiveness of the intervention. SPARSHA, a CBO is the recepient of fund and implementor. MA/SA a Delhi based initiative provides inputs for artistic component. Participants of the meeting included community members, folk artists, people living with HIV and opinion makers including the Project Director of the West Bengal State AIDS Prevention and Control Society (WBSAP&CS) and the Director of the NICED. The 'Greater Involvement of People Living with AIDS' (GIPA)-Coordinator and the Joint Director of WBSAP&CS were also present in the meeting.

NICED PENSIONERS' WELFARE ASSOCIATION (NPWA)

NPWA was formed on April 4, 2008. The objective is to create a platform on which the re,tired employees of NICED can assemble to interact with each other. Initially 40 (forty) pensioners' became members. Now the total number is 58 and the number is gradually increasing. Formation of NPWA was possible only due to the active encouragement and cooperation of Dr. G.B. Nair, Director.

Till date, two Annual General Meetings had been held. Different problems of the retired employees were placed before the Director most of which have been solved through discussion with the Director and cooperation of the present staff of NICED.

In the year 2008 and 2009, Vijaya and Deepavali Sanmelan were held when different cultural programmes like Magic show, songs, recitation were performed and everyone present exchanged greetings.

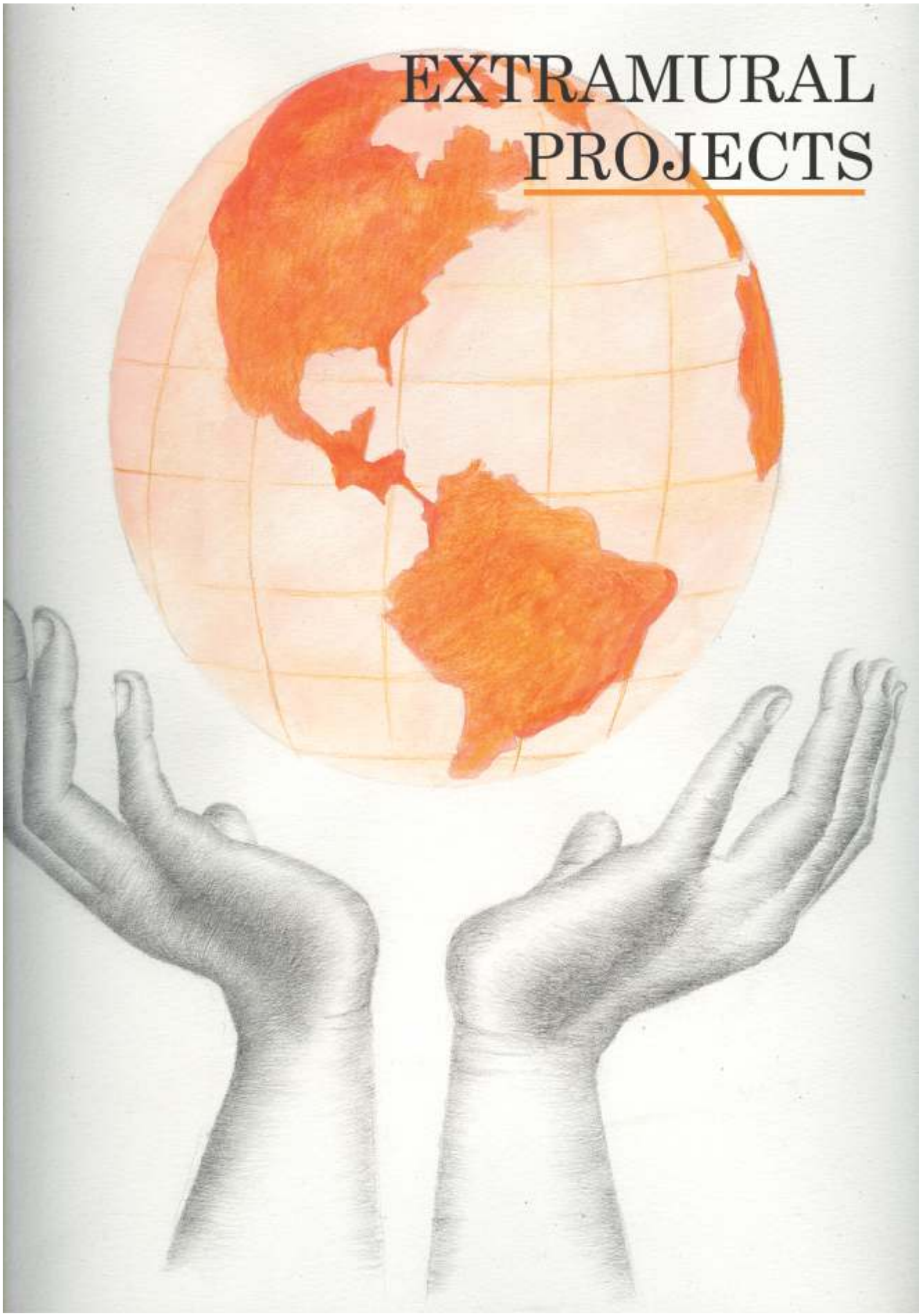
On January 8, 2010 amongst the natural surroundings of Bidhan Sishu Uddyan at Bidhan Nagar/ Kakurgachi a family picnic was organized. 25 members participated in the picnic most of them with their spouse. Dr. Nair & Mrs. Nair graced the picnic by their presence and inspired all. Hope our organization will prosper in the time to come.

Members of NPWA enjoying Magic Show of NICED Vijaya and Deepavali Sanmelan 2009.

Magic show by our member Shri Kajal Kanti Majumder - at Dr. B.C. Deb Auditorium.

Picnic of NPWA members with Dr. Nair & Mrs. Nair on January 8, 2010.

EXTRAMURAL PROJECTS



EXTRAMURAL PROJECTS

Project Title	:	Studies on Emerging and Reemerging Infectious Diseases
Investigators	:	G. B. Nair, T. Ramamurthy, T. Krishnan, S. Ganguly, M. K. Bhattacharya, M. Chawla-Sarkar, N. S. Chatterjee, A. K. Mukhopadhyay, H. Koley, S.S. Das, R.K. Nandy, S. Basu
Funding Agency	:	Okayama University, Okayama, Japan
Duration	:	2007-2010
Project Title	:	Evaluation of Anti-Typhoid and Anti-Diarrhoeal Activity of three Ethnomedicinal Plants of Tribal use from different parts of India.
Investigators	:	Shanta Dutta and Debprasad Chattopadhyay, ICMR Virus Unit, Kolkata
Funding agency	:	ICMR
Duration	:	2008-2009
Project Title	:	Molecular mechanism of enterotoxigenic *Escherichia coli* adherence in the intestine: host-pathogen relationship
Investigator	:	Nabendu Chatterjee
Co-Investigator	:	T. Ramamurthy
Funding Agency	:	Department of Atomic Energy, Govt. of India
Duration	:	2008-2011
Project Title	:	A study on differentiation-induced regulation of the immune response related genes in the intestinal epithelial cells
Investigator	:	Santasabuj Das
Funding agency	:	ICMR (Immunology task force)
Duration	:	2007-2010
Project Title	:	Biomedical Informatics Center of ICMR
Investigator	:	Santasabuj Das
Funding agency	:	ICMR (Immunology task force)
Duration	:	2007-2010
Project Title	:	Identification and Distribution of HIV-1 Encoded MicroRNAs in North-east Indian Population
Investigator	:	Santasabuj Das
Funding agency	:	ICMR
Duration	:	2006-2009
Project Title	:	A randomized controlled trial of the bivalent killed whole cell oral cholera vaccine in Eastern Kolkata, West Bengal, India
Principal Investigator	:	G. B. Nair
Co Investigators	:	D.Sur, B.Manna, S.Kanungo, S.K.Niyogi, B.L.Sarkar
Collaborating Agency	:	International Vaccine Institute, Seoul, Korea
Funding Agency	:	Bill and Melinda Gates Foundation, USA
Duration	:	2006-2009
Project Title	:	Diarrheal Disease in Infants and Young Children in Developing Countries
Investigator	:	Dipika Sur
Co- Investigators	:	T. Ramamurthy, B. Manna and S. Kanungo
Collaborating Agency	:	Centre for Vaccine Development, University of Maryland, USA
Funding Agency	:	Bill and Melinda Gates Foundation, USA
Duration	:	2007-2011

Project Title	:	Surveillance for dengue fever in eastern Kolkata, West Bengal, India
Principal Investigator	:	Sekhar Chakrabarty
Co-Principal Investigator	:	Dipika Sur
Co-investigators	:	Suman Kanungo, Byomkesh Manna, Shanta Dutta. Shyamalendu Chatterjee, Provash Sadhukhan
Collaborating Agency	:	Paediatric Dengue Vaccine Initiative, Seoul, Korea
Funding Agency	:	Bill and Melinda Gates Foundation, USA
Duration	:	2008-2010

Project Title	:	Regulation of Mucosal Immune Response by Synthetic Peptides of Porin: A Candidate Adjuvant
Investigator	:	Tapas Biswas
Co-investigator	:	Ratna Biswas
Funding agency	:	Department of Biotechnology, New Delhi, Govt. of India
Duration	:	2006 -2008

Project Title	:	Implementation of novel genotyping assay to understand complex HIV-1 epidemic in north-eastern and eastern parts of India.
Investigator	:	Sekhar Chakrabarti
Funding agency	:	Department of Biotechnology, Govt. of India
Duration	:	2006-2009

Project Title	:	Possible role of host genetics in relation to infection, progression and pathogenesis of HIV/AIDS. (Collaborative project with NII, India)
Co-Principal Investigator	:	Sekhar Chakrabarti
Funding agency	:	DBT/ICMR
Duration	:	2008-2011

Project Title	:	Establishment of Hospital based rotavirus surveillance for disease and strains and Noroviril infections in pediatric acute gastroenteritis and asymptomatic children
Investigator	:	Triveni Krishnan
Funding Agency	:	ICMR/ CDC
Duration	:	2005-2009

Project Title	:	Multisite Monitoring of Influenza Virus Strains in India- Phase I
Investigator	:	Mamta Chawla-Sarkar
Funding Agency	:	ICMR/CDC
Duration	:	2004-2009

Project Title	:	Enhanced Surveillance of Severe Respiratory Infection in Sadar Hospitals of Malda, Murshidabad and Birbhum districts
Investigator	:	Mamta Chawla-Sarkar
Funding Agency	:	DHS, Govt. of West Bengal
Duration	:	2009-2010

Project Title	:	Novel strategies to combat cholera
Investigator	:	Ranjan Kumar Nandy
Funding Agency	:	Indian Council of Medical Research (ICMR), Govt. of India
Duration	:	2009-2012

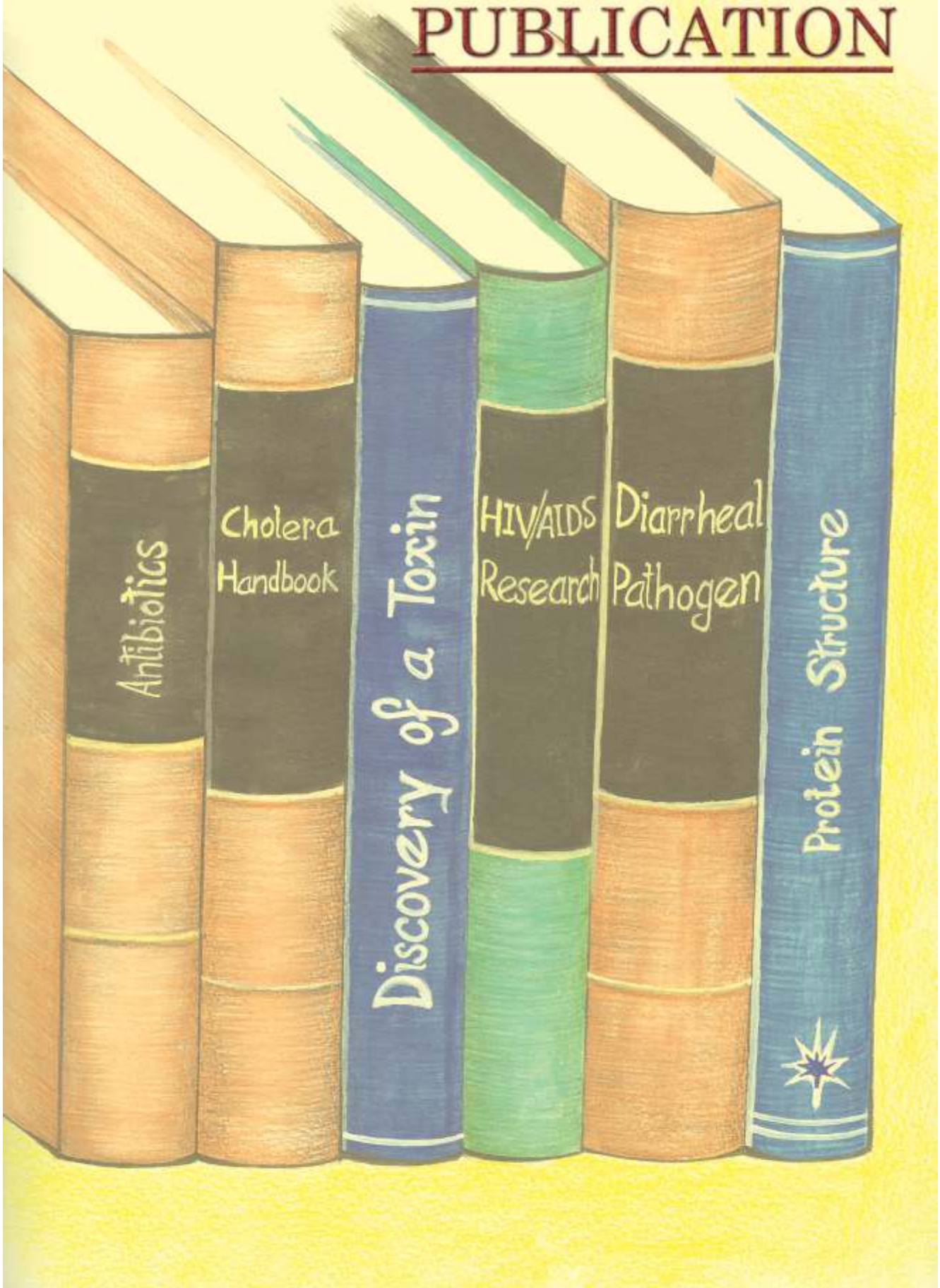
Project Title	:	A multicenter, double-Blind, randomized study to compare the safety and efficacy of Prulifloxacin versus placebo in the treatment of acute gastroenteritis in adult travelers. Novel strategies to combat cholera.
Investigator	:	Ranjan Kumar Nandy
Funding Agency	:	University of Texas, Houston, USA
Duration	:	2009-2010

Project Title	:	Comparative analysis of *luxO*, the quorum sensing master regulator, among O1, O139 and non-O1, non-O139 *V. cholerae* strains
Investigator	:	Ranjan Kumar Nandy
Funding Agency	:	Department of Biotechnology (DBT), Govt. of India
Duration	:	2007-2010

Project Title	:	Impact of Climate Change on Diarrhoeal Diseases in India - Phase I Study
Investigator	:	Anup Palit
Funding Agency	:	WHO assisted NICED-TERI collaborative project
Duration	:	2007-2010

Project Title	:	Elucidation and analysis of Biological Function(s) of *Helicobacter pylori* Restriction-Modification systems
Investigators	:	D. N. Rao (IISc, Bangalore) Asish Kumar Mukhopadhyay
Funding Agency	:	Department of Biotechnology (DBT), Govt. of India
Duration	:	2009 – 2011

PUBLICATION



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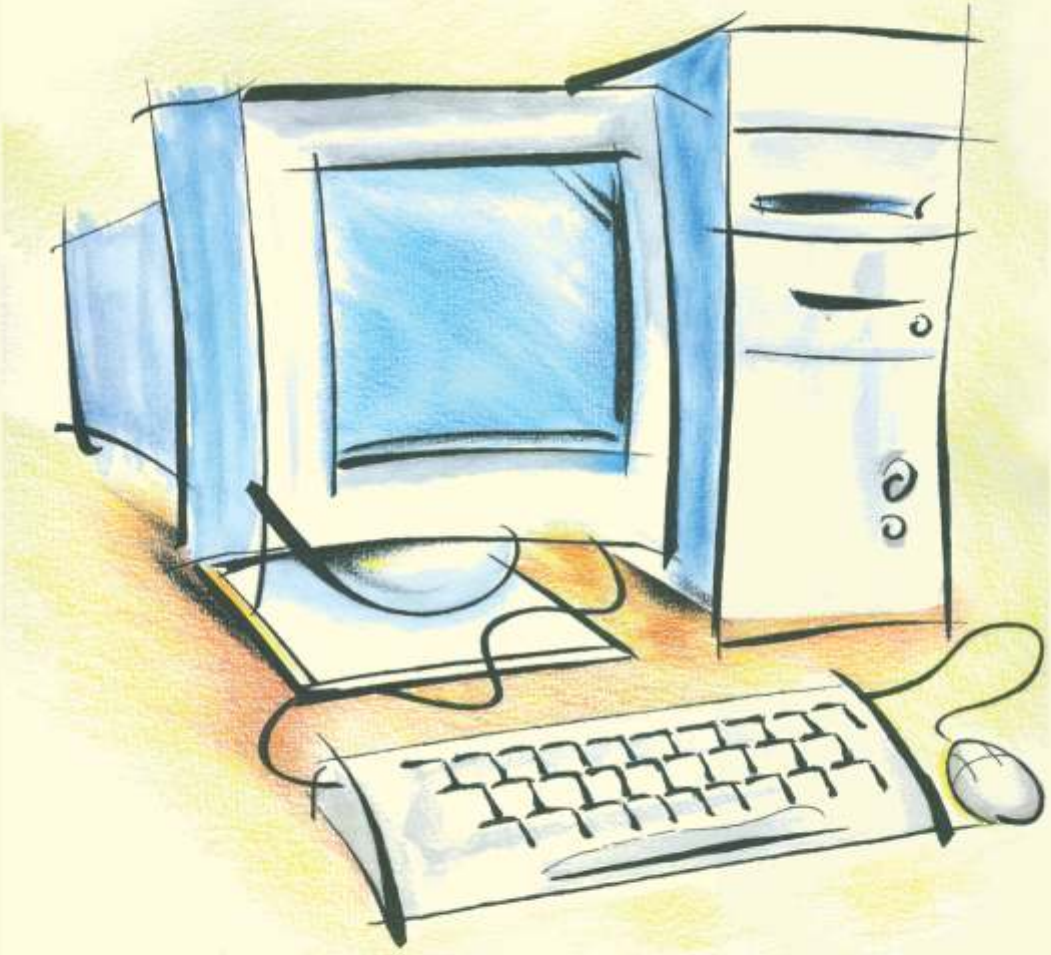
ADMINISTRATION



ADMINISTRATION

Administration provides operational support to the Office of the Director through activities, which include procurement and purchase of equipments, chemicals and stationery, fixing of fiscal responsibilities, budget preparation and execution, personnel administration, mailroom functions and supplies and, in short, for the management of human and material resources of the Institute. The primary objective of the Administration of the NICED, as in any other research organization is to promote and ensure smooth and uninterrupted execution of the reseach mandate of the Institute.

S. Karmakar
Administrative Officer

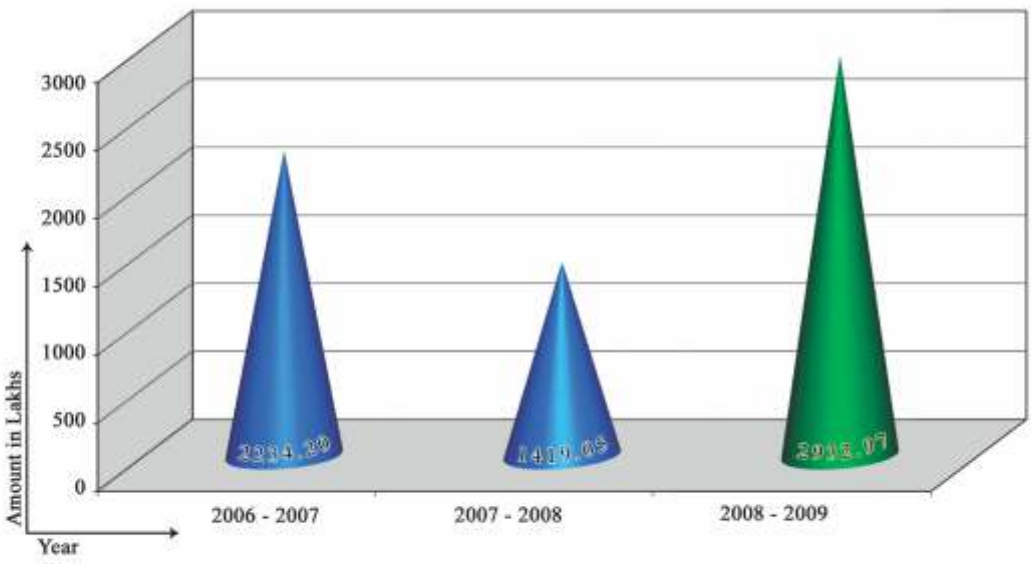
The Administration was involved in the following tasks:

- Supervision and coordination of Staff activities.
- Recruitment of Staff
- Conduct orientation programs for new employees
- Disbursement of salaries and maintenance of leave records
- Staff training and development, preparation of job descriptions, staff assessments and promotions
- Preparation and maintenance of budgetary and inventory controls and make recommendations to management
- Maintain management information systems (manual or computerised)
- Review and answer correspondence
- To provide secretarial or executive services for committees.

T. K. Chandra
Accounts Officer

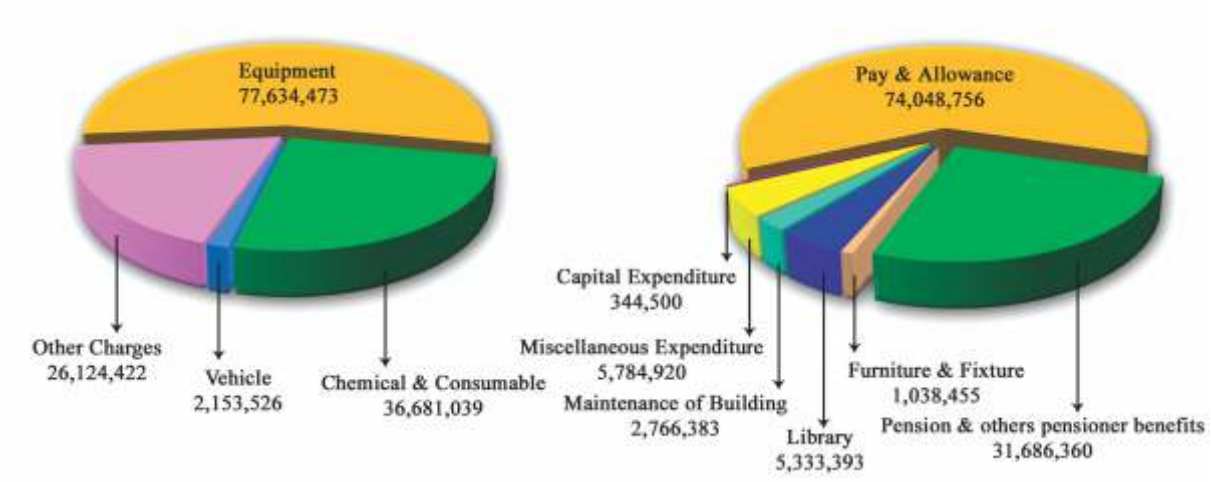
With the expansion of the Institute, the workload of the Administration, especially the Accounts and the Store and Purchase Sections of the Institute, has grown manifold over the years. The Institute is receiving liberal assistance from different Government, non-Government and International Agencies, e.g., IVI, WHO, UNICEF, DST, DBT, CSIR, CDC etc. in the form of more than 51 extramural projects along with Okayama project. Two new buildings have also been built up in I.D. & B.G. Hospital campus under the Institute to accommodate the expansion in activities. The Administration has no alternative but to manage this workload with the existing staff that has remained more or less the same over the years. The Institute has initiated the scheme of reorganizing the functioning of the entire Accounts and Establishment/Personnel sections through computerization and networking. All major Divisions of the Institute are proposed to be computerized.

Grants Received from ICMR



Year wise grant received (Rs. in Lakhs) from ICMR

2008-2009
Expenditure Breakup



Research & Development

Infrastructure

COMMITTEES OF THE INSTITUTE

1. INSTITUTIONAL ETHICS COMMITTEE

Honorable Justice Pinaki Chandra Ghose is a sitting judge of the Calcutta High court and the Executive Chairman of the State Legal Services Authority, West Bengal. He is the Vice President of Managing Committee of the Ramakrishna Mission Institute of Culture, Golpark. During his professional days he was, and is, connected with various Philanthropic Societies and charitable Institutions.

Dr. Dilip Mahalanabis, a pediatrician and clinician scientist par excellance is the Director of Society for Applied Studies, Kolkata. His path-breaking works on ORT earned him worldwide acclaim. He is the recipient of many prestigious awards for his contribution on the development and implementation of ORT. The prominent ones include Pollin Prize in Pediatric Research 2002 by the University of Columbia and Cornell, USA and Prince Mahidol Award 2006 in the field of Public Health.

Prof. Subir Kumar Dutta an eminent Consulatant Pathologist was the Former Dean, Faculty of Medicine, Calcutta University prior to which he held the post of the Head of the Department of Pathology, University College of Medicine, Calcutta University. He is the recepient of Eminent Teacher Award 2003 conferred on him by Calcutta University.

Prof. Biswapati Mukherjee was the Head of the Department of Pharmacology, University College of Medicine, Dr. B. C. Roy Postgraduate Institute of Basic Medical Sciences, University of Calcutta. He also graced the post of Professor and Executive Director of the newly formed S. N. Pradhan Centre for Neurosciences under the same university. His works on medicinal plants of India, marine natural products, diabetes, wound healing, ayurvedic metal preparations and acupuncture has earned him nationwide repute.

Prof. Asoke C. Ghose an eminent scientist was the Professor in Microbiology, the Bose Institute, Kolkata. Before joining the Bose Institute, he worked as Deputy Director in the National Institute of Cholera and Enteric Diseases, Kolkata. Being an elite scientist, he is a member of several scientific societies.

Prof. Mrinmoy Ghosh was the Acting Principal of I. D & B. G. Hospital, Kolkata. Presently he is a Consultant Physician, Professor and Head of Medicine of I. D. & B.G. Hospital.

Prof. Mrinal Kanti Chatterjee is the Principal of Dr. B. C. Roy Memorial Hospital for Children & B. C. Roy Polio Clinic and Hospital for Cripple Children. He is the President of Indian Academy of Paediatrics (West Bengal Chapter).

Mohammed Abdul Wohab is the founder Director of the Southern Health Improvement Samity (SHIS), Bhangar, South 24 Parganas, West Bengal. He has worked relentlessly for the welfare of the poor and downtrodden in the Sunderbans areas of West Bengal. His works towards humanity has brought him laurels the most notable amongst which are Mother Teresa Lifetime Achievement Award 2009 and Unsung Heroes of Compassion 2009 from the hands of His Holiness Dalai Lama in San Francisco, USA.

Mr. Amitrajit Ukil is a senior journalist (special correspondent) with The Telegraph, a Kolkata daily. He has authored several articles on Injecting Drug Users (IDUs) in Manipur and spread of HIV. Apart from articles on HIV and AIDS, he has authored several articles on health related ethical issues.

Mrs. Debolina Sarkar is a Lecturer in Human Rights in the Loreto College Kolkata prior to which she held the post of a Lecturer at the Ramkrishna Sarada Mission Vivekananda Vidyabhavan, Degree College for Women, Dum Dum, Kolkata.

Dr. Phalguni Dutta was the Head of Clinical Division of National Institute of Cholera & Enteric Diseases, Kolkata and is working as an Emeritus Medical Scientist (ICMR) post retirement. He has received many fellowships and awards and was an Editor, Indian Journal of Public Health and the Divisional Editor of The Child and Newborn.

2. SCIENTIFIC ADVISORY COMMITTEE

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Kolkata – 700 010

3. ANIMAL ETHICS COMMITTEE

Prof. (Dr.) T.N. Naik
Retired as Scientist F of NICED
Presently: Professor, National Institute of Science Education & Research
Bhubaneswar, Orissa (Chairman)

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Former Director of Medical Education & Ex-officio Secretary
Ministry of Health, Govt of West Bengal (Member)

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Scientist F, NICED, Kolkata-700010

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Professor, Dept of Physiology, University of Calcutta
Kolkata-7000009 (Member)

Dr. R. Chakraborti
Retired College Teacher, (Member)

Dr. Renuka Sengupta
Member / CPCSEA

Mrs Banani Ghosh
Member / CPCSEA

Dr. R.K. Sarkar
Veterinary Officer & In-charge Animal House, NICED. (Retired on January 31st, 2009)
Kindly note that we are in a process to recruit Veterinary Officer soon. In his absence Dr. Tapas Biswas, M.Sc. Ph.D, Scientist E will act as a Member Secretary.

4. INSTITUTIONAL BIOSAFETY COMMITTEE (IBC):

Constitution of the Institutional Biosafety Committee :

Dr. J. Rajan Vedasiromoni, Scientist F
IICB, Kolkata, Representative of DBT, Govt. of India

Dr. S. Chakrabarti, Scientist F
Director's nominee

Dr. S. S. Das, Scientist C
Biosafety Officer

Dr. S. Dasgupta, Associate Professor
External Member, Bose Institute, Kolkata

Dr. T. Ramamurthy, Scientist E
Member

Dr. N. S. Chatterjee, Scientist D
Member

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M. Ali Khan, Helper to Mechanic

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G.N. Patra, Laboratory Technician
K. Biswas, Laboratory Technician
K.C. Tudu, Laboratory Technician
S.R. Balmiki, Head Watchman
P. Turi, Head Sweeper
S. Hari, Lab. Attendant
N.C. Mondal, Sweeper

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R. J. Mukherjee, Technical Officer
A. R. Das, Care-taker
S. Parui, Electrician
A. Sarkar, Plumber
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Kanu Dey, Laboratory Assistant
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